Alexander Galkin,
Nadiia Gorchakova,
Ganna Zaychenko,
Olena Golembiovska,
Larisa Bondarenko,
Oleksandra Dmytrenko,
Maryna Arkhypova

EFFICIENCY AND SAFETY ISSUES OF MODERN MULTI-COMPONENT HERBAL MEDICINES

Monograph

National Technical University of Ukraine
“Igor Sikorsky Kyiv Polytechnic Institute”
Bogomolets National Medical University

Efficiency and Safety Issues of Modern Multi-Component Herbal Medicines

Monograph

Editors: Alexander Galkin, Nadiia Gorchakova

Approved by
the Academic Council of the National Technical University of Ukraine
“Igor Sikorsky Kyiv Polytechnic Institute”
and the Academic Council of the Bogomolets National Medical University

Kyiv
Igor Sikorsky Kyiv Polytechnic Institute
2024

UDC 615.32.015+ 615.017 + 615.038(02)
E27

Approved by:
the Academic Council of the National Technical University of Ukraine "Igor Sikorsky Kyiv Polytechnic Institute" (the minutes No. 10, November, 6th, 2023) and the Academic Council of the Bogomolets National Medical University (the minutes No. 4, November, 30th, 2023)

The author's team:
Alexander Galkin, D.Sc., Prof., ***Nadiia Gorchakova***, D.Sc., Prof., ***Ganna Zaychenko***, D.Sc., Prof., ***Olena Golembiovska***, Ph.D., ***Larisa Bondarenko***, D.Sc., ***Oleksandra Dmytrenko***, ***Maryna Arkhypova***

Reviewers:
Alexander Reznikov, D.Sc., Prof.,
Academician of the National Academy of Medical Science of Ukraine,
Corresponding Member of the National Academy of Science of Ukraine,
State Institution "V. P. Komisarenko Institute of Endocrinology and Metabolism"

Tetiana Garnyk, D.Sc., Prof.,
Association of Specialists in Traditional and Alternative Medicine of Ukraine

Iryna Kudryavtseva, D.Sc.,
STADA-Ukraine

E27 **Efficiency and Safety Issues of Modern Multi-Component Herbal Medicines** : Monograph / A. Galkin, N. Gorchakova, G. Zaychenko et al. ; Editors : A. Galkin, N. Gorchakova. – Kyiv : Igor Sikorsky Kyiv Polytechnic Institute, Publ. house "Polytechnica", 2024. – 264 p.
ISBN 978-966-990-104-0

The monograph summarizes research on the safety and effectiveness of multicomponent herbal medicinals in the treatment of gynecological, hepatobiliary and cardiovascular diseases, as well as herbal antivirals etc. The work covers evidence-based phytotherapy, pharmacotherapy, safety profiles of herbal combinations, and drug development based on them.

This monograph is an important resource for researchers and health professionals interested in the benefits of herbal medicine.

UDC 615.32.015 + 615.017 + 615.038(02)

ISBN 978-966-990-104-0

CONTENT

The monograph is dedicated to the 125th anniversary of the National Technical University of Ukraine "Igor Sikorsky Kyiv Polytechnic Institute"

INTRODUCTION

Humanity has long used the medicinal potential of plants. Phytopreparations are widely used both in traditional and evidence-based medicine. Medicinal plants are the basis for the development of medicines that are used to treat diseases of all organs and systems. The rapid development of medical chemistry and organic synthesis in the second half of the 20th century somewhat overshadowed the successes of pharmacognosy and phytopharmacology. However, herbal medicines continued to prove their safety and effectiveness. It is worth mentioning the winner of the Nobel Prize in Physiology and Medicine in 2015, Tu Youyou, for the development of drugs based on artemisinin, which is isolated from the *Artemisia annua* and is used to treat malaria. With the rapid development of pharmaceutical chemistry at the turn of the 21st century, the issues of standardization of herbal preparations, which are currently being successfully solved by researchers, became relevant. A permanent trend is a complex approach to the pharmacotherapeutic design of herbal medicines, which involves the combination of several medicinal plants or the inclusion of individual chemical substances (synthetic or natural origin) in the composition of the finished dosage forms. It's obvious that such an approach complicates the task of proving the safety and effectiveness of such medicines, but opens new perspectives in the treatment of current human diseases.

This monograph is a summary of the work of the author's team on substantiating and proving the safety and effectiveness of various phytocompositions, which are medicinal products (fixed combinations) or medical devices. Our efforts were focused on solving the following problems: the use of herbal preparations for the treatment of gynecological diseases with endocrine genesis, disorders of the hepatobiliary system, and cardiovascular diseases.

Particular attention is paid to the development of herbal antiviral drugs, the safety and effectiveness of restveratrol preparations, as well as medical devices containing phytoextracts.

With a focus on evidence-based medicine, the monograph encompasses a wide range of herbal medicine topics. It investigates the utilization of medicinal plants in endocrine gynecology, shedding light on the pharmacotherapeutic substantiation of their use in this specialized area. Additionally, it explores the safety aspects of combining ursodeoxycholic acid, taurine, and artichoke extract in diseases of the hepatobiliary system, providing valuable information for clinicians and researchers in this field.

The monograph also addresses the efficacy profile of a homeopathic combination for the prevention and treatment of influenza and acute respiratory viral diseases. By analyzing the current safety data of complex herbal medicines with sedative and cardioprotective actions, it offers valuable insights into the potential benefits and risks associated with these treatments.

Moreover, the monograph investigates a perspective fixed combination for the treatment of hepatobiliary system diseases, substantiating their pharmacotherapeutic properties and assessing their pharmaceutical quality profile. It also explores the current state of antiviral herbal preparations development, examining the potential applications of plant-based compounds in combating viral infections.

The assessment of plant extracts based medical devices in the form of rectal use suppositories is another important aspect covered in this monograph. By providing a biological assessment of these innovative medical devices, it contributes to the understanding of their effectiveness and potential applications.

Furthermore, the monograph dives into the pharmacological substantiation of new drug elaboration based on resveratrol, a natural compound with diverse therapeutic properties. It explores the possible mechanisms of resveratrol's influence on the reproductive system in ovariectomized rats, shedding light on its potential applications in this area.

Lastly, the monograph investigates the differences in NO/SH-mechanisms of neuron damage in experimental VCD-hypoestrogenemia under the impact of resveratrol. By exploring these mechanisms, it enhances our understanding of resveratrol's potential neuroprotective effects and its implications for neuronal health.

Overall, the chapters of this monograph contribute to our understanding of the efficiency and safety issues surrounding modern multi-component herbal medicines. The comprehensive analysis presented within this work serves as a valuable resource for researchers, healthcare professionals, and individuals interested in the intersection of herbal medicine, pharmacotherapy, and patient care.

The authors believe this monograph will be helpful for specialists in pharmacology, pharmacy, pharmacognosy, biopharmacy, and other healthcare fields. Readers of this monograph will gain valuable insights into the therapeutic potential, safety considerations, and future directions of multi-component herbal medicines, ultimately contributing to the advancement of evidence-based healthcare practices and the well-being of individuals.

The authors extend sincere appreciation to Dmitry Kozak for invaluable assistance in English translation and to Elena Striga for sharing the findings of her dissertation research, which greatly contributed to the preparation of this monograph.

CHAPTER 1.
PHARMACOTHERAPEUTIC SUBSTANTIATION OF THE MEDICAL PLANTS USE IN ENDOCRINE GINECOLOGY

Gynaecological diseases are female reproductive system diseases manifested with different signs and symptoms. Gynaecological problems in women are quite common. Moreover, the number of women with complaints of genital diseases increases significantly every year. This is due to the variety of factors both internal and external environment of the female body.

All gynaecological diseases are divided into 3 groups, taking into account the causes provoking a particular disease. The diseases associated with infection in women belong to the first group; the second group includes the endocrine system pathology and hormonal disorders of the female organism; the third group comprises diseases accompanied by the hyperplastic or dystrophic changes and neoplastic processes.

Gynaecological diseases caused by various hormonal or endocrine shifts manifest by symptoms of menstrual irregularities. In addition, dysfunctional uterine bleeding may occur as a result of hormonal abnormalities, leading to secondary infertility in women, which requires treatment [1].

The neurometabolic endocrine syndrome is a polyetiological complex syndrome characterised by a disbalance of the hypothalamic-pituitary regulatory mechanisms, followed by metabolic, menstrual and fertility disorders. About a third of all patients with this syndrome are those with menstrual and reproductive dysfunctions associated with excessive weight.

The neurometabolic endocrine syndrome pathogenesis is based on the hypothalamus dysfunction with subsequent pathological regulation of the pituitary, adrenal glands and ovaries influenced by the above-mentioned factors [2].

A violation of regulatory mechanisms occurs in the hypothalamus due to the pathology of the tropic releasing hormones of the pituitary gland and neurotransmitters, associated with their impaired synthesis or degradation, and also

with an alteration in the receptor apparatus of the hypothalamic structures, i.e. the number of receptors and/or their sensitivity to the regulatory agents.

The hormonal profile in the syndrome shows increased levels of adrenocorticotropic hormone, luteinizing hormone, prolactin, cortisol, testosterone, insulin, and 11-oxycorticosteroids; a slight decrease in oestradiol concentration with a significant decrease in progesterone levels; and fluctuating levels of 17-ketosteroids, follicle stimulating hormone and somatotropic hormone excretion, all within the age normal range. The metabolic changes are accompanied by abnormal carbohydrate and lipid metabolism, hyperglycaemia or reduced glucose tolerance; hypercholesterolaemia, hypertriglyceridaemia, dislipoproteidaemia with increased low and very low-density lipoproteins and an increased atherogenic index [3-4].

Gynaecological diseases include neoplastic processes, not necessarily malignant, cystic masses in the ovaries, uterine myomas, structural changes in the cervix like pseudoerosion, erosion and ulcerative changes are possible.

Medicinal plants are a traditional raw material for drug manufacturing. Nowadays one third of drugs are derived from herbal raw materials. High efficiency of phytotherapy, confirmed by the centuries-old experience, determines the wide use of medications based on plant-derived raw materials in clinical practice. Phytopreparations are attractive to many clinicians because of a minimum number of side effects during their administration and no interactions with other medications [5-6].

A phytopreparation is a finished medical product with a trade name, containing as its active ingredient a plant, parts of plant material or their combinations in raw condition or processed form. It should be particularly emphasised that phytotherapy is of the utmost interest because of its near-total absence of adverse reactions and the potential for its use in different age groups. Clinical experience and literature data prove that adverse reactions to herbal medicinal products are five times less frequent than to other pharmacological agents; in addition, herbal medicinal products have significantly fewer contraindications,

therefore they can be used for longer periods of time and therapeutic effect is achieved more slowly [5–6].

The medicinal plants can be indispensable in the treatment of many gynaecological diseases. Various plants are used to treat dysmenorrhoea, uterine bleeding, and climax. The medicinal plants most abundantly used in gynaecology are calendula flowers; seeds, roots and leaves of parsley; flowers and leaves of chaste tree (*Vitex agnus-castus*); flowers and leaves of meadowsweet (*Filipendula ulmaria*); herbs and flowers of yellow bedstraw (*Galium verum*), etc. [5–6].

1.1. General characteristics of frequently used plant substances in gynaecology

Calendula (*Calendula officinalis* L.) is an annual plant in the family Asteraceae.

The medicinal properties of calendula have been used for millennia in traditional and conventional medicine. The raw materials are anthodia and flower ligules, administered in the form of infusions and tinctures.

The anthodia contain β-carotene; lycopene, violaxanthin and rubixanthin; neolycopene A; citraxanthin; flavochrome; flavoxanthin, and chrysanthemaxanthin. The total amount of these carotenoids in the marginal flowers is about 3 %. Moreover, the anthodia contain ascorbic acid, essential oil (about 0.02 %), resins (about 3.44 %), mucilage (up to 4 %), including nitrogenous mucilage (up to 1.5 %), albumins (0.64 %), acids – malic (6.84 %), pentadecyl and traces of salicylic acids; and minor amounts of alkaloids [7–9].

Calendula preparations have anti-inflammatory, bactericidal, hypotensive, sedative, and cardiotonic action. Bactericidal properties of calendula are significant against a number of pathogens, especially staphylococci and streptococci. The pharmacological activity of calendula tincture, juice and powder continues to be investigated [10]. Calendula preparations are widely used in in traditional and conventional medicine.

Clinically, it has been defined that its infusion, juice, powder and extract reduce intoxication, eliminate dyspepsia (belching, nausea, vomiting, pressure sense in the epigastric area), and improve sleep and appetite.

Calendula preparations in the form of ointment or emulsion are used mainly as an anti-inflammatory agent to treat minor wounds, cuts, bruises, gangrene, burns, frostbites, furunculosis, sycosis, and impetiginous eczema.

In gynaecological practice, these preparations are administered for the treatment of cervical erosions, trichomonal colpitis in the form of vaginal douche with solution of calendula tincture. Infusion and tincture of the plant is successfully used in amenorrhoea and oligomenorrhoea as the menstrual cycle regulators. In enemas, calendula is prescribed in the treatment of proctitis and paraproctitis. Calendula is widely used in the oral cavity and throat diseases, gingivitis, periodontitis, and thrush in children. Calendula tincture is often used to treat tonsillitis as a gargle, either alone or in combination with sulfanilamides and antimicrobial agents [11].

In periodontitis, calendula is used by oral irrigation before and after dental deposit removal in order to put turundae abundantly wetted in the concentrated solution of calendula tincture into the pathological gingival pockets. At home, instead of toothbrushing in periodontitis, it is advisable to rinse the mouth using 2 % solution of calendula tincture. Patients are also recommended to take vitamins and general tonic agents. As a result of the treatment carried out the inflammatory symptoms in catarrhal gingivitis are suppressed in patients and gingival discharge stops with subsequent healing of the lesional tissues. The treatment outcomes using calendula preparations were persistent.

Calendula tincture is also used in blepharitis. In patients with severe persistent recurrent squamous blepharitis there was a significant improvement after the very first procedures, and there was a complete clinical recovery at the end of treatment (about 20 applications) in the overwhelming majority of patients. The therapeutic value of calendula preparations is definitely related to the topical protective effect on the damaged tissues.

As a general tonic agent, calendula is used for dystrophic processes in the mucous membranes of the digestive tract. The plant is quite effective for a variety of gastrointestinal diseases, such as gastritis, colitis, enterocolitis, and cholangitis. There is some evidence that it has the ability to increase appetite. Calendula is commonly used for inflammatory liver diseases (jaundice, hepatitis) and spleen diseases. Infusion, tincture and decoction of calendula flowers act as diuretic and diaphoretic in the bladder diseases (calculus and sand formation), scrofula, rickets, nervous fever, dizziness, bronchitis, gastritis, gastric and duodenal ulcer. Calendula preparations are also used to treat heart diseases accompanied with palpitations, shortbreathing, and oedema. By strengthening the cardiac function, it helps to reduce swelling. This is also facilitated by the diuretic and diaphoretic properties, which are typical for the plant. Calendula tincture was clinically well tolerated by patients. Due to its low toxicity, the maximum dosage has not been established [7–9].

Common toadflax (*Linaria vulgaris* Mill.) is a perennial plant in the figwort family.

It contains the alkaloid peganin, flavonoid glycosides, linarin, which splits into acacetin and rutinose; neolinarin, which forms pectolinarigenin and rutinose after splitting; and pectolinarin. The herb contains phytosterol, N-tricantane and small amounts of ascorbic acid. The alkaloid peganin makes a significant contribution to the pharmacological action of common toadflax [12–13].

The toxicological and pharmacological properties of alkaloid peganin contained in the common toadflax have been studied comprehensively [10]. The alkaloid peganin has been found to cause general agitation and increase motion activity in animals.

Apart from alkaloid peganin, common toadflax contains flavonoids. The sum of toadflax flavonoids is low-toxic and has a considerable therapeutic window. Toadflax tincture has been found to increase blood pressure, simultaneously increase pulse wave and have a pronounced inotropic effect that occurred 3–5 minutes after its administration. Toadflax tincture also stimulates smooth muscles and increases

urinary excretion by 27–34 %, lasting for up to 2 days after termination of the drug administration. The latter is due to its stimulating effect on the heart.

Toadflax galenical preparations have long been used in patients with intestinal atony. The use of toadflax preparations in the clinical settings proved that toadflax acts as a laxative gently and reliably without any adverse reactions. The effect of toadflax preparations was also observed in chronic constipation. Toadflax is low-toxic; no adverse reactions were noted even during its long-term administration.

Clinical trials have been conducted on toadflax since 1958 after peganin was studied and its valuable pharmacological properties were discovered [14].

The alkaloid peganin was analysed in post-surgery atony, paresis and paralytic intestinal obstruction (after removal of thrombosed lower extremity veins, spinal compression fractures). Usually after 2 days there was independent evacuation of gas and intestinal peristalsis improved. In some cases the effect occurred after 2 hours. The efficacy of peganin in this respect was superior to other similar drugs. An administration of peganin in patients with muscular dystrophy showed that it improved the patients' general state, increased muscle strength and range of active motions in the limb joints; sometimes it was observed the restoration of previously lost tendon reflexes, improvement of the muscular electroexcitability indices, and in some cases a decrease in urinary creatinine. The peganin efficacy in myopathies was manifested by a considerable increase in the amplitude of electrical oscillations together with subjective sensation of increased muscle strength, restoration of tendon reflexes that were absent for several years, and increased movement volume. A higher preparation efficacy was noted after intramuscular injections. By contrast, peganin did not cause adverse reactions inherent to the galantamine. There were also no serious cardiovascular complications after pachycardine and glutamic acid administration. An adverse reaction with the use of peganin was laxative action [12–15].

In traditional medicine, common toadflax is widely used as a diuretic, laxative, for jaundice, heart disease, and as an anthelmintic agent.

Thanks to the biological activators contained in common toadflax, its aqueous infusion and extract relieve aching pain in nephrolithiasis, act as diuretic factors in uric acid diathesis, especially in phosphaturia. The large amount of organic acids contained in common toadflax is responsible for the acidic urine in salt diathesis and inflammatory conditions in the urinary tract. With transition of alkaline urine to acidic, the excretion of uric acid crystals falls dramatically [16].

A small amount of common toadflax is used for headache with vomiting (Meniere's syndrome) and nocturnal urinary incontinence. Herb tea made from common toadflax is popularly drunk in case of liver disease. In combination with oak bark and water pepper herb, common toadflax preparations are used in haemorrhoids in the form of an ointment with pork lard. When the ointment is administered per rectum, there is pain relief and inflammation reduction observed. For topical use, toadflax infusion is usually applied for washing, compresses for haemorrhoids, furunculosis, ulcers, and various skin diseases [12–13].

Meadowsweet (*Filipendula ulmaria* L.) is a perennial herbaceous plant in the family Rosaceae.

The flowers contain up to 0.2 % essential oil, salicylic acid, colouring agents and tannins, 4 to 9.7 % flavonoids, higher fatty acids, and wax. Its leaves and shoots contain up to 11 % protein, 3 % fat, 33 % fibre, up to 6 % ash, and up to 300 mg % vitamin C. Moreover, some phenolcarboxylic acids and their derivatives (caffeic, ellagic), 3.6 to 16.8 % of tannins, 9.6 to 10.7 % of flavonoids, catechins were found. The meadowsweet roots, in addition to the mentioned above components, contain methyl salicylate essential oil, and due to this can replace the chemical methyl salicylate. In domestic and Western European traditional medicine, the underground and aerial plant parts are used. Meadowsweet has antispasmodic and astringent effects in the gastrointestinal diseases, as well as wound-healing, haemostatic, diaphoretic, and diuretic activities. Owing to salicylic glycoside, the plant is used in the treatment of rheumatism, colds, and gout. Decoction from the underground plant parts and the flowers is treated nervous disorders, hypertension, and coughs. It is recommended as an antihelminthic and analgesic agent [17–20]. In Tibetan and

Mongolian traditional medicine, the aerial part is used for the pulmonary and digestive diseases, as well as a choleretic, hair-strengthening, and cosmetic agent. Meadowsweet is known as a haemostatic and astringent agent, normally used in the form of an infusion. The herbal tincture has been established to have antibacterial properties and can promote faster healing of wounds, ulcers, and burns. The meadowsweet roots are used to treat rheumatism, upper respiratory tract inflammation, haemorrhoids, urinary tract inflammation, to wash purulent wounds, ulcers, and furuncles. Decoction of rhizomes and roots are administered for leukaemia, diarrhoea, haemorrhoids, rheumatism, digestive disorders and gynaecological diseases, which are as follows: endometriosis, mastopathy, bleeding, and post-parturient complications. Topically, this decoction is used to treat ulcers, wounds, furuncles, and for vaginal douches in leukorrhoea [20].

Yellow bedstraw (*Galium verum* L.) is a perennial herbaceous plant in the family Rubiaceae.

The chemical composition of yellow bedstraw has not been studied. It is only known to contain glycoside asperuloside, ascorbic acid, and pigments. It is known to have diuretic, anti-inflammatory, analgesic, haemostatic, depurative, wound-healing, and sedative effects [21–23].

Yellow bedstraw is widely used in traditional medicine. Infusion of its herb together with flowers is administered for diarrhoea, gastritis, jaundice, inflammatory kidney and liver diseases, oedema, various nervous diseases such as epilepsy, hysteria, convulsions in children, and also for skin diseases. In these cases, it is prescribed to drink fresh yellow bedstraw juice. In dysentery with abdominal cramps, its herbal powder is administered. Decoction of the yellow bedstraw herb is also used for baths, washings, lotions and compresses in psoriasis. For scrofula, abscesses and furuncles, it is used an ointment of herb (the herb and flowers rubbed with butter). For rapid healing of burns, ulcers and bleeding wounds sprinkle them with powdered flowers of yellow bedstraw. A pronounced anti-inflammatory effect was observed in the treatment of acute pyelitis and acute cystitis [23–24].

Garden parsley (curly) (*Petroselinum sativum* L.) is a perennial herbaceous plant in the family Apiaceae.

Its seeds, a little less often its roots and leaves, are used for medicinal purposes. The seeds of parsley plant contain essential oil (approximately 2.4–3.2 %), apiol (18 %), myristicin (10 %), allyltetramethoxybenzene (38 %), flavonoids (1.12–1.87 %), apiin (approximately 1.4 %), luteolinoapiosidoglycoside, fatty oil (glycerides of petroselinic and petroselidonic acids), bergaptene, and coumarin. Apiol and myristicin enhance smooth muscle contraction. Parsley roots contain essential oil (about 0.05–0.08 %), apiol, myristicin, flavonoid (apiin, grafeobioside A), inosit, mucilaginous compounds, resins, and sugars. Garden parsley leaves contain carotene, luteolin, apigenin, and ascorbic acid. Due to the diuretic and antispasmodic properties peculiar to parsley, its preparations are used popularly as a strong diuretic and diaphoretic agents in nephrolithiasis, other urogenital diseases, and cardiogenic oedema. The seeds of the plant are usually used for the treatment of the urinary diseases. The diuretic properties are determined by the essential oil and flavonoids. Given that the biologically active substances are much more abundant in the seeds than in the roots, the effect of seed preparations is much stronger. Parsley essential oil directly irritates the renal tubules, resulting in increased urine excretion, dilates blood vessels and reduces blood pressure [25–26]. The diuretic properties of flavonoids are closely related to their antispasmodic action. For example, apiin relieves spasm of the isolated rabbit intestinal loop in experiment slightly weaker than papaverine. Parsley essential oil and seed tincture at high doses affect the central nervous system function. Excessive doses increase uterine contractility, cause premature menstruation, and may initiate abortion. Low in essential oil aqueous extracts do not have such strongly-pronounced properties. In traditional medicine, decoction of the parsley seeds or roots is used as an agent to stimulate appetite and enhance digestion. It is drunk in diseases of the kidneys, bladder, cramps and intestinal spasms, flatulence, and menstrual cycle regulation. The crushed plant seeds were used in the past against malaria instead of quinine. Fresh parsley leaves are applied to the affected areas in abscesses, bruises, mosquito bites

and bee stings to reduce inflammation and pain. Freshly pressed leaf juice is also used for this purpose. The crushed seeds are rubbed into the scalp in alopecia and pediculosis. Parsley is used for cosmetic purpose. Decoction of its roots is used to wash the face against tanning. Strong decoction of its roots mixed with lemon juice is rubbed morning and evening into freckles and dark spots on the skin. Chewing parsley leaves interrupts bad breath after eating garlic. Parsley is a non-poisonous plant and is well tolerated by the human body [27–28].

Celery (*Apium graveolens* L.) is a biennial herbaceous plant in the family Apiaceae. Its roots, herb and seeds are used for medicinal purposes in traditional medicine. The chemical composition of celery has not been studied in details, only that it is known to contain a large amount of potassium and sodium salts, glycosides, oxalic acid, purines, essential oil, and a small amount of vitamins C, B1, B2, and PP. The highest vitamin content is in juvenile leaves (vitamin C and carotene predominate). Celery is used in traditional medicine as a diuretic, depurative, and antiallergic agent. Celery has long been noted for its ability to increase general body tone and enhance physical and intellectual work capacity [29–30].

In traditional medicine in many countries, a celery root and leaf infusion is used as an antiflatulent for meteorism, diuretic for swelling and laxative for constipation. Its tinctures are also administered in kidney and bladder diseases, gout, polyarthritis, and as an antiallergic agent in urticaria fever, lichen, and other skin diseases. Infusion of the plant seeds is used against flatulence in intestinal atony; it is particularly effective in dysmenorrhoea and amenorrhoea [26]. Celery is used for tumours accompanied by inflammation, ligneous phlegmon, and for bruises as a resolving and analgesic agent. The celery root and leaf infusion is used to wash purulent wounds and ulcers. Freshly fragmented leaves or an ointment made from mashed leaves and butter or sunflower oil are also can be applied [31].

Chaste tree (V*itex agnus-castus* L.) belongs to the family Verbenaceae.

The medicinal raw materials are leaves, flowers, fruits, branches, less often bark. The raw material is collected in the usual manner: flowers during the flowering stage, branches and leaves during the whole plant vegetation, bark in spring or

autumn, fruits as they ripen. The raw material allows to air dry, fruits dry in dryers at a temperature no higher than 40 °C. The main groups of biologically active substances of *Vitex agnus-castus* include iridoids, flavonoids, diterpenes, progestins, essential oils, and ketosteroids [32]. In chemical composition, flavone derivatives predominate: kaempferol, vitexin, quercetagetin and casticin; and apigenin, luteolin, cinaroside are found slightly less. Flavanones (oryantin, isocampferid) were also detected. The flavonoids isolated from the raw material of *Vitex agnus-castus* have a wide spectrum of pharmacological action: antitumor (apigenin, casticin), dopaminergic (vitexin), hypoglycaemic (luteolin), antioxidant, anti-inflammatory, etc. Ferulic, caffeic and chlorogenic acids have been isolated from the *Vitex agnus-castus* raw material, which probably determine high anticonvulsant and sedative activity of chaste tree extracts. One of the main biologically active substances in plant raw material of *Vitex agnus-castus* are iridoids. Among the iridoid glycosides isolated from leaves and fruits of chaste tree, the marker of genus agnusid is of great importance. A particularity of agnuside isolated from plants of the genus *Vitex* L. is its oestrogen-like along with powerful fungicidal activities [32]. Small amounts of progesterone, hydroxyprogesterone, testosterone, epitestosterone and androstenedione are present in the leaves and flowers of *Vitex agnus-castus*. It is possible that the above biologically active substances complement the action of vitexin and agnuside, creating a powerful complex with oestrogen-like systemic action.

Vitex agnus-castus has been used in medicine for over two thousand years. In traditional medicine, decoction and tincture of fruits, leaves and bark have long been treated liver and spleen diseases, infertility, mastopathy, fibroid in women, impotency in men, malaria. The fruit is used as a spice, a substitute for pepper. Clinical studies have proved that the *Vitex agnus-castus* fruit relieves premenstrual syndrome (in the corpus luteum deficiency syndrome) and menopausal symptoms. Because of its dopaminergic effect, *Vitex agnus-castus* is administered for failure to produce breast milk, swelling and pain in the mammary glands. Side effects with its use are rare and include rash, headache, and increased menstrual bleeding [33–34].

The plant extract has progesterone-like effects, promotes the production of follicle-stimulating hormone and stimulates the release of luteinising hormone. V*itex agnus-castus* contains recombinant dopamine receptors, has dopaminergic activity, and inhibits prolactin secretion by binding D2-dopaminergic receptors in the pituitary gland. Its anti-inflammatory, antimicrobial, sedative, bactericidal, antifungal and analgesic properties have been confirmed. The *Vitex agnus-castus* extract reduces prolactin levels, which in turn regulates the levels of sex hormones and gonadotropins. Prolactin influences a human emotional behaviour. Increased hormone concentrations can lead to menstrual irregularities and mastopathy. *Vitex agnus-castus* (chaste tree) and other medicinal plants are used both independently and as part of combined drugs [35–36].

1.2. Use of herbal medicinal preparations in endocrine gynaecological pathology

Based on the analysed literature data, we proposed pharmacotherapeutic characteristics of the medicinal plant combinations used in endocrine gynaecology (Table 1.1.).

Many plants have a wide range of therapeutic action, that is, in fact, are polytropic. This makes it possible to choose and recommend herbal preparations for treatment most suitable for a particular patient based on the disease nature and the severity of the concomitant pathological processes.

This is possible both for individual plants and for their combinations. Such an approach is especially important in rehabilitation, anti-relapsing and preventive phytotherapy, when there is a need for a combination of specific and non-specific components. The individualization of treatment, taking into account the characteristics of a particular organism, living conditions and disease nature, forms the basis of the phytotherapy adequacy principle.

Tazalok oral drops is a herbal non-hormonal preparation with a complex action, which due to synergistic effects of the medicinal plants that make up its

composition and have hormone-regulating, antiproliferative, anti-inflammatory, sedative and restorative effects.

Table 1.1 – Use of herbal medicinal preparations in endocrine gynaecological pathology

Composition	Indications
Vitex agni casti fructuum extract (Vitex agni casti fruit extract)	Used in women in menstrual irregularities associated with corpus luteum deficiency syndrome, premenstrual syndrome, mastodynia (mastalgia)
Vitex agnus-castus (chaste tree) *Caulophyllum thalictroides* (blue cohosh) *Cyclamen europaeum* (alpine viola) *Strychnos ignatia* *Iris versicolor* (purple iris) *Lilium tigrinum* (tiger lily)	Symptomatic agent used for premenstrual syndrome, menstrual irregularities, fibrocystic mastopathy and infertility caused by corpus luteum deficiency syndrome
Vitex agnus castus (chaste tree) *Zingiber officinale* (ginger) *Trigonella foenum-graecum* (fenegriek) *Malus sylvestris* L. (European crab apple)	Administered for women with menstrual irregularities, fibrocystic mastopathy, and infertility. It has analgesic, antitumour, and immunomodulatory effects.
Filipendula vulgaris Moench (meadowsweet root) *Petroselini radix* (curly parsley root) *Apiumi radix* (celery root) *Galii herba* (yellow bedstraw herb) *Linariae vulgarae herba* (common toadflax herb) *Vitex agnus-castus herba* (chaste tree herb) *Flores Calendulae* (calendula flowers)	Used in menstrual irregularities, premenstrual syndrome, algodysmenorrhoea, dysmenorrhoea, fibrocystic mastopathy, retention ovarian cysts. In the combined therapy for endometrial hyperplasia, uterine fibroids, endometriosis, polycystic ovarian syndrome.

The pharmacotherapeutic effect of the preparation is ensured by the active agents in its composition, extracted from the mixture of medicinal substances (meadowsweet, curly parsley, celery root, yellow bedstraw, Vitex agnus-castus, calendula flowers).

Flavonoids of biologically active substances of the preparation are similar in structure to endogenous oestrogen, but do not exhibit oestrogen-like activity; they have the ability to competitively bind to oestrogen (androgen) receptors in excess of oestrogen (androgen) or such hormone receptors in the target organs, change the activity of the aromatase enzymes systems, preventing the conversion of androgens to oestrogens. Thus, the above plant mixture exhibits selective antioestrogenic activity, leads to rhythmic production and normalisation of the gonadotropic hormones balance, helps to reduce prolactin levels and increase progesterone levels, eliminates the imbalance between oestradiol and progesterone, and normalises the second phase of the menstrual cycle. The formulation demonstrates significant effects on the glandular tissue and stromal elements of the mammary glands, ovaries, and uterus. It also displays anti-proliferative and anti-inflammatory properties, and prevents the development of dysplastic processes in these tissues. In polycystic ovarian lesions, the medication facilitates to soften the cyst capsule, reduces its tension through resorptive effect, enhances the function of the intact part of the ovary, improves active resorption of cystic fluid, and resolves painless, dense nodules in the mammary glands. This medication has antispasmodic properties, analgesic and general sedative effects. The biologically active molecules of the preparation have the ability to induce and enhance apoptosis, block the growth factor action, and suppress angiogenesis.

Tazalok, oral drops, has been used effectively since 2008, both as monotherapy and in combination with other drugs [37–38]. The benefits and safety of its use compared to other medications have been confirmed by many clinical trials. An open-label trial compared the Tazalok efficacy and tolerance in female patients aged 18–35 years who had premenstrual syndrome (PMS) [39]. Most women did not report cyclic breast tenderness after the treatment. After the end of the study, 37.83 % of the women showed a decrease in palpatory signs through reduced density, heaviness and tissue tension, as well as a decrease in palpation tenderness. After 12-week treatment cycle, the general Musa index reduced almost in half, indicating that the allocated therapy was effective in eliminating PMS

symptoms. The PMS symptoms severity, characterised by an increase in 5 out of 8 criteria of the Menstrual Distress Questionnaire, was reduced to 4 symptoms, i.e. to a mild and hardly noticeable level. The trial results indicate the efficacy of the plant mixture contained in the investigational agent for the treatment of the symptoms of premenstrual syndrome, especially the components related to fluid content, which gives grounds to recommend it for the treatment of this pathology. This trial has demonstrated the preparation efficacy in patients with premenstrual syndrome. The preparation was well tolerated by the female patients and did not cause changes in the laboratory blood and urine parameters or serious adverse reactions.

A clinical trial studied the combination of Tazalok oral drops and Oxyprogesterone capronate showed its efficacy in the treatment of simple endometrial hyperplasia as a medication, promoting rapid menstrual cycle recovery and favourable tolerability that gives grounds to recommend it for clinical use in this pathology. Most patients rated the tolerability of the preparation as good. No adverse reactions were observed [40].

Data on the treatment of women with PMS using this medication [41] suggest its benefit with respect to positive changes in normalisation of hypophyseal and ovarian hormones (oestradiol and progesterone) after 6-month treatment. A decrease in serum follicle-stimulating hormone and prolactin is characteristic. At the same time, there is an increase in luteinizing hormone. A six-month preparation administration in the complex therapy (vitamins, micronutrients and sedating medications) of PMS in 88% of women leads to elimination of pathological symptoms of this pathology and normalisation of gonadotropic and ovarian hormones.

The experience of treating mastalgia in female patients with dyshormonal breast disease [42] showed the efficacy of the received complex therapy with this medication as high in 91.8 % of women suffering from fibrocystic mastopathy with a severe pain syndrome. Monotherapy was evaluated positively in 79.6 % of female patients. There were 8.2 % of female patients in the main group and 20.4 % in the

control group dissatisfied with the result. The dynamics of the general clinical parameters indicated the absence of general toxic and sensitising effects of the preparation.

A study of the efficacy of a preparation comprising *Vitex agnus-castus* on dysmetabolic disorders in perimenopausal and premature menopausal women associated with fibrocystic changes in the mammary glands [43] showed a pronounced positive effect on the correction of metabolic disorders, psychoemotional state and structural changes of the mammary glands. There was established a significant reduction in the severity of psycho-emotional signs of menopausal syndrome, regular menstruation regained in some patients, decrease in the breast pain frequency and intensity; and women also reported improved sleep and sense of calmness, comfort, and decrease in body weight. After 3-month treatment in the mammary glands, according to the ultrasound examination provided over time, there was a decrease in the glandular tissue density and after 6 months there was a reduction in the number of cysts and their diameter. All of that allows the combined therapy to be used as an alternative to the hormone replacement therapy in the perimenopause and premature menopause.

Current non-hormonal treatment options for polycystic ovarian syndrome in obese women have shown that the studied preparation administered in the complex therapy leads to improvement of hormonal background, lipid and carbohydrate metabolism, normalisation of the menstrual cycle with preservation of the effect after treatment discontinuation [44–45]. In a study of the preparation effects in the treatment of women with metabolic syndrome (MS) and polycystic ovarian syndrome, the development of which is common in those with MS, it has been confirmed that adding the study preparation to a lifestyle change programme leads to statistically significant restoration of natural ovulatory cycles in women with MS, and to restoration of the endometrial histological structure in accordance with the actual cycle day as well [46-47].

The thyroid function is closely connected with the other endocrine organs. For example, the ovary responds to thyroid hormones. The thyroid gland influences on

such processes as the sex hormone synthesis, ovulation and corpus luteum function. A woman with thyroid dysfunction may experience menstrual irregularities, lack of ovulation, infertility or miscarriage.

Given the frequent combination of female genital dyshormonal diseases (uterine fibroids, polycystic ovaries, ovarian cysts, endometriosis, mastopathies, etc.) with thyroid disorders, it is advisable to prescribe herbal medicinal product with basic thyroid therapy.

The therapeutic effect of Tazalok oral drops on the thyroid gland is due to the meadowsweet flavonoids and the yellow bedstraw iridoids, which have a positive effect on the lymphoid tissue functional state of the thyroid gland, activating its work. The levels of thyroid hormones, triiodothyronine and thyroxine, have been normalised while taking the preparation, resulting in elimination of clinical manifestations, such as irregular menstrual cycle, menorrhagia, mastalgia, and dysmenorrhoea [48].

The use of a phytopreparation for the correction of luteal-phase defect in women of reproductive age with hypothyroidism has been studied [49]. The treatment efficacy using the preparation was noted in 97 % of those studied and only 3 % of women with severe anovulation were administered an additional pharmaceutical treatment. The study demonstrated that the phytopreparation is effective in improving women's quality of life, and the increase in progesterone concentration during the therapy allows it to be recommended as an agent with phytogestagenic activity.

In a study evaluating combined gestagen therapy using the phytopreparation in the treatment of multiple dyshormonal benign reproductive organ disorders associated with thyroid pathology, the combined therapy provided a positive clinical effect in 90 % of cases, in contrast to gestagen monotherapy (76.7 %) [50]. Due to the versatile therapeutic effects of the phytopreparation, an improvement or complete recovery of the hormonal regulation mechanisms of the menstrual cycle and thyroid gland was observed during the therapy period.

Uterine leiomyoma remains one of the most pressing challenges in modern gynaecology for now as the most common benign tumour in women. Sex hormones together with oestrogen and progesterone receptors in the uterus play an important role in tumour development. It is believed that the main role in the occurrence and growth of fibroids belongs to the synergistical effect of oestrogen, growth factors and immunoreactive insulin on the myometrium. At the same time, several trials indicate an increase in myometrial mitotic activity under the influence of progestins. The principal direction in the treatment of uterine tumours in gynaecological practice is to reduce the oestrogenic effect on cellular proliferation, achieved by reducing the sex hormone synthesis in the ovaries or by blocking oestrogen receptors in the target organs. A phytopreparation with antioestrogenic and anti-proliferative effects has been used in the treatment of female patients with uterine leiomyoma who have been scheduled for myomectomy with preservation of fertility [51]. The study demonstrated a certain suppressive effect on the uterine proliferation processes and a stabilising effect on the leiomyocyte apoptosis. The clinical trial data are consistent with results of the experimental preclinical studies. In the latter, it was proved that flavonoids, terpenoids and phytosterols contained in the plants (meadowsweet, common toadflax, parsley, celery), which make up the phytopreparation, are characterized by antioestrogenic activity. This may explain one of the preparation's mechanisms of action with regard to normalising oestrogen-progestin balance in female body [49]. At the same time, this mixture does not fully utilise the potential of phytotherapy for the neurometabolic endocrine syndrome, which could be achieved by the addition of *Vitex* leaves that would broaden the spectrum of phytotherapeutic effects.

High antiproliferative activity is known for yellow bedstraw, common toadflax, meadowsweet, parsley, celery, calendula and chaste tree, which aims to reduce the prolactin levels [14]. It is the increased concentration of prolactin that is the main cause of proliferative processes in the mammary glands, leading to the neoplasm formation. The decrease in prolactin levels is due to a dopaminergic effect and contributes to the mammary duct narrowing, proliferative processes reduction

and formation of the connective-tissue component. The indicated composition and proportion of the new medicinal plant raw materials will allow the spectrum of pharmacological and therapeutic action to be extended by complementing and enhancing each other.

The described phytocomposition with pronounced anti-proliferative function reduces blood filling and swelling of the mammary glands to a greater extent, reduces pain syndrome, and reverses changes in the mammary gland tissue.

Due to the harmonious combination of pathogenetic effects and symptomatic action, medicinal plants, in various combinations, have been used for centuries to treat menstrual disorders and various gynaecological diseases in women. The time-tested patterns of using a mixture of medicinal plants, meadowsweet, celery, parsley, calendula, common toadflax, yellow bedstraw, contributed much empirical experience to traditional medicine in the treatment of endocrine gynaecological diseases, which laid the ground for the formation of conventional phytotherapy schemes with similar compositions of herbal preparations, whose effectiveness became the subject of research and further development of scientific medicine.

CHAPTER 2.
SAFETY OF THE COMBINED USE OF URSODEOXYCHOLIC ACID, TAURINE AND ARTICHOKE EXTRACT IN DISEASES OF THE HEPATOBILIARY SYSTEM

Chronic hepatobiliary diseases are among the most widely spread human diseases, second to only atherosclerosis. Based on WHO data, there are over two billion people worldwide suffering from liver disease that is 100 times greater than the HIV infection prevalence. Over the last twenty years, there has been a clear trend towards an increasing incidence of the hepatobiliary diseases worldwide. There has been an increase in the hepatobiliary pathology incidence at a young age observed and four to seven times more frequently in women than men. According to WHO experts, one in five women and one in ten men in Europe have hepatobiliary disorder [5, 52].

The high prevalence of biliary pathology is evidenced by the fact that cholecystectomy is the most frequent operative intervention among abdominal surgeries. It has caused concern that the number of patients with cholelithiasis is increasing in young adults and paediatric patients. Biliary diseases are closely connected to hepatic dysfunction. The synthesis of cholesterol-oversaturated bile by the liver against a background of reduced content of bile acids considerably raises the risk of gallstone formation, as well as gallbladder cholesterosis [5, 52].

In recent years, there has been a search for polyfunctioning agents. This is conditioned by the increase in the number of patients with multimorbid pathologies. Currently, a doctor encounters with patients who have more than one pathology. According to present knowledge, the number of patients with multimorbid pathology who consult a doctor is around 80 %. Multimorbidity is often caused by the anatomical proximity of the affected organs, common pathogenesis, and cause-effect relationship. This is especially relevant to the hepatobiliary system and conditions stemming from metabolic disorders, including metabolic syndrome, diabetes mellitus, and atherosclerosis. The liver, the major organ of metabolism, occupies a

central place in the development of these diseases. Multimorbidity always involves the use of several intermediates, leading to polypragmasy, therefore, the use of natural safe agents, which have a multifunctional normalising effect on the disease pathogenesis, allow reducing the amount of preparations and avoiding the adverse effects of polypragmasy, is advisable [53–54].

At the preliminary stages, a pharmacotherapeutic design of a combination drug containing ursodeoxycholic acid 150 mg, artichoke leaf extract 200 mg and taurine 300 mg as active ingredients has been made and may be suitable for the treatment of dyspeptic disorders in functional biliary diseases, hypokinetic biliary dyskinesia and gastritis with bile reflux [55–57]. Consequently, it is important to scientifically establish the safety of co-administration of ursodeoxycholic acid, taurine and artichoke extract in hepatobiliary diseases.

2.1. Biochemical characteristics of the active ingredients of the combined use of ursodeoxycholic acid, taurine and artichoke extract

Ursodeoxycholic acid (UDCA) is one of the endogenous bile acids synthesised during the normal bile acid metabolism in humans [58–60]. It is a chenodeoxycholic acid epimer and represents a hydrophilic, non-cytotoxic bile acid. UDCA, a milder form of bile acid, is a naturally occurring constituent of human bile, comprising approximately 1-5 % of the overall bile acid content in the human body. It has been used as a drug in the world medicine for more than 30 years, including more than 20 years in Ukraine [58–60].

Originally prescribed for dissolving gallstones and managing reflux gastritis, UDCA has evolved to become the established treatment approach for cholestatic liver conditions that have an autoimmune element, including primary biliary cirrhosis (PBC) and primary sclerosing cholangitis (PSC). In the National Drug Formulary of Ukraine it belongs to the pharmacotherapeutic group of drugs used in liver and biliary diseases, section – bile acids and derivatives (ATC code A05AA02) [58–60].

A modest quantity of UDCA remains consistently present in human bile. When taken orally, it diminishes the cholesterol saturation of bile, thereby retarding the absorption of cholesterol within the small intestine and decreasing the release of cholesterol into the bile. Obviously, the cholesterol dispersion and formation of liquid crystals result in partial dissolution of the gallstone cholesterol.

According to a contemporary view, in hepatitis and cholestatic diseases, UDCA is believed to cause an effect due to the relative substitution of lipophilic, detergent-like toxic bile acids for hydrophilic cytoprotective non-toxic UDCA, and also due to an improvement in the secretory capacity of hepatocytes and immunoregulatory processes [58–62]. It stabilises hepatocyte and cholangiocyte membranes and has a direct cytoprotective effect. By reducing cholesterol absorption in the intestine and other biochemical effects, it has a hypocholesterolaemic effect. The administration of the ursodeoxycholic acid preparation, Ursodexan, contributed to reduction of sonographic signs of hepatic steatosis, choledoch duct diameter, atherogenic lipids in the blood serum, and increase of high-density lipoprotein cholesterol level [65].

UDCA inhibits cell death induced by harmful bile acids. Thanks to its highly polar molecule, UDCA can create non-toxic mixed micelles when combined with apolar (harmful) bile acids. This property diminishes the capacity of gastric refluxate to harm cell membranes in cases of biliary reflux gastritis and reflux esophagitis. In addition, UDCA forms double molecules capable of incorporating into cell membranes, stabilising them and making them immune to the action of cytotoxic micelles. It reduces bile cholesterol saturation by suppressing its absorption in the intestine, inhibiting its synthesis in the liver and reducing its secretion into bile; increases cholesterol solubility in bile, forming liquid crystals with it; reduces the lithogenic index of bile. The outcome is the dissolution of cholesterol gallstones (due to a change in the cholesterol-to-bile acid ratio in the bile) and the prevention of new calculus formation (due to a reduction of cholesterol content in the bile). Moreover, UDCA induces bicarbonate-rich choleresis, which results in increased bile passage, and stimulates the excretion of toxic bile acids through the intestine. Administration

of ursodeoxycholic acid preparations in cholestatic liver diseases and gallbladder pathology promoted normalisation of physiological properties of bile and improvement of hepatobiliary secretion, restoration of hepatocyte cytoskeleton membrane fluidity, elimination of morphological signs of cholestasis [66].

The immunomodulatory effect of ursodeoxycholic acid is based on the inhibition of HLA antigen expression on hepatocyte and cholangiocyte membranes and normalisation of natural killer lymphocyte activity [52–66]. UDCA has the potential to delay the advancement of fibrosis in individuals with primary biliary cirrhosis, cystic fibrosis, and alcoholic steatohepatitis. Additionally, it can lower the risk of developing esophageal varices.

The second component of the preparation is **taurine**, a sulfonic acid formed from the amino acid cysteine [60, 67–68]. Taurine is normally found in small amounts in human and animal tissues and bile [16–18]. It is synthesised in the body by the enzymatic oxidation of the sulfhydryl group of cysteine by cysteine deoxygenase to cysteinesulfinic acid with subsequent decarboxylation of cysteinesulfinic acid to hypotaurine and oxidation of the latter to taurine.

Taurine forms conjugates with bile acids in the liver (via acetylation with them at the amino group), and the conjugates formed (e.g. taurocholic and taurodeoxycholic acids) are part of bile and, as surface-activated substances, help to emulsificate fats. It plays a role in lipid metabolism, enhances energy and metabolic processes, and promotes healing in dystrophic conditions and situations characterized by substantial disturbances in tissue metabolism [66–68]. Under the systemic influence, taurine also has hepatoprotective, cardiotonic and hypotensive properties [69–72]. It is used in cardiovascular insufficiency, cardiac glycoside poisoning and in type 1 and 2 diabetes mellitus. The positive effect of taurine in chronic heart failure and type 2 diabetes mellitus is explained by its endothelioprotective properties. Against the background of taurine administration, an increase in the nitric oxide blood level and a decrease in endothelin-1 and the implementation of a hypolipidaemic effect were noted [73]. Taurine is often given as a component of complex medications. Pharmacotherapeutic group is amino acids

(ATC code S01XA21). Like UDCA, it has been used as a drug in the world medicine for more than 30 years, including more than 20 years in Ukraine [60].

The third pharmacologically active ingredient in its composition is an **artichoke extract from the *Cynara scolymus* leaves**. It is a herbal medicinal product and belongs to the pharmacotherapeutic group "Other drugs for bile therapy" (ATC code A05AX10). It has choleretic, diuretic, hepatoprotective and hypolipidaemic actions. It increases biliary excretion, promotes excretion of nitrogen-containing substances (urea, creatinine), toxins, reduces the lipids and total cholesterol content in the blood, as well as reduces feeling of stomach fullness and relieves spasm. The pharmacological impact is a result of a combination of bioactive compounds found in the medication, including cynarin, chlorogenic acid, ascorbic acid, carotene, B-group vitamins, and inulin. The primary active component, cynarin, exhibits choleretic properties. The cholekinetic effect is found to a lesser degree. Ascorbic acid, carotene, vitamins B1 and B2 and inulin in artichoke help to normalise metabolic processes. The indications for the use of artichoke extract are the dyspeptic and bile outflow disorders, gallbladder hypokinesia, chronic hepatitis, chronic intoxications, chronic kidney disease, nephrolithiasis, uraturia, atherosclerosis, and obesity (as part of complex therapy) [60]. Like UDCA and taurine, artichoke extract has been used as a drug in the world medicine for more than 30 years, including more than 20 years in Ukraine [60].

2.2. Pharmacological safety of the combined use of ursodeoxycholic acid, taurine and artichoke extract

UDCA is a native component synthesised in a living cell in a certain amount and is an absolutely essential component of normal bile acid metabolism. UDCA is considered to be a well-tolerated medication. Until recently, there has been no in-depth pharmacological safety assessment of UDCA, although individual academic papers have investigated the toxic potential of ursodeoxycholic acid in different models in a series of safety trials planned to evaluate the potential organ toxicity of the compound, impact on reproductive function and body development, genotoxicity

and carcinogenicity. When extrapolated to the recommended doses for human with the purpose of clinical use of UDCA, there have been established guaranteed safety reserves [76].

Many authors point out the lack of evidence that high doses of UDCA affect the liver, except in the terminal stages of primary biliary cirrhosis, when cirrhosis decompensation has been observed in individual cases associated with the UDCA use [76], and consider UDCA to be a medication the relative safety of which has been well confirmed by preclinical and clinical trials.

Taurine, like UDCA, is a native component synthesised in a living cell in a certain amount and is an essential component of normal amino acid metabolism. Taurine, a sulfur-containing amino acid, is indispensable for numerous essential biological functions in the body. These include its influence on synaptic transmission in the central nervous system, its cardiotropic effects, its roles as an antioxidant and anticonvulsant, its ability to stimulate energy production and tissue repair processes, its protective action against cataracts in the eyes, its role in reducing cholesterol synthesis, and its capacity to bolster the immune system. Endogenous synthesis of taurine varies greatly between individuals depending on dietary patterns, the amount of protein intake and the availability of cysteine in food [77]. The main conclusion from multi-faceted studies conducted prior to the 1970s is that taurine showed no significant toxic properties with different dosing regimens. It is next to impossible overdose the taurine-based preparations due to the reliable excretion pathways of this natural amino acid. This conclusion has been confirmed by the entire history of taurine administration on hundreds of thousands of people. High doses of exogenously introduced taurine can negatively affect the nervous system and trigger psoriasis [78]. Elevated plasma concentrations of somatotropic hormone in some epileptic patients during the taurine administration at the dose of 50 mg/kg body weight per day may indicate the ability of this amino acid to stimulate the hypothalamus and alter neuroendocrinal function, which may be a side effect of consuming increased doses of taurine. When taurine is administered in combination with various components found in artichoke extract, like caffeic acid, it can lead to

heightened diuretic effects and increased elimination of water and salts from the body. This effect is particularly notable in children and young adults [78].

Artichoke extract is one of the few herbal medicines for which clinical and experimental studies have complemented each other. Both experimental and clinical effects have been verified during the development of artichoke-based herbal medicines. Artichoke extract is well tolerated and has virtually no severe side effects at the recommended doses [79]. The use of artichoke as a food product in various countries for hundreds of years has confirmed its safety, with the exception of allergic reactions. Such allergies must be considered a contraindication for its oral administration. Due to its choleretic effect, the extract should not be taken by patients with bile duct obstruction. Artichoke leaf extract is used as a safe natural dietary supplement and antioxidant to complement the routine medical practices.

The virtual lack of side effects and the high safety level of artichoke extract have been confirmed by a number of clinical and non-clinical studies [80].

2.3. Pharmacodynamic interactions of the combined use of ursodeoxycholic acid, taurine and artichoke extract

Pharmacodynamic drug interactions are related to the following main mechanisms: receptor binding competition, drug kinetics alteration at the site of action, effect on synaptic transmission, and interaction of drug actions if they cause opposite effects. UDCA does not exert its direct effects immediately via a receptor-mediated mechanism and, therefore, does not compete for its own receptors with other drugs. However, as mentioned above, UDCA is chemically similar to steroid hormones and can therefore interact with their nuclear receptors [81–84]. UDCA binds to glucocorticoid receptors and changes their activity [85] proportionally with its concentration [84]. UDCA enhances glucocorticoid-induced expression of the tyrosine aminotransferase gene, which is blocked (by inhibition of protein kinase C) by the transcription inhibitor sphingosine [92]. Glucocorticoid, mineralocorticoid, progesterone and androgen receptors interact with UDCA in a similar way and with marked crossed activity. UDCA activates the nuclear glucocorticoid and

mineralocorticoid receptors [81] and inhibits progesterone and oestrogen receptors [86]. Although UDCA does not directly affect synaptic transmission, however, this compound inhibits mitochondrial membrane depolarisation and ion channel formation, so an indirect influence is not excluded either by this mechanism or by inhibiting the formation of reactive oxygen intermediates, cytochrome C release, caspase activation and cleavage of nuclear poly(ADP-ribose)polymerases [87]. UDCA has no drug antagonists.

Besides all of the above, as mentioned previously, like other bile acids, UCDC conjugates with other active ingredient of the drug, taurine. Conjugation is a necessary step in realising the pharmacological effects of both compounds, as the introduction of a sulfogroup into the UDCA tauroconjugate increases its ionisation, detergent action, solubility, and reabsorption. Thus, the interaction of UDCA with taurine leads to an increase in their pharmacological activity. It is commonly believed that the favourable effect of taurine regarding the atherogenesis degree reduction is mainly determined by its binding to UDCA. As a result, cholesterol, triglycerides, low-density lipoproteins and lipid peroxidation products are reduced simultaneously with increase in glutathione levels [92].

The interaction of UCDC with taurine brings certain specificity to realization mechanisms of pharmacological effect [93, 88], as the formed complex can interact with more cellular targets than the parent compounds. For example, tauroursodeoxycholic acid (TUDCA) realizes its choleretic effect mainly through the integrin α5 β1-mediated pathway [88]; moreover, significant conformational changes occur in the main integrin molecule region responsible for its activation. The results of other studies comparing the pharmacological effects of UDCA and TUDCA have also shown an increase in biological activity as a result of conjugation.

The possibilities of pharmacodynamic interactions for taurine are not limited with this. Since the main biological mechanisms of action of this amino acid, besides participation in the bile acid conjugation, are neuromodulation (agonist of gamma-aminobutyric acid, GABA, and glycine); neuronal and synaptic membrane stabilization; influence on distribution of extra- and intracellular calcium ion flows;

osmoregulation; retinoid and xenobiotic conjugation; anti-oxidative action, taurine can (as an agonist) modify the GABA and glycine effects on cells, indirectly affect the functioning of transmembrane cell channels, synaptic transmission, modify the effects of compounds that are antagonists of GABA and glycine or can form conjugates with taurine [88, 89]. Involving the above mechanisms, taurine is able to lower blood pressure and enhance the antihypertensive drug effects (captopril, enalapril, lisinopril, etc.).

As for the third ingredient, artichoke extract, currently, there is some evidence that it may potentiate the cholesterol-lowering effects of other drugs, particularly statins, by enhancing their effect [90].

The native origin of all the active ingredients of the preparation explains its relatively good safety profile. The human body has efficient systems for catabolism and excretion of excessive amounts of these compounds, which make it impossible to overdose them.

The co-use of ursodeoxycholic acid, taurine and artichoke extract will contribute to the additive hepatotropic effect of these agents. Based on the literature data presented, it is possible to predict the safety of using a fixed composition consisting of ursodeoxycholic acid, taurine, and artichoke extract.

CHAPTER 3.
EFFICACY PROFILE OF THE HOMEOPATHIC COMBINATION FOR INFLUENZA AND ACUTE RESPIRATORY VIRAL DISEASES TREATMENT AND PREVENTION

An acute respiratory viral infection (ARVI) manifests with a general intoxication syndrome and primarily affects the respiratory mucosa. This type of infectious disease, which primarily affects the upper respiratory tract, spreads rapidly and easily. ARVI can be caused by a wide variety of viral pathogens, totaling more than 200 types, spanning different nosological groups such as influenza viruses, parainfluenza, adenoviruses, respiratory syncytial viruses, coronaviruses, enteroviruses, rhinoviruses, picornaviruses, herpesviruses, and more. These infections account for roughly 90 % of all infectious diseases and represent a significant medical and socioeconomic challenge. On average, adults experience ARVI symptoms at least 2-3 times a year, while children may face them 6-10 times annually [94-96].

Despite the abundance of medications available, the challenge of treating influenza and ARVI in both children and adults remains a pertinent issue, with treatments primarily focusing on alleviating symptoms. The course of the illness is largely influenced by an individual's immune response, which can vary significantly. Modern pharmaceuticals often come with a high risk of adverse effects, prompting a growing interest among both healthcare professionals and patients in alternative treatment methods. This interest has led to a notable increase in the use of homeopathic remedies and herbal medicines, emphasizing a more comprehensive approach to treatment.

Ethnoscience and non-traditional medical practices can complement conventional treatments and preventive measures for influenza and ARVI, working in harmony with antiviral drugs, antihistamines, and detoxification agents. These alternative methods can also stand alone, particularly in the treatment of uncomplicated cases. For instance, “anti-cold” homeopathic remedies (HR) can be

employed right from the onset of the illness, when symptoms like elevated temperature, signs of intoxication, and inflammation are present [97-98].

To make homeopathic treatments more accessible as a primary healthcare option, comprehensive homeopathic remedies have been developed. These remedies can be prescribed by medical professionals and are also suitable for independent use by patients. Ukraine's pharmaceutical market features approximately 200 complex (multicomponent) homeopathic medicines from well-established national and international companies.

It is therefore essential to establish a scientific basis for the safety and effectiveness of a tablet-form complex homeopathic formulation. This formulation includes *Aconitum napellus* D6, *Ammonium bromatum* D4, *Atropa belladonna* D6, *Bryonia* D6, *Cinchona pubescens* D6, *Echinacea* D3, *Hydrargyrum bicyanatum* D8, *Rhustoxicodendron* D6. This formulation is designed to boost natural immunity in cases of influenza and respiratory diseases while also helping normalize the functional state of the immune system and upper respiratory tract.

3.1. Characteristics of the pathogenesis of influenza and acute respiratory viral diseases

Influenza is the result of an infection caused by one of three types of circulating RNA influenza viruses, which are classified as types A, B, or C [99]. Influenza C virus typically leads to respiratory illnesses that are relatively milder in comparison to those caused by the A and B viruses [100]. According to the World Health Organization (WHO), approximately one-third of the global population experiences acute respiratory infections each year [101]. The unpredictability of influenza epidemics is primarily attributed to the antigenic variability of these viruses, which can result in partial or complete alterations in group and strain determinants. The rapid spread of the disease over short periods is facilitated by a brief incubation period, airborne transmission, the high susceptibility of individuals to these viruses, and the absence of immunity in the population against new antigenic variants of the virus.

A pivotal aspect of the development of influenza, as well as viral infections in general, is the damage inflicted upon the vascular system. This damage results from the toxic effects of the virus and is characterized by increased permeability of blood vessels, fragility of their walls, and impairment of microcirculation. These vascular alterations play a central role in the emergence of neurological syndromes. Furthermore, a complex of functional disorders within the nervous system can be attributed to damage to the autonomic nervous system and the diencephalon region, which includes the hypothalamus and pituitary gland. This region boasts the highest degree of vascularization and plays a crucial role in neurovegetative, neuroendocrine, and neurohumoral regulation.

In the pathogenesis of influenza infection, immune mechanisms also hold significant importance, particularly T-lymphocytes and their subpopulations, as well as natural killer cells (NK). The suppression of the T-system and NK functional activity is a characteristic feature of severe forms of the infection, leading to prolonged viral persistence and the development of secondary bacterial complications [99].

Influenza stands apart from other ARVIs due to its pronounced toxic effects. In contrast, parainfluenza, adenoviral, and respiratory syncytial infections typically exhibit weak toxic symptoms, even in cases of high fever. These symptoms may even be absent in rhinovirus infections.

While symptoms related to upper respiratory tract issues with systemic involvement are the most commonly observed in these infections, severe non-pulmonary manifestations are also quite common. These severe manifestations can encompass conditions such as myocarditis, rhabdomyolysis, encephalitis, hypovolemic shock with either hyperthermia or hypothermia, and more [102].

It's important to note that secondary bacterial pneumonia, often caused by drug-resistant *Staphylococcus aureus,* is a significant contributor to infant deaths associated with the flu [103].

Individuals at the highest risk of developing complications related to influenza include infants and young children, the elderly, those with compromised

immune systems, and people with specific chronic heart, lung, or neurological conditions [104].

3.2. Efficacy profile of the homeopathic product

The utilization of homeopathic treatment methods has been broadened thanks to the creation of official laboratory-developed formulations, which facilitate the achievement of a swift therapeutic outcome. In contrast to classical homeopathy, integrated homeopathy employs conventional medical terminology and is founded not on homeopathic principles but rather on conventional clinical diagnoses [105].

Complex homeopathic remedies that incorporate both biogenic and mineral components are commonly used, especially in the treatment of colds and flu. For instance, trials assessing the effectiveness and safety of an anti-cold homeopathic medication (comprising *Echinacea angustifolia, Eupatorium perfoliatum, Aconitum, Belladonna*) have yielded promising results [106].

These clinical trials involved 1050 outpatients suffering from colds who received the test drug for a duration of 8 days. The study was conducted across 64 outpatient clinics, with general practice therapists who were trained in the use of homeopathic medicines participating. Doctors and patients assessed tolerance, adherence to the treatment regimen, and its efficacy using diaries. The results indicated that this homeopathic complex medication demonstrated a positive therapeutic effect in approximately 84 % of patients, with ratings of “good” and “very good” efficacy recorded in 84.9 % of all patients. This complex homeopathic medicine was found to be safe and effective for the treatment of colds in both children and adults.

In another set of tests involving this anti-cold complex homeopathic remedy, its effects were compared to traditional methods of treating colds and flu, which typically involve antihistamines, antitussives, and nonsteroidal anti-inflammatory drugs [107]. It's important to note that this trial was non-randomized, and the treatment lasted for a maximum of 2 weeks. A total of 85 medical institutions specializing in general and homeopathic practices in Germany participated in the

trial, and it involved 397 patients with cold symptoms and upper respiratory inflammation.

Patients who received homeopathic treatment did not use additional analgesics, antibiotics, or anti-inflammatory medications. However, they were allowed to pursue non-pharmacological treatments like taking vitamins, thermal therapy, etc. The effectiveness of the treatments was assessed by evaluating the level of symptomatic improvement, which encompassed factors such as fatigue, general feeling of illness, cold-related symptoms, shivering, joint pain, overall disease severity, sum of all clinical variables, presence of elevated temperature, and duration of symptoms.

Both treatment approaches, traditional and homeopathic, yielded notable symptomatic relief. Notably, a significantly higher proportion of patients ($P < 0.05$) experienced improvement within 3 days when treated with homeopathic therapy (77.1 % as opposed to 61.7 % in the control group) [107].

The developed medication falls within the category of complex homeopathic remedies designed for the treatment of colds and flu. While comprehensive evidence-based pre-clinical and clinical studies of the entire homeopathic complex may not have been conducted, the scientific literature does contain data regarding the pharmacological effectiveness of its individual components at various concentrations, potencies, and administration regimens.

Aconitum napellus and *Atropa belladonna* are commonly used components in various complex homeopathic remedies to treat upper respiratory tract infections, typically at different dilution levels (6X, 12X, 30C, 200C, 200CF, 10,000CF) [108].

The efficacy of aconite homeopathic preparations in alleviating throat pain, itching, inflammation, and reducing fever has also been studied in experiments involving mice [109]. The objective of this study was to assess the impact of two different doses of aconite homeopathic preparations on the body temperature of mice receiving the medicine at six different time intervals over a 24-hour period.

Female BALB/c mice were placed in six chambers (with six mice in each) with an air temperature of 24 ± 3 °C, humidity of 60 ± 4 %, and a 12-hour light/dark

cycle, but with a 4-hour staggered onset of light between chambers. This allowed for testing at one specific time followed by testing at six different times (2, 6, 10, 14, 18, and 22 hours after the onset of light). Rectal temperatures were measured both at the beginning and 1 hour after oral administration of either a placebo or two doses of homeopathic aconite (6C and 30C) in these six experimental regimens.

The study revealed a circadian rhythm in the effects of both doses of homeopathic aconite preparation (6C and 30C). It was also established that the time of day significantly influenced the outcomes of both doses of homeopathic aconite and other homeopathic treatments. This finding underscores the importance of considering the optimal dosing and treatment time to maximize the desired results while minimizing adverse effects.

The application of new methodological approaches has allowed for a more evidence-based examination of the homeopathic effects of aconite [110-111].

Typically, the action of ultra-high dilution homeopathic medicines is not directly monitored. However, an attempt was made to selectively observe specific autonomic responses to homeopathic medicines in healthy individuals using the Medical Analyzer System Medical Device Analyzer, developed by the Electronics Division of the Bhabha Atomic Research Center in Mumbai, India [111]. The primary goal of this study was to document the impact of homeopathic remedies on the physiological variability of heart rate and blood flow.

In this study, pre- and post-interventional variabilities of cardiac rhythm and blood flow spectra were recorded in 77 volunteers using a medical analyzer. The effects were investigated following the administration of homeopathic preparations containing the theese ingredients: *Aconitum napellus* (6C, 10M), *Arsenicum album* (200C, 1M), *Gelsemium sempervirens* (200C, 1M), *phosphorus* (200C, 1M), *Pulsatilla nigricans* (200C), and *sulfur* (200C, 1M), with a comparison to a placebo control. Significant changes were recognized as an increase in the amplitude of any real peak by 100% or a decrease by 50 % [111].

A group of researchers conducted a series of studies to investigate the effects of homeopathic preparations containing aconite on various models of anxiety

behavior in rodents [110]. These studies involved fifteen pilot experiments, and some of the findings were corroborated by multiple research laboratories.

Furthermore, there was a study on the impact of homeopathic preparations containing aconite on leukocyte cells [112, 113]. Specifically, a Brazilian complex homeopathic preparation containing *Aconitum, Thuya, Bryonia, Lachesis*, and *Arsenicum* was examined [113]. Previous research had indicated that this medication led to an increase in the number of leukocytes. Since the bone marrow is the primary site for blood cell formation, the *in vitro* effects of the drug on bone marrow cells in mice were investigated.

The homeopathic medicine Canova, which contains *Aconitum*, has been found to have several effects on macrophages [113]. It suppresses the production of tumor necrosis factor TNF-alpha by macrophages, which subsequently enhances the activity of NAD(P)H-oxidase and nitric oxide synthase iNOS in these macrophages. This increase in activity is due to the increased generation of reactive oxygen species and active nitrogen forms.

In this context, it's important to note that the activity of cytochrome oxidase and peroxisomes in macrophages is inhibited by nitric oxide (NO). Since both NO and superoxide (O_2^-) are produced simultaneously, it can lead to the formation of peroxynitrite ($ONOO^-$).

These findings shed light on the mechanisms underlying the stimulation of immune functions when using *Aconitum*-containing homeopathic medicines, particularly in terms of the cytotoxic action of macrophages. In essence, these medicines appear to modulate macrophage activity in a way that enhances immune responses.

Research on the effects of homeopathic preparations containing aconite has also been conducted on humans [114, 115]. Although healthy individuals often report experiencing a response to homeopathic remedies, the mechanisms behind such reactions remain unclear. To investigate the short-term effects of the homeopathic preparation *Aconitum napellus* C30 on healthy volunteers, a study was conducted [115].

In this double-blind, placebo-controlled, cross-study, 33 individuals were randomly selected, and 27 were included in the analysis. The study consisted of two 7-day treatment periods, each comprising a 3-day intake of the homeopathic preparation *Aconitum napellus* C30 followed by a 4-day washout period. One group initially received *Aconitum napellus* C30 and then a placebo, while the other group received both preparations in reverse order. Signs and symptoms were recorded, counted, and assessed before and after each administration. Statistical analysis of the collected data was performed using the Wilcoxon-Mann-Whitney test. The results of the study demonstrated statistically significant differences between *Aconitum napellus* C30 and the placebo ($p = 0.004$) [115].

A prospective observational study was conducted, involving one homeopath and four conventional Ear, Nose, and Throat (ENT) practitioners [114]. The study aimed to compare two methods of treating acute otitis media in pediatric populations. Group A received treatment with individual homeopathic remedies (*Aconitum napellus, Apismellifica, Belladonna, Capsicum, Chamomilla, Kaliumbichromicum, Lachesis, Lycopodium, Mercurius solubilis, Okoubaka, Pulsatilla, Silicea*), while Group B received nasal drops, antibiotics, mucolytics, and/or antipyretics. The primary evaluation criteria included the duration of pain, duration of fever, and the number of relapses within one year. Secondary evaluation criteria encompassed improvement within 3 hours, results of audiometry and timpanometry, as well as the necessity for additional therapy. These parameters were described descriptively.

The study involved 103 children in Group A and 28 children in Group B, ranging in age from 6 months to 11 years in both groups. Regarding the duration of pain, the average duration was 2 days in Group A and 3 days in Group B. It's noteworthy that in terms of the duration of therapy, the median was 4 days for Group A and 10 days for Group B. This difference arises because antibiotics are typically administered over 8-10 days, while homeopathic remedies can be discontinued earlier from the outset of treatment. Among the children in Group A who received homeopathic remedies, 70.7 % experienced no relapses during the

year, and 29.3 % had a maximum of 3 relapses. In contrast, in Group B, 56.5 % remained free of relapses, while 43.5 % experienced a maximum of 6 relapses. Out of the 103 children in Group A, only 5 subsequently required antibiotics. Homeopathic treatment alone led to recovery in the remaining 98 patients.

Medications based on belladonna are used as widely as homeopathic preparations containing aconite [108, 116]. The efficacy of homeopathic preparations containing belladonna has also been studied in animal experiments [117-119]. For example, research was conducted on the effects of homeopathic mono preparations of plant origin, which included *Atropa belladonna* and *Rhus toxicodendron* in three different dilutions (potencies), on interstitial humoral transport in healthy laboratory animals (mice). This was evaluated by measuring the rate of lymphotropic label secretion from the mesentery using Oyvin's method, which involves vital biomicroscopy of the intestinal mesentery in small laboratory animals. These homeopathic mono preparations demonstrated a dose-dependent inhibitory effect on interstitial transport and lymphatic drainage in the tissues of healthy mice [119].

In another study, six different homeopathic remedies (*Arnica montana* D4, *Apis mellifica* D4, D30, *Atropa belladonna* D4, *Hamamelis virginiana* D4, *Lachesis* D6, D30, *Phosphorus* D6, D30) were investigated in animal experiments involving two experimental edema models (carrageenan-induced edema and autologous blood-induced edema) and two routes of administration (sub-plantar and oral administration) [117]. This study involved 720 Sprague Dawley male rats weighing 170-180 grams. Saline solution and indomethacin were used as controls. Edema levels were measured using a plethysmometer before and at various times after edema induction. Data analysis was performed using ANOVA and Student's t-test. The results of these experiments suggested that further research is needed to better understand the anti-edema effects of certain homeopathic remedies.

A study aimed at determining the effectiveness of a homeopathic complex preparation containing belladonna on the symptoms of acute viral tonsillitis in children in South Africa was conducted [120]. This was a randomized, double-

blind, placebo-controlled pilot study that lasted for 6 days. The study involved thirty patients, aged 6 to 12 years, who were diagnosed with acute viral tonsillitis and were recruited from a primary school.

In this study, participants in the treatment group were instructed to take two tablets of the homeopathic preparation (containing *Atropa belladonna* D4*, Calcarea phosphoricum* D4*, Hepar sulphuris* D4*, Kalium bichromat* D4*, Kalium muriaticum* D4*, Mercurius protoiodid* D10*, Mercurius biniodid* D10) four times a day, while the placebo group received lactose tablets. Pain intensity was measured using the Wong-Baker FACES Pain Rating Scale, and changes in tonsillitis signs and symptoms were evaluated using a Symptom Grading Scale.

The results of the study indicated that the treatment group experienced a statistically significant improvement in various symptoms compared to the placebo group. These improvements included reduced tonsillitis-associated pain, pain on swallowing, erythema and inflammation of the pharynx, and the size of the tonsils. The homeopathic preparation used in this study exhibited significant anti-inflammatory and pain-relieving qualities in patients with acute viral tonsillitis, and no adverse effects were reported by any of the patients involved in the study.

Another study aimed to assess the efficacy of homeopathic remedies in preventing and treating migraines in children [121]. This was an observational, prospective, open, non-randomized, multicenter study conducted in 12 countries worldwide. The study investigated various homeopathic medicines, including *Ignatia amara* (25 %; 9C), *Lycopodium clavatum* (22 %), *Natrum muriaticum* (21 %), *Gelsemium* (20 %), *Pulsatilla* (12 %; 15C), *Belladonna* (32 %; 9C), *Ignatia amara* (11 %; 15C), *Iris versicolor* (10 %; 9C), *Kalium phosphoricum* (10 %; 9C), *Gelsemium* (9 %; 15C and 30C). The study involved 59 doctors trained in prescribing homeopathic preparations and 168 children aged 5-15 years.

The study evaluated the intensity, frequency, and duration of migraine attacks for a 3-month period prior to inclusion and compared them with the same parameters during a 3-month treatment and follow-up period. Data were collected through questionnaires completed by physicians and patients or their

parents/guardians. The results indicated that the frequency, severity, and duration of migraine attacks significantly decreased during the 3-month follow-up period ($p < 0.001$). Homeopathic preparations were used for preventive medication in 98 % of cases. The most commonly used preventive medicines included *Ignatia amara* (25 %; 9C), *Lycopodium clavatum* (22 %), *Natrum muriaticum* (21 %), *Gelsemium* (20 %), and *Pulsatilla* (12 %; 15C). Homeopathic monotherapy was used for migraine attack treatment in 38 % of cases, with the most frequently used medicines being *Belladonna* (32 %; 9C), *Ignatia amara* (11 %; 15C), *Iris versicolor* (10 %; 9C), *Kalium phosphoricum* (10 %; 9C), and *Gelsemium* (9 %; 15C and 30C). These results demonstrated the efficacy of homeopathic preparations in preventing and treating migraine attacks in children [28].

As previously mentioned, *in vitro* studies of complex homeopathic preparations containing *Bryonia* were conducted on immune system cells, which showed an increase in the number of leukocytes and stimulated macrophage activity [112-113].

Homeopathic remedies based on *Bryonia* have demonstrated anti-inflammatory and analgesic effects in both animal and human studies [121-123].

In animal studies, the anti-inflammatory and analgesic effects of homeopathic remedies containing *Bryonia* were demonstrated [121]. These studies included the investigation of homeopathic mono-preparations such as *Aconitum* D4, *Phytolacca* D1, *Bryonia* D, and D4, *Lachesis* D8, and *Mercurius solubilis* D4 in fifty cows with acute mastitis. Promising results were obtained, particularly in the treatment of *Escherichia coli* mastitis.

Human studies have also confirmed the anti-inflammatory and analgesic effects of homeopathic remedies containing *Bryonia* [122-123]. In one study, homeopathic remedies containing *Apis mellifica* 9CH and *Bryonia* 9CH were used to suppress inflammation and reduce pain during postpartum lactation suppression [122]. This double-blind, placebo-controlled study involved 71 patients who received basic treatment, including naproxen and fluid restriction. Patients receiving homeopathic remedies containing *Apis mellifica* 9CH and *Bryonia* 9CH

exhibited significant suppression of lactation pain, which was the primary outcome of the study ($p < 0.02$). These findings support the anti-inflammatory and analgesic properties of Bryonia-containing homeopathic remedies.

A study conducted in Gauteng, South Africa, found that homeopathic complexes containing Bryonia and Toxicodendron were equally effective in suppressing inflammation and reducing the pain syndrome associated with osteoarthritis [123]. This study was a six-week, randomized, double-blind, placebo-controlled pilot study, conducted within a private physiotherapy practice. The study involved 30 male and female participants aged 45-75 years who were receiving physiotherapy treatment for osteoarthritis of the lumbar spine.

Both the intervention group and the control group received standard physiotherapy as part of their treatment. In addition to physiotherapy, the treatment group received a homeopathic complex containing the following ingredients at 6CH potency: *Arnica montana, Bryonia alba, Causticum, Kalmia latifolia, Rhus toxicodendron,* and *Calcareafluorica*. The control group received a placebo.

The results of the analysis showed that the group receiving the homeopathic complex exhibited significant improvements in reducing the severity of the pain syndrome and manifestations of inflammation compared to the control group. It's important to note that the study's sample size was relatively small, which limits the conclusiveness of the results. Nevertheless, the findings suggest that the combination of homeopathic complex therapy and physiotherapy may significantly improve the symptoms associated with osteoarthritis.

Research on the efficacy of homeopathic mono- and complex preparations based on *Echinacea* extracts has primarily been conducted through human trials. In the past decade, a significant number of controlled clinical trials have examined the preventive or therapeutic immunomodulatory effects of homeopathic preparations containing *Echinacea*, either as monotherapy or in combination with other plant extracts. These trials include 26 controlled clinical trials, of which 18 were randomized and 11 were double-blind. Among these trials, 6 tested three different mono-extracts, while 20 examined complex homeopathic remedies. These studies

focused on various aspects, including 19 trials studying the effectiveness in preventing or treating infections, 4 trials examining the reduction of adverse effects during antitumor therapy, and 3 trials investigating the modulation of various immune parameters. The collective findings from these controlled clinical studies suggest that homeopathic preparations containing Echinacea extracts can be effective immune modulators [124].

Furthermore, another group of researchers conducted studies on the immunomodulatory activity of homeopathic preparations containing Echinacea extracts on healthy volunteers. This study involved 134 healthy volunteers, ranging in age from 18 to 40 years, with 18 women and 116 men included. The homeopathic complex preparations used in this study contained *Echinacea angustifolia* D1 and D4, two alcohol extracts of *Echinacea purpurea* roots, and an *Echinacea pallida* extract. The primary criterion for evaluating the effectiveness of the immunomodulatory action was the relative phagocytic activity of polymorphonuclear neutrophils (PNG), assessed through various cytometric methods, as well as the number of leukocytes in peripheral venous blood.

The use of complex homeopathic remedies containing *Echinacea* extracts has found widespread application in otolaryngology, with 163 out of 833 respondents reporting positive effects from their use. These remedies have been reported to improve the quality of surgical care [125-127].

Research into the immunomodulatory and anti-inflammatory activity of homeopathic preparations containing *Toxycodendron* extracts has primarily been conducted *in vitro*. One study used primary cultures of preosteoblast cells from MC3T3-E1 mice to investigate different homeopathic dilutions of *Rhus toxicodendron* extract. The stimulation of cells by different concentrations of *Rhus toxicodendron* extract resulted in an increased expression of mRNA for cyclooxygenase-2 (COX-2). The application of 30X *Rhus toxicodendron* extract caused the most pronounced increase in mRNA expression. Additionally, prostaglandin E2 (PGE2) production significantly increased with 30X *Rhus toxicodendron* extract compared to other homeopathic dilutions. However, changes

in the levels of COX-2 protein expression differed from changes in mRNA expression. The greatest effect on COX-2 protein production was observed with the use of 30C *Rhus toxicodendron* extract compared to other dilutions. The production of nitric oxide (NO) significantly decreased in MC3T3-E1 cells when exposed to homeopathic dilutions of *Rhus toxicodendron*. This suggests that the homeopathic remedies had a dual effect on cells, involving both an increase in cyclooxygenase-2 expression and the inhibition of NO production, thereby modulating inflammation [128].

Similar experiments were conducted on the culture of mouse chondrocyte cells. The study assessed the effects of different homeopathic dilutions (4X, 30X, 30C, and 200C) of *Rhus toxicodendron* extracts on the expression of type II collagen, a marker protein of chondrocytes, and COX-2. The expression of collagen type II and COX-2 was evaluated using various biochemical and immunological methods, including reverse transcription polymerase chain reaction (RT-PCR), quantitative PCR (qRT-PCR), and immunoblot assays. Stimulation of the cell culture with various concentrations of *Rhus toxicodendron* increased the mRNA expression of cyclooxygenase-2, with the most prominent effect observed with *Rhus toxicodendron* 30X. Homeopathic dilutions of *Rhus toxicodendron* 4X, 30X, and 30C inhibited the expression of collagen type II and induced the dedifferentiation of chondrocytes. Additionally, treatment with *Rhus toxicodendron* 30X significantly increased PGE2 release compared to other homeopathic dilutions [129].

The results of several studies suggest that homeopathic remedies containing *Rhus toxicodendron*, both as mono-preparations and when included in complex homeopathic remedies like Zeel comp. N, exhibit anti-inflammatory effects. These remedies were shown to inhibit the synthesis of leukotriene B4 (LTB4) and PGE2 by enzymes like 5-lipoxygenase (5-LOX) and cyclooxygenase 1 and 2 (COX 1 and 2) in human cell culture studies. Complex homeopathic remedy Zeel comp. N and its individual constituents, including Arnica montana, Sanguinaria canadensis, and

Rhus toxicodendron, displayed inhibitory actions on the production of LTB4 by 5-LOX and the synthesis of PGE2 by COX 1 and COX 2 enzymes *in vitro* [130].

Furthermore, the immunomodulatory and anti-inflammatory activity of homeopathic remedies containing Rhus toxicodendron extract has been confirmed in animal experiments [131-135]. This suggests that these remedies have the potential to modulate inflammation and immune responses *in vivo*.

Homeopathic preparations of *Cinchona officinalis*, when included in complex homeopathic remedies, have also demonstrated immunomodulatory and anti-inflammatory effects [105, 136]. Additionally, studies on HepG2 human cell culture indicated antioxidant activity in D4 homeopathic preparations of *Cinchona officinalis* [137].

In vitro testing of the homeopathic complex Oligoplex and its components, such as *Mercurius cyanatus* D5, *Echinacea angustifolia* D1, *Ailanthus glandulosa* D3, *Ammonium bromatum* D3, *Baptisia tinctoria* D3, and *Euspongia officinalis* D2, revealed bactericidal activity against various clinical isolates, including *Streptococcus pyogenes, Streptococcus agalactiae, Streptococcus pneumoniae, Staphylococcus aureus,* and *Enterococcus faecalis*. This bactericidal activity was found to be higher than that of vancomycin, especially for *Mercurius cyanatus* [138].

Overall, the results of these studies suggest that the components of complex homeopathic medicinal products, both *in vitro* and *in vivo*, have significant anti-inflammatory, analgesic, immunomodulatory, and bacteriostatic activities across a wide range of potencies.

The components of the developed homeopathic product, including extracts of aconite, belladonna, chininum sulphuricum, as well as mineral ingredients, have a long history of use in homeopathic practice both in Ukraine and around the world. These ingredients have been utilized as anti-cold and immunomodulating mono- and complex homeopathic preparations for over a century, dating back to the 1830s.

One notable aspect of homeopathic preparations is their safety profile, as the active ingredient concentrations used in these remedies are extremely low and

considered non-toxic. This makes them suitable for use in a wide range of individuals without significant side effects or safety concerns.

The primary purpose of the developed homeopathic product is for the treatment and prevention of acute respiratory diseases and influenza. It is designed to enhance the body's natural protective responses and provide relief from the common symptoms associated with acute respiratory infections and flu, including symptoms like headache, runny nose, sneezing, sore throat, fever, and body aches. Additionally, the product aims to promote a quicker recovery from these illnesses.

Given the long history of use and the generally favorable safety profile of homeopathic remedies, this product offers an alternative approach for individuals seeking relief from cold and flu symptoms and aiming to support their immune system during these infections. However, it's important for individuals to consult with a healthcare professional or homeopath before starting any new treatment, especially if they have underlying medical conditions or are taking other medications.

CHAPTER 4.
CURRENT SAFETY DATA OF THE COMPLEX HERBAL MEDICINE WITH SEDATIVE AND CARDIOPROTECTIVE ACTIONS

Pharmacovigilance represents the branch of pharmacological science within modern healthcare that is dedicated to safeguarding patients' well-being during drug usage, with the overarching goal of reducing illness and fatalities. Marketing authorization holders bear the responsibility of establishing, furnishing, and ensuring the effectiveness of their own pharmacovigilance systems, which is an essential prerequisite for the availability of medicines in the market. Presently, the prevailing trend in developed countries' pharmacovigilance efforts involves a shift from a reactive approach (detecting and responding to cases) to a proactive one (strategically planning and preventing issues).

Within the pharmacovigilance system, three pivotal documents focus on quality management of organizational structures and the handling of pharmacovigilance data. The first document is the risk management plan, a comprehensive record delineating the risk management system's intricacies, with a strong emphasis on planning pharmacovigilance actions and risk minimization. The second document, the periodic safety update report (PSUR), is centered around the analysis of accumulated and time-specific safety and efficacy data for a particular medicinal product, with a primary goal of evaluating the benefit-to-risk ratio. Finally, the third critical document is the addendum to the clinical overview, which critically assesses the existing benefit-to-risk profile of a specific medicinal product. This evaluation is based on data from the PSUR, as well as safety and efficacy data gathered since the initial registration or the most recent re-registration of the medicinal product.

Proactive management strategies enable the effective mitigation of risks associated with drugs, benefiting both the general population and the operations of pharmaceutical companies and regulatory bodies alike [94, 139-141].

With the rising use of herbal medicines, there has been a corresponding increase in the reporting of suspected toxicity and adverse events. These undesirable reactions can arise from several factors:

(i) Side effects: These are typically detectable through pharmacodynamic assessments and are often predictable.

(ii) Reactions stemming from overdose, prolonged usage, tolerance, or dependence/addiction: These can be identified through pharmacodynamic or pharmacovigilance methods.

(iii) Hypersensitivity, allergic reactions, and idiosyncratic responses: These are detectable through pharmacovigilance efforts.

(iv) Mid-term and long-term toxic effects: These include issues such as liver, renal, cardiac, and neurotoxicity, as well as genotoxicity and teratogenicity. These effects can be identified through *in vitro* and *in vivo* toxicological studies or through pharmacovigilance efforts.

It's important to note that many herbal products available on the market have not undergone comprehensive testing for their pharmacological and toxicological properties. Consequently, pharmacovigilance plays a crucial role in identifying and addressing unwanted reactions, ensuring the safety of those who use these products [142].

One of the herbal preparations widely utilized to provide support for the cardiovascular system during periods of stress, as well as in the management of neurocirculatory dystonia, cardiac neurosis, and as a part of comprehensive treatment for conditions like arterial hypertension, angina pectoris, and arrhythmia, is a multifaceted medicinal product known as Carvelis. This product is available in the form of oral drops and is provided as a solution by Dr. Gustav Klein GmbH & Co. KG in Germany. The active pharmaceutical components of this medication include:

1. Extract from a mixture of hawthorn leaves, flowers, and fruit (*Crataegi folii cum flore*, *fructus extractum*).
2. Herb extract of canine nettle (*Leonuri herbae extractum*).
3. Melissa herb extract (*Melissae herbae extractum*).

4. Valerian root extract (*Valerianae radix extractum*).

The primary objective of this study was to conduct a comprehensive evaluation of the existing scientific data concerning the balance between the benefits and risks associated with this complex herbal medicine, which possesses both sedative and cardioprotective effects.

4.1. Pathophysiological and epidemiological characteristic of nosological forms for the treatment of which a phytopreparation is prescribed

Support for the cardiovascular system at nervous tension (stress). Stress is a generalized response of the body to a powerful external stimulus that exceeds the norm, triggering a corresponding reaction from the nervous system. There are various types of stress, including informational, emotional, physiological, and post-traumatic stress. During periods of stress, the sympathetic nervous system becomes active, leading to increased production of "stress" hormones, elevated heart rate, and heightened blood pressure. These physiological changes can result in organic alterations in the heart, potentially leading to conditions like arrhythmias and ischemia. Additionally, stress sets the stage for thrombosis, the onset, or progression of atherosclerosis, and coronary heart disease. This complex interplay of physiological and pathomorphological changes contributes to the development and advancement of many cardiovascular diseases. There exists a direct correlation between stress levels, average daily blood pressure, and heart rate [143].

The inclination towards experiencing stress, of varying degrees of severity, is a characteristic that applies to every individual, regardless of their socio-economic status. In the United States, as many as 90 % of adults encounter periodic episodes of stress, with 60 % facing stress-inducing factors 1-2 times a week, and 30 % experiencing them on a daily basis. Remarkably, in two-thirds of cases, visits to healthcare professionals are prompted by diseases and conditions rooted in the impact of stressors. The annual economic expenditure for treating and rehabilitating individuals affected by stress-related issues in the United States is estimated to be around $300 billion [144].

Data from the World Trade Center Health Registry were used to analyze adult enrollees from New York State who had no prior history of cardiovascular disease at the time of enrollment (n = 46,346). This data was linked with New York State's hospital discharge-reporting system.

The study found an increased risk of hospitalization related to cerebrovascular or cardiovascular diseases, particularly among women (adjusted hazard ratio 1.32, 95 % confidence interval [CI] 1.01 to 1.71). However, this increased risk was not observed in men (AHR 1.16, 95 % CI 0.97 to 1.40). It's important to note that all patients in this study had post-traumatic stress disorder. Among those who were either evacuated from the World Trade Center or involved in its restoration, there was a notably high rate of hospitalization for cardiovascular diseases in men, whereas the data for women were less clear. This observation primarily pertained to the diagnosis of “ischemic heart disease”. Furthermore, stress was associated with an elevated rate of hospitalizations for cerebrovascular disorders in men but not in women [145].

In a separate study, the Japan Collaborative Cohort Study for the Assessment of Cancer Risk examined the impact of stress perception on health outcomes. This study involved over 70,000 participants aged 40 to 79 years, with exclusion criteria including a history of stroke, ischemic heart disease, or cancer. Among women experiencing severe stress, the risk of death from stroke and coronary artery disease was twice as high as those not reporting severe stress, and the risk of any cardiovascular disease was 1.5 times higher. In men, while the effect was less pronounced, chronic stress was still associated with an increased risk of myocardial infarction [146].

Another study, conducted by different authors, reported the findings of a 20-year survival analysis of patients with coronary heart disease (CHD) who were consistently exposed to emotional stress [10]. The researchers discovered that among the group of patients subjected to persistent and pronounced stress, the survival rate was notably lower after just 3 years compared to the groups with no stress or low-intensity stress. Furthermore, this difference became even more pronounced after 10 and 20 years of observation.

It's worth noting that various treatments are employed to manage stress. These treatments include the use of medications such as antidepressants, neuroleptics, nootropics, and tranquilizers. Additionally, vitamin therapy involving vitamins B and C is utilized. Non-pharmacological approaches like aromatherapy, engaging in sports, and practicing yoga are also employed as strategies to address and alleviate stress [148-150].

Neurocirculatory dystonia (NCD) is a neurogenic symptom that arises due to various triggering factors. It is characterized by unstable pulse and blood pressure readings, chest pain (cardialgia), breathing difficulties, autonomic dysfunction, muscle and vascular tone irregularities, and reduced tolerance to normal physical activity and stressful situations. NCD results from an imbalance in the autonomic nervous system (ANS), with the specific form of NCD depending on the predominant activity of either the sympathetic or parasympathetic part of the ANS [151].

NCD is not considered a standalone illness but rather a condition often associated with a range of symptoms, primarily affecting the cardiovascular, nervous, and respiratory systems. It is most frequently diagnosed in young individuals, who may later develop various cardiovascular issues. This higher prevalence among the younger population is attributed to the discrepancy between physical development and the slower maturation of the neuroendocrine system. Contributing factors to the development of NCD include intoxication, acute and chronic infections, fatigue, regular sleep deprivation, poor dietary habits, physical overexertion, frequent changes in sexual partners, and exposure to stress. Additionally, a genetic predisposition can play a role in some cases.

There are three recognized types of neurocirculatory dystonia: cardiac, hypertonic, and antihypertensive. When symptoms become particularly intense, leading to constant irritability or sleep disturbances, various treatments are recommended. These may include medications like phenobarbital, valerian-based preparations, oxazepam, and other tranquilizers. Medicinal treatments can also involve valerian and motherwort-based preparations, tranquilizers such as tofisopam,

antidepressants like amitriptyline, nootropic drugs including piracetam, and cerebral angiocorrectors such as vinpocetine [152].

Cardiac neurosis is classified as a functional disorder affecting both the cardiovascular and nervous systems. Its pathogenesis is primarily associated with the diminished regulatory function of the cerebral cortex, leading to disruptions in the functions of the subcortical centers of the autonomic nervous system. Stress, mental strain, and physical exertion often serve as triggers for cardiac neurosis. Stress, in particular, can lead to a sudden release of adrenaline, which can have adverse effects on the heart and its function. The consumption of coffee, alcohol, and smoking also plays a significant role in disrupting the autonomic regulation of the cardiovascular system. It's important to note that individuals of all age groups can be at risk of developing this condition [153-155].

The most common symptom of cardiac neurosis is chest pain, often radiating to the left arm and shoulder blade. Even mild agitation can lead to irregularities in heart rhythm (arrhythmia), sensations of “heart fluttering”, palpitations (tachycardia), or a strong pulsation felt throughout the body. Other possible symptoms include breathlessness or a feeling of breathlessness, a mild increase in body temperature (subfebrile), fluctuating blood pressure, digestive disturbances like vomiting and nausea related to eating, wheezing, and abdominal pain. General weakness, fatigue even with minor physical exertion, headaches, signs of asthenia (lack of energy), sleep disturbances or drowsiness, sudden sensations of heat or cold, facial and chest redness, increased sweating, cold and numb extremities, tingling sensations, and a sense of impending doom or tearfulness may also manifest [153-155].

During the acute phase of treatment, the use of tranquilizers and beta-blockers is typically recommended. Subsequent medical therapy aims to alleviate the signs of cardiac neurosis, promote relaxation, and alleviate unpleasant symptoms. It may include herbal preparations and a combination of vitamins and minerals, particularly those high in magnesium and B-group vitamins. Research suggests that suitable herbal choices for treatment may include hawthorn, valerian, motherwort, passiflora, mint, yarrow, marigold, and others [154-155].

Hypertension, particularly in stages 1 and 2, can be influenced by stress. Stress-induced arterial hypertension refers to a sudden rise in blood pressure due to psycho-emotional factors. It can affect individuals with previously normal blood pressure as well as those with pre-existing hypertension [143]. This condition is notably prevalent among young urban dwellers, with a substantially lower likelihood of occurrence in rural areas. In older individuals, stress can trigger irregular heart rhythms, and it also increases the risk of heart attacks and strokes.

During periods of stress, various physiological parameters undergo changes: heart rate increases, respiration becomes more rapid, smooth muscle tone is altered (including in the gastrointestinal tract), pupil diameter shifts, blood pressure rises, and blood glucose concentration elevates. These changes collectively prepare the body for survival in high-stress situations. However, when stress becomes a daily occurrence, the mechanisms responsible for the cardiovascular system's function in stressful conditions are consistently activated, leading to pathological changes in organs involved in regulating blood circulation. Over time, this can result in a gradual increase in blood pressure [156-157].

A striking example of the impact of prolonged stress is seen during the earthquake in southern Italy, where the constant exposure to stress factors led to a significant and sustained increase in blood pressure. On average, systolic arterial pressure increased by 20 %, while diastolic arterial pressure rose by 46 % [158]. This underscores the potent influence of chronic stress on blood pressure regulation.

Angina pectoris, a form of coronary heart disease, is primarily characterized by the presence of pain symptoms. It is more commonly observed in mature and elderly individuals, affecting both males and females. However, the incidence varies with age, with different risk periods for each gender. Angina is primarily a result of atherosclerosis in the coronary arteries. Sudden angina attacks can be triggered by factors such as excessive stress, physical exertion, smoking, excessive alcohol consumption, exposure to a smoky or dusty environment, and extremely cold temperatures. Typically, the pain occurs in the early morning hours. Various meteorological and geomagnetic factors, high humidity, and fluctuations in

atmospheric pressure can also provoke angina pain. Remote risk factors that accelerate and exacerbate angina development include conditions like diabetes mellitus, obesity, a sedentary lifestyle, frequent mental stress, alcoholism, and a diet high in salty, fatty, and refined carbohydrate-rich foods. Symptoms of angina can often be relieved with the use of nitroglycerin [159-160].

Certain medicinal plants containing antiatherosclerotic substances have been identified as beneficial in managing angina and coronary heart disease. These plants include corn (specifically the female inflorescence and young silk), hawthorn (both flowers and fruits), rose hips, mountain arnica flowers, Jacob's-ladder roots, elderberry leaves, black chokeberry fruits, common nettle, garlic, beet, and carrots. Herbal infusions are not typically used to alleviate pain but rather to provide a prophylactic effect. Therefore, they are often used over an extended period, usually in combination with other medications or treatments [161-162].

Jitters, or nervous excitement, is a biological process driven by nerve impulses that activate various components of the body. This process of excitation occurs in all organs composed of nervous and muscular tissue, as well as in glands. One characteristic feature of muscle excitation is the contraction of the muscle. In nerve cells, excitation leads to the generation of nerve impulses, while glandular cells release secretions. An essential property of excitation is its ability to travel along nerve fibers, establishing a physiological connection between all systems and elements within the organism, promoting their functional integration. To effectively alleviate anxiety and nervous excitement, the use of specific medicinal herbs is recommended [163].

Current data on phytopreparation safety and estimation benefit/risk ratio. Autonomic dysfunction represents a significant challenge in modern medicine. Symptoms of neurocirculatory disorders, combined with various manifestations of autonomic imbalance, are observed in a substantial percentage of patients, including those under the care of therapists and family doctors (25-30 %) and cardiac patients (30-50 %) [163].

It is now widely accepted that NCD is an integral component of the clinical presentation of neurosis, which, in turn, is often the primary cause of NCD. Many

forms of neurosis accompanied by NCD symptoms are now considered as part of a broader spectrum of pathologies known as psychosomatic disorders [164].

The pathogenesis of psychosomatic diseases is intricate and multifaceted. It involves crucial aspects of neurohumoral regulation, including structures in the CNS such as the hypothalamic-pituitary and limbic systems, regulatory centers in the cerebral cortex, and adrenal glands. However, chronic stress is the central factor in the development of psychosomatic pathology. Chronic stress plays a pivotal role in initiating a complex chain of events leading to psychosomatic diseases, primarily affecting the nervous or cardiovascular systems, digestive system, or other systems [165].

The most prevalent clinical presentation of NCD is the cardiovascular syndrome. NCD with hypersympathicotonia, constant or crisis-driven increases in blood pressure, and severe tachycardia holds the greatest clinical significance. This form of NCD carries an elevated risk of developing conditions such as coronary artery disease, hypertension, arrhythmias, and cerebral circulation disorders. Therefore, the appropriate pharmacotherapy for this variant of NCD is particularly relevant [166].

Effective treatment of autonomic dysfunction often involves a combination of traditional somatic therapy (e.g., antihypertensive drugs, coronary agents, beta-blockers, proton pump inhibitors) and neuropharmacological agents that target the central mechanisms of dysregulation.

Psychoemotional imbalance is a common feature of autonomic vascular dystonia, significantly affecting the quality of life and contributing to a pathogenetic “vicious circle” of stress-induced CNS disorders. The normalization of both the psychoemotional sphere and vegetative disorders can be achieved through appropriate therapeutic agents with sedative and vegetative stabilizing properties, with phytopreparations playing a prominent role.

In recent years, combined phytopreparations that aid in normalizing central regulatory mechanisms associated with the development of psychosomatic pathology have gained popularity. These preparations stabilize the relationship between the cortex and subcortical structures, which are disrupted in autonomic vascular dystonia

and psychoemotional imbalance. Key advantages of herbal preparations include their ability to combine proven, effective combinations of biologically active substances in one dosage form, reduce the need for multiple medications while maintaining or increasing treatment effectiveness, enhance patient and physician compliance, and improve the economic accessibility of treatment [167-169].

One such phytopreparation is Carvelis (Dr. Gustav Klein GmbH & Co. KG, Germany), available in the form of oral drops. It contains the essential components mentioned, including extracts of hawthorn leaves, flowers, and fruits; motherwort herb; melissa leaves; and valerian root. Carvelis is indicated for the treatment of various psycho-related disorders, including NCD, cardiac neurosis, stages 1-2 arterial hypertension, stages 1-2 angina, stress-induced arrhythmias, and as pharmacoprophylaxis for managing excitement, stress, and emotional lability in chronic stress.

Hawthorn is a plant that boasts a rich composition of vitamins and other biologically active substances. In its berries, you can find various vitamins, including A, K, C, and E, as well as B-group vitamins. Hawthorn berries also contain saponins and flavonoids, starch and fructose, organic acids, essential oils, pectin, choline, and sorbitol. Some of the substances found in hawthorn are exceptionally rare in other edible fruits and plants, making it unique. For instance, ursolic acid, found in hawthorn, possesses antimicrobial properties and promotes vasodilation [170-171].

Preparations made from hawthorn have a mild diuretic effect, exhibit antitumor activity, and have a positive impact on cardiac stimulation. The acids in hawthorn fruit are beneficial for the skin and have rejuvenating effects. The use of hawthorn berries promotes active cell regeneration. The therapeutic properties of hawthorn fruit are particularly pronounced in the cardiovascular system. Substances from hawthorn berries dilate coronary vessels, ease the work of cerebral arteries, and improve blood oxygen saturation, leading to reduced dizziness, headaches, stabilized sleep, and decreased daytime fatigue. Hawthorn has been proven to have hypotensive, coronary-dilating, antiarrhythmic, and cardioprotective effects. Its unique characteristic lies in its regulatory and stabilizing action, which is effective in various manifestations of

cardio neurosis, such as tachycardia, cardiac discomfort, cardialgia, and blood pressure instability. Additionally, hawthorn is an effective cardiotonic agent with pronounced antihypoxic and antioxidant effects [170-172].

The substances found in hawthorn berries strengthen blood vessels, lower harmful cholesterol levels, and improve blood coagulation, reducing the risk of developing atherosclerotic plaques. Adverse effects are minimal at recommended dosages. The primary contraindication is individual intolerance. It should be used with caution during pregnancy and breastfeeding, as hawthorn can increase uterine tone. Special care is needed for patients in the post-stroke period and individuals with severe kidney disease. In such cases, consultation with a healthcare provider is essential [173-174].

Data from 687 individual patients treated with quantified hawthorn extract or a placebo in ten studies were analyzed. The study evaluated the effect of treatment on patients' physiological parameters and disease symptoms, as well as their relationship with symptom severity and gender. The study found that the reduction in the severity of typical symptoms (reduced exercise tolerance, exertional dyspnea, weakness, fatigue, and palpitations) was associated with more active treatment, particularly in patients with more severe symptoms. However, there was a weak correlation between improvements in physiological parameters and symptoms. Therefore, the effect of hawthorn extract treatment on physiological outcomes and typical symptoms was modulated by symptom severity. Across all differences, the benefits were similar in both male and female patients with reduced physical capacity due to early chronic heart failure [175].

Fourteen clinical trials were reviewed in the Cochrane database, all of which were randomized, double-blind, placebo-controlled studies using hawthorn leaves and flowers. These trials involved 855 patients with chronic heart failure (Classes I-III according to the New York Heart Association classification). The results showed that the use of hawthorn extract significantly improved maximum workload, exercise tolerance, and the pressure-heart rate product (an index of cardiac oxygen consumption). Additionally, symptoms like dyspnea and fatigue were significantly

alleviated with hawthorn extract treatment compared to a placebo. No data on mortality or cardiac diseases were reported, except in one study that mentioned deaths without further details. Side effects of hawthorn were rare, mild, and transient and included symptoms like nausea, dizziness, and minor gastrointestinal complaints [176].

Clinical trials conducted in European Union countries involving 1,780 patients did not observe acute toxicity. Between 1992 and 2011, approximately 810 million doses of dry extract from hawthorn leaves and flowers were sold in the European market [177].

Motherwort is a plant known for containing several beneficial substances, including alkaloids, glycosides, polysaccharides, saponins, tannins, essential oils, and vitamins. Its mechanism of action is attributed to its activation of gamma-aminobutyric acid (GABA) receptors, as well as its role in normalizing neurotransmitter balance. This includes increasing the activity of inhibitory GABA-ergic systems and weakening the activating catecholaminergic systems in the brain through modulation of neurotransmitter release, reuptake, and receptor binding [178].

In addition to its central sedative, mild anti-anxiety, and hypnotic effects, motherwort preparations offer valuable benefits in the context of vegetative-vascular dystonia. These include antispasmodic, antihypertensive, and anti-anginal effects, as well as increased cardiac output and a reduction in heart rate [179].

Officially, motherwort is prescribed in cases of neurosis (in various forms), hysteria, myocarditis, cardiosclerosis, and cardiovascular insufficiency of degrees I and II within conventional medicine. In traditional medicine, it is used for various nervous disturbances, anxiety, hypertension, and to improve sleep and overall well-being. While there are no significant contraindications, it is not recommended for individuals with hypotension. Prolonged use can occasionally lead to allergic reactions or complete intolerance, although this is quite rare [180-181].

Motherwort is well-known and widely utilized in Chinese medicine. Reviews [182-183] provide an overview of its chemical composition, emphasizing its biological activity and ethnopharmacology. The authors describe mechanisms that could underlie its protective effects in cardiovascular bypass, such as the inhibition of intracellular

active forms of oxygen (ROS) and various cellular mechanisms involved in preventing apoptosis. Additionally, the authors suggest that motherwort's unique modulation of intracellular calcium homeostasis may explain its cardiovascular protective effects.

Motherwort, as an herbal substance, has been used in the European Union for over 30 years and has been included in official pharmacopeias for more than 50 years. Continuous use in the form of herbal tea has been recorded for almost 400 years. For example, in Lithuania, 98 thousand packets of 25 ml tincture were packaged in 2008, and over 350,000 packages were sold from 2003 to March 2009 [184].

Valerian is a plant that contains over 120 chemical components, with essential oils, valpotriates, amino acids, salts of organic acids, phenolic compounds, and alkaloids being among the most important from a clinical perspective [185]. Its primary effect is central nervous system sedation, which helps restore emotional balance, alleviate headaches, reduce anxiety, and improve overall productivity. Valerian also has a positive impact on the cardiovascular system, making it suitable for conditions characterized by increased heart rate, angina pectoris, and heart pain. The substances found in the root of the valerian plant have a vasodilatory effect.

In Germany, from the 1980s to the 1990s, approximately 300 clinical trials on combined herbal preparations were conducted, including 10 preparations based on valerian. According to evidence-based medicine criteria, all valerian-containing preparations were found to be clinically effective in treating various forms of therapeutic and neurological conditions, especially psychosomatic diseases of cardiological and gastroenterological profiles [186].

Valerian is generally considered safe; however, at high doses or when exceeding the recommended course of treatment, individuals may experience unpleasant side effects such as reduced alertness, drowsiness, lethargy, and gastrointestinal disturbances. Prolonged use beyond two months or substantial doses beyond recommendations can lead to gastrointestinal issues, depression, drowsiness, nausea, and headaches. Valerian should be avoided by individuals with intolerance to its components and those suffering from enterocolitis [187-188].

An open, prospective cohort study conducted in Germany compared valerian with the homeopathic preparation Neurexan in 409 individuals suffering from insomnia. The study evaluated sleep duration and insomnia based on daily patient records over 14 days, with sleep quality assessed after 28 ± 1 days. After 14 days of treatment, both therapies showed improved sleep duration compared to baseline, with reported side effects including headache, dizziness, paradoxical stimulation, anxiety, and cardiac abnormalities [189].

Another prospective, uncontrolled, non-interventional observational study conducted in Germany included 807 patients with various symptoms, including anxiety, sleep disturbances, fatigue, and gastrointestinal disorders, who were treated with Neurexan or various valerian extracts. Symptoms improved in both treatment groups, and compliance with the treatment was high. No side effects were reported in the valerian extract group [190].

A study aimed to determine whether a valerian-lemon balm combination could improve sleep in menopausal women. The research used a clinical randomized testing approach and found that valerian-lemon balm supplements could enhance sleep duration and quality in menopausal women with sleep disturbances. Furthermore, no adverse effects were reported during the treatment [191].

Valerian root preparations have a high degree of use in EU member states, with sales exceeding 50 million units in 2002, and nearly 50 % of these sales occurring in Germany. Side effects associated with valerian root consumption are primarily related to the gastrointestinal tract, such as nausea and abdominal colic (frequency unknown). In the EU, traditional use of valerian and its preparations is considered to pose minimal risks due to minimal side effects [192].

Melissa, also known as lemon balm, is a plant that contains various beneficial compounds, including organic acids, saponins, flavonoids, resins, tannins, essential oils, copper, manganese, iron, potassium, selenium, zinc, magnesium, calcium, and vitamins from groups B and C.

One notable component of Melissa is amber acid, which acts as a potent natural antioxidant. It effectively protects cells in the brain and peripheral organs from the

damaging effects of free radicals, which are often associated with chronic stress. Melissa also possesses properties as a psychoemotional stabilizer and cognitive activator, potentially improving mood and cognitive functions. This effect may be attributed to its modulation of the cholinergic and monoaminergic systems in the brain. Additionally, Melissa has spasmolytic (muscle-relaxing) properties [193].

Long-term use of Melissa-based preparations can lead to several benefits, including the reduction of arterial pressure, relief from nervous tension (e.g., night tremors), diuretic effects, and the normalization of heart rhythm. Melissa is known to rapidly and effectively lower heart rate, potentially contributing to an extended lifespan. Furthermore, Melissa can influence various metabolic processes in the body, enhance liver and brain functions, alleviate feelings of heat, and assist in bile elimination [194].

While there are no strict contraindications for using Melissa-based medications, there are some situations where its use is not recommended by medical professionals. These include individuals with hypotension (low blood pressure) and individuals engaged in activities that require heightened concentration, motor skills, or rapid psychological reactions, such as driving a motor vehicle [194-195].

Melissa is a traditional herbal remedy, and its use is based on a long history of usage for specific indications. Traditional uses for Melissa include its use as an herbal preparation for alleviating mild symptoms of mental stress and insomnia, as well as for the symptomatic treatment of minor gastrointestinal complaints, such as abdominal distension and flatulence [194-195].

4.2. Complex action of phytopreparation characteristics and risk assessment

The optimization of pharmacotherapy for vegetative-vascular dystonia, including neuro-circulatory dystonia, can indeed be achieved through the use of combined preparations containing hawthorn, motherwort, melissa, and valerian. These herbal components have demonstrated a high degree of safety when used in therapeutic doses. The side effects associated with these herbs are typically limited to individual

intolerance or excessive doses, resulting in increased sedation, drowsiness, weakness (for valerian, motherwort, and melissa), or minor dyspeptic disorders, hypotension, and bradycardia (for hawthorn). Adjusting the dosage can help alleviate these side effects.

Combined phytopreparations can be prescribed to a range of patients, including young people with functional disorders (autonomic dysfunction syndrome) or chronic fatigue syndrome, as well as elderly patients with age-related cardiovascular changes and decreased exercise tolerance, where active therapy (such as antihypertensive medications or symptomatic treatment for coronary heart disease) may not be immediately necessary. These preparations can also enhance the effectiveness of complex therapy for conditions like arterial hypertension, coronary artery disease, and heart failure, due to the presence of hawthorn. Additionally, individuals prone to excessive vegetative reactions to social stimuli, significant physical activity accompanied by palpitations and shortness of breath, or meteosensitivity can benefit from using these preparations either regularly or on an as-needed basis [196].

The combined phytopreparation provides a comprehensive, multimodal effect on the nervous system, cardiovascular system, and vasculature. It offers sedative, antihypertensive, antiarrhythmic, coronarolytic, cardioprotective, and vegetative-stabilizing effects. This combination of effects is essential for the successful treatment of various manifestations of vegetative dystonia, addressing both central nervous system and cardiovascular system involvement. Importantly, these preparations do not typically lead to addiction, withdrawal syndromes, or excessive sedation, allowing patients to maintain their social activity throughout treatment.

Although the preparation consists of individual herbal medicines, it should be considered as an active drug with a synergistic effect when used in combination, rather than merely a collection of individual herbs (Table 4.1).

The use of medicinal herbs in phytopreparations for improving sleep and addressing conditions related to psycho-emotional stress has a long history of traditional use. Scientific studies conducted over the past 20 years, involving thousands of participants, have provided evidence supporting the positive effects of valerian in the treatment of sleep disorders [197]. The European Medicines Agency has also

recognized the traditional use of herbal medicinal products containing *Leonurus cardiaca* L., herba, *Crataegus* spp. (hawthorn), and *Melissa officinalis* L. in addressing nerve stress, heart-related complaints, and sleep disturbances [177, 184, 192, 195].

Table 4.1 – Basic safety issues

Nature of the risk factors	Unfavorable (side) effects
Important identified risks	Reactions of hypersensitivity Depression and other disorders accompanied by inhibition of the central nervous system Pronounced arterial hypotension Bradycardia
Important potential risks	Severe liver dysfunction
Lack of information	The use of preparation during pregnancy and breastfeeding Application of the drug to children

Conditions related to psycho-emotional background disturbances, such as mood disorders and depression, can significantly reduce an individual's efficiency and quality of life, impacting both the patient and their loved ones. Studies have shown that these conditions, much like cardiovascular diseases, are becoming increasingly prevalent. For example, approximately 10 % of the total U.S. population, or about 19 million people, suffer from depression. According to the WHO, depressive disorders accounted for 40 % of all recorded mental illnesses worldwide in 2000. In developed countries, health authorities are actively seeking ways to address this issue. While modern antidepressants can be effective, their success rate does not exceed 70 %, and some individuals are resistant to these drugs. This has led to an intensive search for new antidepressants.

Phytotherapy, which involves the use of medicinal plants, offers a promising approach to addressing these psycho-emotional conditions. Phytotherapeutic remedies generally have fewer side effects and produce milder effects compared to pharmaceutical drugs, making them suitable for use in the early stages of conditions or for prevention. Sedative medications, which are available over-the-counter and are

often used without a doctor's prescription, play a significant role in supporting emotional well-being [198-199].

The prevalence and impact of conditions such as neurocirculatory dystonia, stress, nervous excitement, and heart neurosis are indeed significant medical and social problems. These conditions can have a profound effect on individuals' well-being and daily functioning.

Phytopreparations like Carvelis, which contain components known for their efficacy in addressing these conditions, offer a potential solution. It's important to consider the risks associated with any treatment, but in the case of phytopreparations, these risks are generally predictable and manageable. Physicians can assess patients for contraindications and carefully monitor their response to treatment. If side effects or adverse reactions do occur, there are often alternative treatments available.

Ultimately, the benefit of using phytopreparations, especially when the risks are understood and manageable, can outweigh the potential drawbacks. Many individuals find relief from their symptoms and an improvement in their quality of life through the use of these herbal remedies. However, it's crucial for patients to work closely with healthcare professionals to determine the most appropriate treatment approach for their specific needs and circumstances.

CHAPTER 5.
PERSPECTIVE FIXED COMBINATION FOR THE TREATMENT OF THE HEPATOBILIAR SYSTEM DISEASES: SUBSTANTIATION OF PHARMACOTHERAPEUTIC PROPERTIES AND PHARMACEUTICAL QUALITY PROFILE

Chronic hepatobiliary diseases represent a significant global health concern, ranking second only to atherosclerosis in terms of prevalence. According to the WHO, over 2 billion people worldwide are affected by liver diseases, making it 100 times more prevalent than HIV infection. Over the past two decades, there has been a noticeable upward trend in the incidence of hepatobiliary diseases. This increase is particularly concerning given that these diseases are now affecting individuals at a younger age and are disproportionately more common in women, occurring 4-7 times more frequently in women than in men. WHO experts estimate that in Europe, one in every five women and one in every ten individuals overall suffer from liver and biliary tract pathologies. This growing burden underscores the need for targeted healthcare strategies and interventions to prevent, diagnose, and treat these conditions effectively, thereby alleviating their impact on individuals and healthcare systems [52, 200].

The high prevalence of gallbladder removal (cholecystectomy) as the most frequently performed surgical procedure in abdominal medicine highlights the widespread occurrence of biliary system disorders. This trend is particularly concerning due to the increasing number of young and even infant patients diagnosed with cholelithiasis, a condition characterized by the formation of gallstones. Biliary system disorders are intricately linked to disruptions in the functional health of the liver. In cases where the synthesis of cholesterol-enriched bile occurs alongside reduced bile acid content, the risk of gallstone formation, particularly cholesterol-based gallstones within the gallbladder, significantly escalates [201-204].

In recent years, there has been a growing emphasis on the development of polyfunctional drugs to address the complex health issues seen in a rising number of patients with polymorphic pathology. Nowadays, medical practitioners frequently

encounter patients who present not with a single ailment but with multiple concurrent health conditions. According to contemporary statistics, approximately 80 % of patients seeking medical attention exhibit polymorbidity, where multiple health issues coexist. The underlying causes of polymorbidity often include the proximity of affected organs, shared pathogenic mechanisms, and causal relationships between different ailments. This scenario is particularly relevant in the context of hepatobiliary diseases and conditions stemming from metabolic disorders such as metabolic syndrome, diabetes mellitus, and atherosclerosis. The liver, being the primary organ for metabolism, occupies a central role in the development of these conditions. Polymorbidity typically necessitates the use of multiple medications, leading to polypharmacy. Therefore, the utilization of natural and safe agents that possess multifunctional properties for normalizing disease pathogenesis is advisable. These agents can help reduce the need for a multitude of drugs and mitigate the potential adverse effects associated with polypharmacotherapy [53, 54, 205-207].

In a previous study, the safety of a pharmaceutical combination containing artichoke leaf extract (200 mg), ursodeoxycholic acid (100 mg), taurine (100 mg), and *Angelica sinensis* root extract (50 mg) was scientifically substantiated. This preparation was formulated for the treatment of dyspeptic disorders associated with functional abnormalities of the biliary system, specifically targeting biliary dyskinesia of the hypokinetic type, as well as gastritis characterized by the reflux of bile [5, 55-56].

General characteristics of active pharmaceutical ingredients

The proposed pharmaceutical composition is a fixed combination of drugs with well-studied medical applications.

Ursodeoxycholic acid (UDCA) is a naturally occurring bile acid in the human body and is an epimer of chenodesoxycholic acid. It is characterized by its hydrophilic and non-cytotoxic properties. UDCA has been used as a medicinal product in global medicine for over three decades, and for more than 20 years in Ukraine [15, 16].

Initially, UDCA was recommended for the dissolution of gallstones and the treatment of reflux gastritis. Today, it is considered a standard treatment for cholestatic

liver diseases with an autoimmune component, such as primary biliary cirrhosis (PBC) and primary sclerosing cholangitis (PSC), among others. In Ukraine, it is classified within the pharmacotherapeutic group of drugs used for liver and bile duct diseases, under the ATC code A05AA02 [60, 208].

UDCA is naturally present in small amounts in human bile. When administered orally, it reduces the saturation of bile with cholesterol by slowing down cholesterol absorption in the small intestine and decreasing cholesterol secretion into bile. This process can lead to the partial dissolution of gallstones as a result of cholesterol dissipation and the formation of liquid crystals. In modern understanding, UDCA is believed to be effective in hepatitis and cholestatic diseases due to its ability to replace toxic, lipophilic bile acids with hydrophilic, cytoprotective, non-toxic ursodeoxycholic acid. It also improves hepatocyte secretory function and modulates immune processes [60, 208].

UDCA stabilizes the membranes of hepatocytes and cholangiocytes and has a direct cytoprotective effect. It reduces cell death caused by toxic bile acids. Due to its highly polar molecule, UDCA can form non-toxic mixed micelles with apolar (toxic) bile acids, reducing their ability to damage cell membranes in conditions like biliary reflux gastritis and reflux esophagitis. Additionally, UDCA forms dual molecules that can be incorporated into cell membranes, stabilizing them and making them less susceptible to the action of cytotoxic micelles. It also lowers cholesterol saturation in bile by inhibiting its absorption in the intestine, reducing hepatic synthesis, and decreasing bile secretion. These actions increase cholesterol solubility in bile, leading to the dissolution of cholesterol gallstones and preventing the formation of new ones. UDCA induces choleresis enriched with bicarbonates, promoting the dilatation of bile ducts and enhancing the excretion of toxic bile acids through the intestines [60, 208].

UDCA also exhibits immune-modulating effects by inhibiting the expression of HLA-antigens on hepatocyte and cholangiocyte membranes, normalizing natural killer lymphocyte activity, and more. It has been shown to slow down fibrosis progression in conditions such as primary biliary cirrhosis, cystic fibrosis, and

alcoholic steatohepatitis. Additionally, it reduces the risk of developing esophageal varices [76, 209-211].

Taurine. The second component of the drug is taurine, which is a sulfonic acid. Taurine is naturally formed in the body from the amino acid cysteine [60, 208]. It is typically found in small quantities in the tissues and bile of both humans and animals [67, 212].

Taurine is synthesized in the body through an enzymatic process that involves the oxidation of the sulfhydryl group of cysteine with the participation of cysteine dioxygenase, leading to the formation of cysteine sulfinic acid:

$$HSCH_2CH(NH_2)COOH \rightarrow HO_2SCH_2CH(NH_2)COOH, \quad (5.1)$$

followed by decarboxylation of cysteine sulfic acid in hypotaurine:

$$HO_2SCH_2CH(NH_2)COOH \rightarrow HO_2SCH_2CH_2NH_2, \quad (5.2)$$

and oxidation of hypotaurine in taurine [213]:

$$HO_2SCH_2CH_2NH_2 \rightarrow HO_3SCH_2CH_2NH_2. \quad (5.3)$$

Taurine forms conjugates with bile acids through acylation of the amino group, leading to the formation of conjugates such as taurocholeic and taurodeoxycholic acids. These conjugates are essential components of bile and act as surfactants, aiding in the emulsification of fats in the intestine. Taurine plays a role in lipid metabolism, enhances energy and metabolism, and promotes tissue healing in conditions characterized by significant metabolic disturbances [60, 67, 208].

Furthermore, taurine exhibits hepatoprotective, cardiotonic, and antihypertensive properties when administered systemically. It is utilized in the treatment of conditions like congestive heart failure, cardiac glycoside poisoning, and both type 1 and type 2 diabetes mellitus [69-72]. Taurine is frequently included in complex pharmaceutical formulations and belongs to the pharmacotherapeutic group of amino acids, with the ATC code S01X A 21. Similar to ursodeoxycholic acid, it has been used as a medicinal product in global medicine for more than three decades [67, 208].

Artichoke leaves extract. The third pharmacologically active substance in the drug is an extract from artichoke leaves (*Cynara cardunculus* var. *scolymus*). This

herbal remedy belongs to the pharmacotherapeutic group of agents used in liver and bile duct diseases, with the ATC code A05AX10. It possesses several beneficial effects, including choleretic, diuretic, hepatoprotective, and hypolipidemic properties.

Artichoke leaf extract promotes increased bile excretion, facilitates the elimination of nitrogen-containing substances like urea and creatinine, aids in the removal of toxins from the body, reduces blood lipid and total cholesterol levels, and alleviates sensations of stomach fullness while relieving spasms. These pharmacological effects are attributed to a combination of biologically active compounds found in the preparation, including cynarine, chlorogenic acid, ascorbic acid, carotene, B-group vitamins, and inulin. Cynarine, the primary active ingredient, is responsible for its choleretic effect, although its cholekinetic effect is less pronounced. Ascorbic acid, carotene, vitamins B1 and B2, and inulin present in artichokes contribute to the normalization of metabolic processes.

Artichoke leaf extract is indicated for use in dyspeptic symptoms, impaired biliary outflow, chronic hepatitis, gallbladder hypokinesia, chronic intoxication, chronic renal insufficiency, urolithiasis, urate-related conditions, atherosclerosis, and obesity as part of comprehensive therapy [215-218]. Similar to UDCA and taurine, artichoke extract has been utilized as a medicinal product in global medicine for more than three decades [67, 208].

Angelica sinensis extract. The fourth pharmacologically active substance in the drug is an extract from *Angelica sinensis*, commonly known as Danggui. This herbal remedy belongs to the pharmacotherapeutic group of other agents applied to functional disorders of the gastrointestinal tract, with the ATC code A03AX20. *Angelica sinensis* (Oliv.) Diels is a traditional medicinal and edible plant that has a long history of use for various purposes.

Angelica sinensis has been traditionally utilized for tonifying, replenishing, and invigorating blood, as well as for relieving pain, promoting intestinal lubrication, and treating irregular menstruation and amenorrhea in females. Additionally, A. sinensis has gained popularity as a health product and is increasingly used in China, Japan, and

Korea [219]. The plant's yellowish-brown root, harvested in the fall, has been a well-known component of Chinese medicine for thousands of years.

Review of biopharmaceutical trials

UDCA is a naturally occurring component in the normal metabolism of bile acids within the body. Its physico-chemical properties and the site of its physiological action suggest that it is best administered orally in capsule or tablet form. Research has shown that the degree of enrichment of bile with UDCA is generally independent of the dosage form or the frequency of daily doses but rather depends on the total daily dose [67, 208].

Nevertheless, ongoing research is exploring new UDCA dosage forms. One such formulation is based on UDCA and is coated with a specially developed capsule film that dissolves in the stomach and releases the active substance only at a pH level of 6.5 or higher [220]. In a study comprising 12 healthy volunteers, serum levels of UDCA were assessed following a single oral dose of 450 mg of UDCA in three distinct dosage forms: enteric-clearing capsules, solid gelatin capsules, and conventional gelatin capsules. The drug was administered subsequent to a meal. The results showed that the area under the curve (AUC, mmol/l) after oral administration of the enteric-coated UDCA capsule was significantly higher (39.0 ± 8.5) compared to conventional capsules (30.5 ± 4.9), both solid and non-enteric capsules (29.3 ± 3.4). Furthermore, the maximum serum UDCA concentration was significantly higher with the enteric-coated capsules compared to the other two formulations, although the time to reach maximum UDCA concentrations in serum was delayed. This suggests that the new capsule releases its contents into the intestine at a stage with the most alkaline pH, which is caused by the secretion of bile and pancreatic secretions. This improved solubility of UDCA at alkaline pH, creating a higher concentration gradient that facilitates passive absorption.

The physico-chemical properties of UDCA have also influenced the design of biopharmaceutical studies for its dosage forms intended for pediatric use [Santoveña et al., 2014]. Due to its very low solubility and low dose (1.5 %), UDCA for pediatric

dosing was formulated as a suspension with minimal excipient usage, avoiding the use of complex additives and those not recommended by the European Medicines Agency (EMA). Stable compositions with specific particle sizes were obtained, which remained stable for 30-60 days.

Clinical studies have explored various dosage regimens of UDCA and their effects on liver function in chronic liver diseases. These studies have provided valuable insights into the optimal dosing for different conditions.

For example, in patients with PBC, PSC, and chronic hepatitis (CH), UDCA was administered at doses of 250, 500, and 750 mg/day for two months, with each patient receiving a specific treatment regimen. Significant improvements in serum marker enzyme levels were observed in all groups at the 250 mg/day dose, which roughly corresponds to 4-5 mg/kg/day. Higher doses (500 mg/day and 750 mg/day) led to further improvements, particularly in PBC patients, but no significant differences were found between the 500 mg/day and 750 mg/day dose levels [221].

In a short-term, randomized, double-blind, controlled cross-study, different UDCA dosing regimens were investigated, including a 3-month course followed by placebo for 3 months or placebo for 3 months followed by a 3-month UDCA course. Additionally, long-term UDCA courses lasting up to 20 months were studied [222]. Dosage regimens of UDCA up to 8.7 mg/kg/day have been studied in double-blind, multicenter trials for PBC [223]. Another randomized study compared three UDCA doses for treating PBC: low (5-7 mg/kg/day), normal (13-15 mg/kg/day), and high (23-25 mg/kg/day) UDCA doses. In this study, no statistically significant differences were observed between the effects of different doses [224].

Higher UDCA doses (25-30 mg/kg/day) have been explored in clinical trials for patients with PSC. These studies have indicated potential benefits for patients with PSC, and further evaluation in long-term, randomized, placebo-controlled trials is warranted [225]. A randomized, double-blind, multi-dose study evaluated the efficacy of UDCA at various doses (300, 600, 900 mg/day) for bile acid metabolism over a 21-day period, with higher average UDCA doses showing greater efficacy [226].

However, not all studies have yielded positive results. A multicenter, double-blind, randomized controlled trial examined the prophylactic use of UDCA (15 mg/kg/day) in patients after liver transplantation but did not confirm the initial optimism regarding the beneficial effects of UDCA prophylactic treatment in preventing acute rejection after liver transplantation [227].

Taurine is a natural component present in the human body, highly soluble in water, and capable of passing through biological barriers. Given that taurine works in synergy with UDCA to exert its hepatoprotective effects, the selection of dosage forms is influenced by its physical and chemical properties. Concerning the specific taurine content in capsules, a series of clinical studies explored various dosing regimens [228-230]. For instance, a combination of taurine at a dosage of 30 mg/kg/day with UDCA at 15 mg/kg/day was administered for a year, resulting in an enhanced pharmacological effect in the treatment of liver disease among patients with severe pancreatic dysfunction and poor nutritional status [228]. Tauroursodeoxycholic acid (TUDCA) at doses ranging from 10 to 13 mg/kg/day, administered for 3 months, was found to induce hepatocyte proliferation in humans [231]. High-dose TUDCA regimens (30 mg/kg/day) during enteral administration were also investigated for pediatric patients [229]. However, the combination of taurine with UDCA was found to be ineffective in preventing or treating cholestasis in newborns.

The third component of the drug is artichoke extract, and its herbal origin and complex chemical composition can affect its antioxidant profile, antioxidant activity, and physical characteristics. Pharmaceutical preparations of artichoke leaves have shown that the antioxidant activity is higher in aqueous fractions compared to lipophilic fractions, and this activity correlates with the total phenol content in the extracts [232-233].

Regarding the quantitative content of artichoke extract in capsules, clinical studies have explored various dosage regimens. In the treatment of functional dyspeptic symptoms, oral administration of artichoke extract (containing 15 % chlorogenic acid, 150 mg extract per capsule) for 60 days resulted in a positive clinical effect, with a 50 % reduction in the total score of all symptoms observed in 38% of patients within

30 days and 79 % within 60 days. Additionally, after 60 days of treatment, patients showed decreased levels of total cholesterol, low-density lipoprotein, and serum triglycerides by 6-8 % compared to baseline values ($p \leq 0.001$), as well as a 13-20 unit per liter reduction in the activity of transaminases and gamma-glutamyltransferase compared to higher initial values ($p < 0.01$) [234]. In a randomized, double-blind, placebo-controlled study of the effects of large doses of artichoke extract on low-density lipoprotein cholesterol in patients with primary moderate hypercholesterolemia, it was found that doubling the daily dose from 3 to 6 tablets for 4 weeks did not result in any additional statistically significant increase in the pharmacological effect [235].

Radix *Angelica sinensis*, derived from the dried root of *Angelica sinensis* (commonly known as Danggui), is a herb extensively used in Chinese medicine for its potential benefits in enriching blood, promoting blood circulation, and modulating the immune system. Additionally, it has been employed in the treatment of chronic constipation among the elderly and individuals with weakened health, as well as for managing menstrual disorders. Research has unveiled various pharmacological properties of Danggui and its active constituents, highlighting its potential as an anti-atherosclerotic, anti-hypertensive, antioxidant, and anti-inflammatory agent. Moreover, it has shown promise in limiting platelet aggregation, reducing the size of cerebral infarctions, and enhancing neurological deficit scores. One of its chemical compounds, butylidenephthalide, exhibits antispasmodic activity *in vitro* and may provide relief from muscle cramps. Over the years, extensive chemical analysis has identified a total of 165 constituents in *Radix Angelica sinensis*, encompassing phenylpropanoids, essential oils, phthalides, terpenoids, alkaloids, sterols, fatty acids, and polysaccharides, among others [236-239].

Pharmacokinetic studies

The available literature does not provide comprehensive information regarding the effects of UDCA, taurine, and artichoke extract on the various enzymes (1A2, 2A6, 2C9, 2C19, 2D6, and 2E1) within the cytochrome P450 system in human biomaterials.

While UDCA has been identified as an inducer of CYP3A, the clinical significance of this effect remains uncertain. Research employing quantitative polymerase chain reaction and Western blot techniques to investigate the impact of ursodeoxycholic acid on proteins involved in bile acid metabolism and xenobiotic detoxification processes in the intestines of both healthy individuals and patients with PBC has revealed that UDCA induces the expression of genes related to crucial intestinal transport proteins. This includes breast cancer resistance proteins (BCRP) and P-glycoprotein, both of which are involved in multidrug resistance. However, there appears to be no direct reversal effect on the enzymes responsible for bile acid metabolism. Additionally, there is some data suggesting that taurine may influence the biosynthesis of specific transport proteins involved in the cytochrome P450 2E1-mediated catabolism of xenobiotics [240-242].

Absorption. UDCA is typically present in the human body as a small fraction of the total bile acids (approximately 5%). Upon oral administration, UDCA is primarily absorbed in the small intestine and, to a lesser extent, in the colon through passive, non-ionogenic diffusion. Absorption is incomplete, with approximately 50 % of UDCA absorbed from the portal blood in its first pass through the liver, where it conjugates with amino acids. Following a single oral dose of 500 mg UDCA in healthy volunteers, peak plasma concentrations ranged from 2.7 to 6.3 μg/ml. The maximum concentration was achieved after 60 minutes, with a second peak concentration observed after 180 minutes. The absorption rates for different doses of UDCA (250 mg, 500 mg, 1000 mg, and 2000 mg) were found to be 60.3 %, 47.7 %, 30.7 %, and 20.7 %, respectively. UDCA binds to plasma proteins to a high extent, with a binding rate of 96-98 % [60, 208].

Taurine, a sulfur-free amino acid, is a natural component of the human diet, but its pharmacokinetics in humans, particularly after oral administration, has only recently received attention. A study on the pharmacokinetics of taurine involved 8 healthy male volunteers with a mean age of 27.5 years (ranging from 22 to 45 years). These individuals were administered an oral dose of 4 g of taurine in the morning. Blood samples were collected at specific time intervals, and the concentration of taurine in

plasma was measured using a modified HPLC method. Following oral administration, taurine was absorbed from the gastrointestinal tract over a period of 1-2.5 hours. The maximum concentration of taurine in plasma (C_{max}) reached an average of 0.69 ± 0.15 mmol, and it was observed around 1.5 ± 0.6 hours after administration (T_{max}). The plasma half-life of taurine varied from 0.7 to 1.4 hours, with an average of 1.0±0.3 hours. The volume of distribution ranged from 19.8 to 40.7 liters, with an average of 30.0 ± 7.6 liters. The clearance/bioavailability ratio ranged from 14.0 to 34.4 l/h, with an average of 21.1 ± 7.8 l/h. The area under the curve (AUC) for the interval 0-8 hours ranged from 116.0 to 284.5 mg/l, with an average of 206.3 ± 63.9 mg/l [243].

In another study, the pharmacokinetic parameters of taurine were investigated in the presence of intravenous injection of 200 mg of taurine in 6 hypertensive patients and 6 healthy volunteers. This study reported a shorter half-life of taurine (3.85 ± 0.05 min) and a larger distribution volume (9.6 ± 3.2 liters). However, it's important to note that this study monitored taurine concentration in blood plasma for only 20 minutes, primarily capturing the alpha phase, which is influenced by the absorption phase in oral administration [243].

The study on artichoke leaf extracts involved 14 healthy volunteers who received doses of two different extracts [77]. Here are the details of the extracts and the findings:

1. First Extract:
- Content of caffeoylquinic acids equivalent to 107.0 mg of carbohydrate.
- Content of luteolin glycosides equivalent to 14.4 mg of luteolin.
2. Second Extract:
- Content of caffeoylquinic acids equivalent to 153.8 mg of caffeic acid.
- Content of luteolin glycosides equivalent to 35.2 mg of luteolin.

In this study, urine and plasma samples were analyzed using a validated HPLC method. The results indicated different absorption and metabolic pathways for various components of the extracts:

Peak concentrations of carbohydrate, ferulic acid, and isofuric acid in the blood plasma were achieved within 1 hour after administration.

On the other hand, the highest concentrations of dihydrofuranic acid and dihydroisopropylacetate were detected approximately 6-7 hours after administration, indicating distinct metabolic pathways for these compounds when compared to the metabolization of caffeoylquinic acids.

The peak concentration of luteolin glycosides in plasma was rapidly reached within just 0.5 hours after administration.

These findings provide insights into the absorption, metabolism, and utilization of different components of artichoke leaf extracts in the human body.

Studies on Angelica sinensis extract have encompassed investigations into its pharmacokinetics in both normal and pathological animals, as well as exploring drug-drug interactions and pharmacokinetics in humans.

Regarding *Angelica sinensis* extract there some works dedicated directly to its pharmacokinetics in normal and pathological animals [244, 245]. Several studies have been conducted to observe both drug-drug interactions and pharmacokinetics [246, 247].

The main pharmacokinetic parameters of *Angelica sinensis* extract have been examined and summarized by Chinese researchers (Table 5.1) [245] using a validated ultra-performance liquid chromatography-triple quadruple mass spectrometry (UPLC-TQ/MS) method to quantify the content of the main constituents in Gui-Hong extracts (ancient and classic formula comprised of *Angelica sinensis* and *Carthamus tinctorius* L.). The results of these studies have revealed the following concentrations of key compounds in Gui-Hong extracts (at a concentration of 0.405 g/ml):

- Hydroxysafflor yellow A (HSYA): 144.075 μg/ml
- Caffeic acid: 3.758 μg/ml
- p-Coumaric acid: 4.350 μg/ml
- Kaempferol-3-O-rutinoside: 33.844 μg/ml
- Ferulic acid: 31.647 μg/ml
- 3-n-Butylphthalide: 1.583 μg/ml

- Ligustilide: 3.175 μg/ml

These findings provide valuable information about the pharmacokinetics and composition of Angelica sinensis extract, shedding light on its potential therapeutic effects and interactions within the human body.

Table 5.1 – Pharmacokinetic parameters of seven components of Gui-Hong extracts (n = 6) [245]

Components	C_{max} (ng/mL)	T_{max} (h)	$T_{1/2z}$ (h)	MRT_{0-t} (h)	AUC_{0-t} (ng× mL^{-1}×h)	$AUC_{0-\infty}$ (ng× mL^{-1}×h)
Hydroxysafflor yellow A	25 ± 9	1.8 ± 0.40	2.60 ± 0.30	3.65 ± 0.27	116 ± 31	127 ± 32
Caffeic acid	20 ± 5	0.75 ± 0.00	2.70 ± 1.20	2.21 ± 0.21	41 ± 3	53 ± 11
p-Coumaric acid	750 ± 29	0.17 ± 0.00	1.04 ± 0.37	0.76 ± 0.11	430 ± 34	434 ± 35
Kaempferol-3-*O*-rutinoside	17 ± 3	0.75 ± 0.00	1.00 ± 0.53	1.73 ± 0.21	31 ± 8	32 ± 8
Ferulic acid	341 ± 34	0.17 ± 0.00	2.38 ± 1.10	1.43 ± 0.19	273 ± 65	312 ± 77
3-*n*-Butylphthalide	21 ± 11	0.17 ± 0.00	7.60 ± 1.73	2.55 ± 0.08	48 ± 9	109 ± 25
Ligustilide	136 ± 25	0.08 ± 0.00	6.33 ± 1.45	5.69 ± 0.76	432 ± 71	463 ± 79

The pharmacokinetic parameters of hydroxysafflor yellow A (HSYA) in blood stasis rats have shown distinct differences compared to non-blood stasis conditions. Specifically, the blood stasis rats exhibited the following pharmacokinetic characteristics for HSYA:

1. Higher C_{max}: This indicates that the peak concentration of HSYA in the bloodstream was higher in blood stasis rats.
2. Longer $T_{1/2z}$: Blood stasis rats had a prolonged half-life of HSYA in their plasma, suggesting that the compound lingered in their circulation for a longer duration.
3. Greater AUC_{0-t} and $AUC_{0-\infty}$: Both measures of the total exposure to HSYA were higher in blood stasis rats, indicating increased bioavailability.
4. Lower T_{max}: Blood stasis rats reached the maximum plasma concentration of HSYA more quickly.

These differences in pharmacokinetic parameters can be attributed to several factors related to blood stasis. It is suggested that HSYA is primarily absorbed through the small intestine. In conditions of poor blood circulation associated with blood stasis, the retention time of HSYA in the small intestine may be prolonged. This prolonged exposure in the intestine could lead to increased absorption of HSYA into the bloodstream, contributing to the higher Cmax and enhanced bioavailability observed in blood stasis rats.

Additionally, slowed blood circulation may reduce the perfusion of the liver, which could result in decreased hepatic metabolism processes such as hydroxylation, methylation, acetylation, and glucuronidation of HSYA. The decreased metabolism of HSYA in the liver further contributes to its increased bioavailability in blood stasis conditions.

These findings suggest that blood stasis can significantly impact the pharmacokinetics of HSYA, leading to altered drug absorption, distribution, and metabolism in the body [6245, 248, 249].

The pharmacokinetic differences observed for various compounds in blood stasis rats compared to normal rats suggest that the condition of blood stasis can significantly impact the absorption, distribution, and metabolism of these compounds. Here is a summary of the pharmacokinetic findings for specific compounds:

1. **Caffeic Acid:**

Blood stasis rats showed higher C_{max}, $T_{1/2z}$, AUC_{0-t}, MRT_{0-t}, and $AUC_{0-\infty}$.

T_{max} was the same in normal and blood stasis rats [245].

Catechol-O-methyltransferase (COMT)-mediated O-methylation is involved in the metabolism of caffeic acid in rat hepatocytes.

The induction of blood stasis by adrenaline hydrochloride or the occlusion of the left anterior descending coronary artery resulted in a reduction of COMT activity in both liver plasma and the heart..

The increased bioavailability of caffeic acid in blood stasis rats may be attributed to decreased liver and blood metabolism [245, 250-252].

2. **p-Coumaric Acid:**

Blood stasis model samples showed higher C_{max}, $T_{1/2z}$, AUC_{0-t}, MRT_{0-t}, and $AUC_{0-\infty}$ for p-coumaric acid.

The absorption of p-coumaric acid was increased, and the elimination process was slowed in pathological states.

Compatibility in rats may also alter the pharmacokinetic behavior of p-coumaric acid [253].

3. **Kaempferol-3-O-rutinoside:**

Blood stasis model samples exhibited higher C_{max}, $T_{1/2z}$, AUC_{0-t}, MRT_{0-t}, and $AUC_{0-\infty}$ for kaempferol-3-O-rutinoside.

Kaempferol-3-O-rutinoside is a flavonoid glycoside with high polarity and large molecular size, leading to its low bioavailability after oral administration [253].

4. **Ferulic Acid:**

Blood stasis rats showed higher C_{max}, AUC_{0-t}, and $AUC_{0-\infty}$ for ferulic acid.

$T_{1/2z}$ and MRT_{0-t} were lower in blood stasis rats [253].

T_{max} was the same in normal and blood stasis rats, suggesting rapid absorption of ferulic acid in blood stasis conditions.

5. **3-n-Butylphthalide:**

Blood stasis model rats showed lower $T_{1/2z}$ and MRT_{0-t}, and higher C_{max}, AUC_{0-t}, and $AUC_{0-\infty}$ for 3-n-butylphthalide.

The $T_{1/2z}$ and AUC_{0-t} of 3-n-butylphthalide had a significant difference in blood stasis model rats.

3-n-Butylphthalide is mainly metabolized by CYP2E1, 2C11, and 3A1/2 in rats, and the altered elimination time may be attributed to changes in these enzymes in blood stasis conditions [253].

6. **Ligustilide:**

Blood stasis model rats exhibited higher C_{max}, AUC_{0-t}, and $AUC_{0-\infty}$, and lower MRT_{0-t} and $T_{1/2z}$ for ligustilide.

The time and extent of absorption of ligustilide were accelerated in blood stasis model rats.

There was no significant difference in ligustilide pharmacokinetics between normal and blood stasis model rats [253].

These findings collectively suggest that the presence of blood stasis alters the pharmacokinetics of these compounds, affecting their absorption, metabolism, and distribution in the body. The specific mechanisms underlying these changes may vary for each compound and involve factors such as metabolic enzyme activity and the physicochemical properties of the molecules.

There is also pharmacokinetic data available in animal models for substances with well-established therapeutic properties [254]. The pharmacokinetic study [255] involving ferulic acid and paeoniflorin in mice after intra-gastric administration of a combination of *Angelica-Paeonia* root powder provides specific pharmacokinetic parameters for ferulic acid. Here are the key pharmacokinetic parameters for ferulic acid observed in the experiment:

T_{peak}: 2.606 ± 0.586 hours

C_{max}: 6.372 ± 1.510 mg/l

$t_{1/2(ka)}$ (Half-life of Absorption): 1.249 ± 0.365 hours

t1/2(ke) (Terminal Elimination Half-life): 2.101 ± 0.665 hours

AUC: 41.399 ± 11.763 mg × h/l

Ke (Elimination Rate Constant): 0.330 ± 0.085 h^{-1} (per hour)

Ka (Absorption Rate Constant): 0.555 ± 0.133 h^{-1} (per hour)

These parameters provide a comprehensive understanding of how ferulic acid is absorbed, distributed, metabolized, and eliminated in the body following intra-gastric administration of the combination *Angelica-Paeonia* root powder in mice. These pharmacokinetic parameters are essential for assessing the bioavailability and behavior of ferulic acid, which is crucial for its therapeutic applications.

Other researchers [256] have also reported a limited oral bioavailability of senkyunolide A in rat studies. They conducted pharmacokinetic investigations into senkyunolide A, a key component found in the essential oil of Rhizoma Chuanxiong (*Ligusticum chuanxiong*), a plant commonly employed for treating cardiovascular ailments in traditional medicine. The study in rats revealed the following results:

1. Intravenous Administration:

Senkyunolide A displayed extensive distribution in the body, denoting a high volume of distribution (Vd/F).

Rapid elimination from the bloodstream was observed, indicating a short half-life ($T_{1/2}$).

The primary route of elimination was strongly indicative of hepatic metabolism, as substantiated by findings from an *in vitro* S9 fraction analysis.

2. Intraperitoneal (IP) Administration:

Senkyunolide A exhibited consistent pharmacokinetics, irrespective of the administered dose, following IP administration.

Rapid absorption (characterized by a short T_{max}) was noted, and a relatively high bioavailability of 75 % was documented.

3. Oral Administration:

Senkyunolide A was rapidly absorbed when administered orally, with a brief T_{max}.

However, its oral bioavailability was notably low, estimated at approximately 8 %.

Factors contributing to this included the instability of the compound in the gastrointestinal tract (accounting for 67 % of the loss) and extensive hepatic first-pass metabolism (accounting for an additional 25 %).

4. Effect of Extract:

The pharmacokinetics of senkyunolide A remained largely unchanged when it was administered as an extract. This suggests that the components within the extract had minimal influence on senkyunolide A's pharmacokinetic behavior.

These findings shed light on the challenges associated with achieving adequate oral bioavailability of senkyunolide A, which is commonly used in traditional medicine for cardiovascular conditions.

Distribution. In healthy individuals, approximately 70 % of unconjugated UDCA binds to plasma proteins [257]. UDCA specifically binds to a site on a protein molecule responsible for binding biliary bile acids in the ileum, known as the ileal bile

acid-binding protein (IBABP). It's worth noting that UDCA also increases the affinity of other human bile acids for the second binding site on IBABP. This interaction with UDCA reduces the cooperative binding effect often observed with major bile acids in humans. Additionally, IBABP plays a crucial role in fully activating the farnesoid-X alpha receptor (FXRα) for bile acids, including ursodeoxycholic acid. However, there is currently no available information on the binding of conjugated UDCA to plasma proteins in both healthy individuals and patients with primary biliary cirrhosis. Since the efficacy of UDCA primarily relies on its concentration in bile rather than in plasma, its serum levels do not serve as an indicator of bioavailability in clinical conditions. While the precise volume of distribution of UDCA has not been determined, it is believed to be relatively small, as the compound is primarily distributed in the bile and small intestine. In bile, the concentration of UDCA reaches its peak within 1-3 hours [258].

Taurine can be obtained from external sources or synthesized within the human body. Once administered, taurine is rapidly absorbed and distributed throughout various tissues. Most notably, taurine accumulates in the brain, retina, heart muscle, liver, and kidneys. Taurine has the capability to cross both the blood-brain barrier and the placental barrier [67].

The pharmacological activity of both artichoke extract and *Angelica sinensis* extract arises from the collective effects of their components. As a result, comprehensive pharmacokinetic studies regarding their distribution in human tissues have not been extensively conducted [60, 208].

Metabolism. Approximately 50-70 % of UDCA from portal blood is initially absorbed by the liver, where it conjugates with amino acids, primarily glycine and taurine. Conjugated UDCA is then excreted into bile and subsequently enters the small intestine. Within the intestine, some UDCA conjugates may undergo reverse processes, leading to deconjugation and reabsorption in the ileum. Additionally, UDCA conjugates can be subjected to dehydroxylation, leading to the formation of lithocholic acid, some of which is absorbed and then sulfated in the liver before being excreted from the body through the bile ducts.

In adults, around one-quarter of bile acids are conjugated to taurine, and a small portion of taurine itself is converted to isethionate with the involvement of either bacterial or tissue enzymes. Subsequent metabolism proceeds with the formation of sulfate, CO_2, water, and ammonia, with the latter being further converted into urea [259].

The components of artichoke extract are extensively metabolized within the human body, giving rise to methylated derivatives of carbohydrate, such as ferulic and iso-fructose acids, as well as hydration products like dihydric and dihydrofuranic acids. Except for dihydrofurylic acid, all these compounds are present in the human body in the form of sulfates or glucuronides. Luteolin, another component, undergoes complete metabolism in the human body, leading to the formation of sulfate or glucuronide metabolites [77].

There are several reports on the metabolism of ligustilide, a key bioactive ingredient in Angelica sinensis extract. One study [260] investigated the pharmacokinetics of ligustilide, administered both in its pure form and within an herbal extract, in rats. After intravenous administration of pure ligustilide, it exhibited extensive distribution (Vd: 3.76 ± 1.23 l/kg) and rapid elimination ($T_{1/2}$: 0.31 ± 0.12 h). Notably, the intravenous clearance (CL) of ligustilide was significantly higher when administered as an extract compared to its pure form (CL: 20.35 ± 3.05 versus 9.14 ± 1.27 l/h/kg, $p < 0.01$; area under the curve (AUC): 0.79 ± 0.10 versus 1.81 ± 0.24 mg × h/l, $p < 0.01$), suggesting significant interaction between ligustilide and components within the extract. Dose-dependent pharmacokinetics were observed after intraperitoneal administration, with a significantly higher dose-normalized AUC at 52 mg/kg compared to 26 mg/kg (1.77 ± 0.23 mg × h/l versus 0.93 ± 0.07 mg × h/l, $p < 0.05$). Oral bioavailability of ligustilide was low (2.6 %), primarily due to extensive first-pass metabolism in the liver. Seven metabolites of ligustilide were identified, with three of them being definitively characterized as butylidenephthalide, senkyunolide I, and senkyunolide H, all of which occur naturally in the herb and are reported to be bioactive.

In another study [261], evidence is presented indicating that caffeic acid (CA) from plant extracts can undergo oxidation by peroxidase/H_2O_2 or tyrosinase/O_2. Mass spectrometry analysis of the metabolites formed in the presence of peroxidase/H_2O_2/glutathione (GSH) revealed the formation of mono- and bi-glutathione conjugates, with bi-glutathione conjugates forming only when GSH was present. In the absence of GSH, hydroxylated products and p-quinones of CA were formed due to peroxidase/H_2O_2 activity. NADPH also supported CA-glutathione conjugate formation in rat liver microsomes, which was inhibited by benzylimidazole, a cytochrome P450 inhibitor. Furthermore, the cytotoxicity of CA towards isolated rat hepatocytes was significantly enhanced by hydrogen peroxide or cumene hydroperoxide-supported cytochrome P450 and was inhibited by benzylimidazole. Cytotoxicity was also markedly increased by dicumarol, an NADPH/oxidoreductase inhibitor. These findings suggest that dihydroxycinnamic acids are metabolically activated by P450 peroxidase activity to generate cytotoxic quinoid metabolites. Metabolism of phenolic compounds has been comprehensively described in several articles [252, 262, 263].

Elimination. UDCA is primarily eliminated through feces, with urinary excretion accounting for less than 1 % of the elimination, except in cases of severe cholestatic liver disease. In healthy volunteers who received a single oral dose of 500 mg ^{14}C-labeled UDCA, approximately 30 to 44 % of the dose is excreted in the feces during the initial three days. This excretion consists of unchanged UDCA (2-4 %), lithocholic acid (37 %), and 7-keto-lithocholic acid (5 %). The half-life of UDCA, as determined using the radioactive label, ranges from approximately 3.5 to 5.8 days following oral administration due to the effective enterohepatic circulation of UDCA in the body. In individuals with severe liver disease, renal excretion becomes a significant route for eliminating bile acids [60, 208].

Following a single oral administration of taurine at a 4 g dose, its plasma concentration returns to the normal range after 8 hours during the elimination phase. Taurine elimination from plasma occurs through a mechanism characterized by first-order kinetics [243]. The total content of taurine in the body is regulated by the kidneys.

Taurine is among the major amino acids excreted in urine since its reabsorption by the kidneys is limited [264, 265]. The daily amount of taurine excreted in urine varies depending on diet but typically falls within the range of 65 to 250 mg (0.5–2.0 mmol).

Metabolic products of artichoke extract are primarily eliminated through the kidneys via urine, and their elimination profile often exhibits a biphasic pattern [77]. Elimination of metabolic products of *Angelica sinensis* extract has been reported in previously described studies [245, 246, 261, 263-265].

Pharmacokinetics in special patient groups. The pharmacokinetics and bioavailability of UDCA do not appear to be influenced by an individual's sex. Additionally, among individuals of European and Mongolian races, there have been no clinically significant inter-racial differences observed in the pharmacokinetic parameters of UDCA. However, there is limited data available regarding the pharmacokinetics of UDCA in individuals of the Negroid race [60, 208].

Pregnant women. There is a lack of valid and well-controlled studies involving pregnant women for UDCA, taurine, and artichoke extract. Due to the fact that the impact on animal reproductive function does not necessarily predict the response in humans, it is not recommended to use these compounds in women who are pregnant or who may become pregnant. If these preparations are used during pregnancy or if a patient becomes pregnant while taking these substances, it is crucial to be aware of the potential risks they may pose to the fetus [60, 208]. It is advisable to consult with a healthcare provider for guidance on the use of these compounds during pregnancy.

There is evidence suggesting that taurine accumulates in the tissues of the mother during pregnancy and is subsequently transferred to the fetus through the placenta as well as to the newborn through the mother's milk. This accumulation can also occur in the brain of newborns. Low levels of taurine in the maternal body can result in reduced taurine levels in the fetus and may contribute to slower growth in offspring [266].

Breastfeeding women. The excretion of orally administered UDCA, taurine, and artichoke extract into maternal milk was not investigated in available studies [60, 208]. Therefore, there is limited information on whether these compounds are

excreted into breast milk when taken orally by lactating mothers. If a nursing mother is considering taking any of these compounds, it is advisable to consult with a healthcare provider for guidance on the potential risks and benefits, as well as alternative options if necessary. The safety of these compounds during breastfeeding has not been well-established.

Older patients (geriatrics). There are no adequate or well-controlled studies of UDCA, taurine, and artichoke extract in the geriatric population [60, 208]. This means that there is limited scientific data available on how these compounds specifically affect older adults.

Children (pediatrics). In the case of UDCA, taurine, and artichoke extract, there is a lack of adequate and well-controlled studies in the pediatric population. Instead, there are only individual studies available that have explored various aspects of the pharmacological action of these compounds in children of all ages, including newborns [267].

For UDCA, limited research has investigated the pharmacokinetic parameters in newborns. This research used an alpha magnetic spectrometer to measure the concentration of sub-therapeutic doses of ^{14}C-labeled UDCA in small fractions of biological fluids. However, due to the small sample size (data from only 3 newborns) and high data variability, definitive conclusions are challenging to draw. Since the absorption of bile acids in the intestine and their passage through the liver's portal vein are still immature processes in young children, the pharmacokinetic parameters of UDCA may differ significantly in the pediatric population compared to adults.

As for taurine, it is considered an essential amino acid in humans, and it is routinely added to many infant nutrition formulas. This addition is a precautionary measure aimed at improving the nutritional quality of formulas for premature infants and children with conditions like cystic fibrosis. Taurine is also believed to have a positive impact on the auditory pathway in the brains of premature newborns, although further research is needed in this area [268, 269].

Pharmacodynamic studies

Upon oral ingestion, UDCA undergoes absorption through passive diffusion in the intestine and active transport in the ileum. In the liver, UDCA forms conjugates with glycine and taurine. These conjugated compounds are subsequently excreted into the bile, where UDCA becomes part of the enterohepatic circulation system. With consistent administration, UDCA gradually becomes the predominant bile acid in the bloodstream, constituting approximately 50 % of the total bile acid pool. This increase in UDCA concentration in the bile pool is dose-dependent [270].

The presence of UDCA in bile results in an enrichment that enhances bile's hydrophilicity while reducing its cytotoxicity. UDCA plays a role in regulating apical secretion within hepatocytes by modulating the phosphorylation and dephosphorylation of transport proteins, thereby activating or deactivating them, ultimately improving the liver's excretory function. Elevated UDCA levels in bile lead to a reduction in the extent of damage to cholangiocytes, decreased portal inflammation, and inhibition of duct proliferation. Furthermore, UDCA promotes the secretion of bile acids and various organic anions, such as glucuronides of bilirubin and glutathione conjugates, effectively preventing cholestasis caused by hydrophobic bile acids. Patients diagnosed with PBC and PSC who receive UDCA treatment exhibit a reduced inflammatory response within their bile ducts. Currently, it is presumed that UDCA operates through three primary mechanisms: protecting cholangiocytes from the cytotoxic effects of hydrophobic bile acids, stimulating hepatobiliary secretion, and shielding hepatocytes from apoptosis induced by bile acids [62, 270-276].

The precise mechanisms underlying UDCA's cytoprotective effects are not yet fully elucidated. However, it is established that the excessive accumulation of toxic bile acids can trigger hepatocyte apoptosis. UDCA acts to suppress this apoptosis by intervening in mitochondrial dysfunction. Laboratory experiments have demonstrated that UDCA can prevent apoptosis induced not only by deoxycholic acid but also by other damaging factors, including ethanol, transforming growth factor, Fas-ligand, and okadaic acid. UDCA's mechanism of action involves reducing mitochondrial

depolarization, subsequently inhibiting the release of cytochrome C and the activation of caspases [62, 270-276].

The mechanism by which UDCA and tauroursodeoxycholic acid (TUDCA) inhibit apoptosis is depicted in Figure 5.1. UDCA interferes with mitochondrial processes, including the inhibition of Bax translocation, the synthesis of reactive oxygen species (ROS), the release of cytochrome *C*, and the activation of caspase-3. According to available data, UDCA may also interfere with the death receptor pathway by inhibiting caspase-3 activation. Additionally, TUDCA suppresses apoptosis processes associated with endoplasmic reticulum (ER) stress by regulating intracellular calcium levels, inhibiting calpain, and activating caspase-12. It's worth noting that UDCA interacts with nuclear steroid receptors (NSRs), leading to the dissociation of NSR/hsp90 complexes and facilitating the nuclear translocation of UDCA/NSR complexes. Once in the nucleus, UDCA modulates the E2F-1/p53/Bax pathway, effectively preventing apoptosis. Furthermore, UDCA reduces levels of cyclin D1 and Apaf-1, further inhibiting the mitochondrial apoptotic cascade [277].

In pharmacological doses, UDCA has a significant impact, reducing the saturation of bile with cholesterol by approximately 40-60 %. It achieves this by inhibiting the absorption of cholesterol in the intestine and suppressing its secretion into bile [274]. UDCA plays a crucial role in diminishing the toxicity of bile acids, which have the potential to harm cell membranes and lead to cholestasis. This protective effect involves several mechanisms, including the inhibition of the absorption of endogenous hydrophobic bile acids from the small intestine and a choleretic effect. The choleretic effect leads to the dilution of endogenous bile acid salts within the bile ducts, providing protection to hepatocytes.

For the treatment of cholelithiasis, the recommended UDCA dose is typically 8-10 mg/kg/day, with larger doses offering no additional benefits. The dissolution rate achieved with UDCA therapy is approximately 1 mm reduction in the diameter of a gallstone per month. It's worth noting that the diameter of gallstones larger than 20 mm can negatively impact the speed of dissolution and the overall success of litholytic therapy. If there is minimal reduction or no significant change in the size of the

gallstone after 6-12 months of UDCA treatment, it may suggest a less favorable prognosis for dissolution. The likelihood of reducing the diameter of large gallstones (greater than 20 mm) or multiple gallstones through litholytic therapy typically does not exceed 40-50 % after the first year of treatment [275].

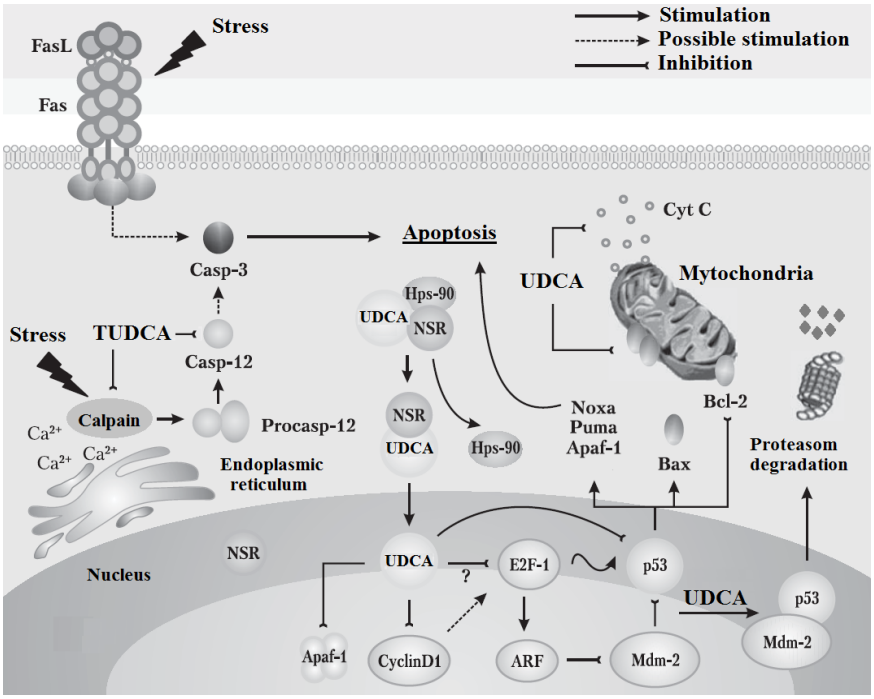


Figure 5.1 – Mechanisms of UDCA and TUDCA inhibition of apoptosis [277]: Apaf-1 – apoptotic protease activating factor 1; ARF – ARF (alternative reading frame) cell suppressor; Bax, Bcl-2 – negative (Bcl-2) and positive (Bax) regulators of apoptosis; Casp-12, Casp-3 – caspases; Cyt C – cytochrome *C*; Fas – Fas cell surface death receptor; Fas L – ligand of Fas- receptor; Hsp-90 – heat shock protein; MDM-2 – mouse double minute 2 homolog; NSR – nuclear steroid receptors; Procasp-12 – procaspase

Gallbladder sludge represents another therapeutic target for UDCA. Its formation can be triggered by various factors such as rapid weight loss, pregnancy, parenteral nutrition, or organ transplantation. Clinical studies have demonstrated the positive effects of UDCA in patients with idiopathic acute pancreatitis and concurrent microcholelithiasis or gallbladder sludge. These patients received UDCA preparations for a period of 3-6 months, leading to the prevention of cholelithiasis and pancreatitis relapses during a 44-month observation period in the majority of cases [62].

Additionally, UDCA exhibits anti-inflammatory properties. Long-term administration of UDCA preparations significantly reduces the occurrence of complications associated with cholelithiasis. Research has shown that UDCA treatment in patients with cholelithiasis results in a decreased incidence of biliary pain and cholecystitis exacerbations over an 18-year period [278]. During the initial four years of UDCA treatment, relapses occurred in less than 10 % of cases, compared to 40 % in the placebo group. Interestingly, this therapeutic effect was not solely dependent on the dissolution of gallstones but was associated with a reduction in overall inflammatory processes within the body. Other studies [273] have indicated that UDCA treatment restores the contractile function of the gallbladder, improves oxidative-reduction processes, and alleviates oxidative stress and inflammation, all of which contribute to positive effects on biliary symptoms regardless of gallstone dissolution.

The reduction in inflammation is thought to be potentially mediated by the antioxidant properties of UDCA, a decrease in prostaglandin E2 concentration, and a reduction in catalase activity. However, in clinical trials involving patients with severe cholecystitis and frequent biliary colic, UDCA treatment for 100 days did not show significant positive effects on the clinical course [272].

UDCA's properties are also utilized in the treatment of liver parenchymal lesions. Recent experimental data have indicated the drug's significant hepatoprotective properties, particularly against the most common toxic agent, alcohol. UDCA has been shown to improve the functional and morphological state of the liver. However, clinical trials involving the administration of UDCA at doses of 13-15 mg/kg/day for 6 months in individuals with progressive alcoholic cirrhosis of the liver (Child-Pugh Classes B and C) did not demonstrate improved survival rates in these patients [271]. Using modern diagnostic methods such as ultrasound elastography of the liver, it was observed that patients who were additionally taking UDCA during abstinence had a more pronounced reduction in organ density [279]. Clinical trials assessing the efficacy of UDCA in alcohol-induced liver damage have not been conducted extensively, making it challenging to determine the drug's exact role in

treating various forms of alcoholic liver disease. This may be attributed to the considerable heterogeneity of clinical variants of alcoholic liver disease and the lack of universally accepted recommendations regarding dosage and treatment duration. Given the diverse biochemical and immunological effects of UDCA, its use is considered feasible in various clinical presentations of alcoholic liver disease.

An important aspect of using UDCA involves the treatment of biliary reflux. The damaging impact of bile acids is influenced by factors such as their concentration, conjugation, and the pH of the environment. Taurine-conjugated bile acids, for instance, remain soluble even at a low pH of 2. Therefore, in environments with very low pH values, only taurine-conjugated bile acids tend to cause damage. Conversely, in situations with high pH values, such as post-surgery stomach conditions, unconjugated bile acids can have a harmful effect. Bile acids, known for their detergent properties, facilitate the solubilization of lipids within the membranes of surface epithelial cells. Soluble conjugated bile acids, typically in the pH range of 2-4, can penetrate into these epithelial cells. Inside the cells, bile acid concentrations can be up to 8 times higher than outside, and this excessive accumulation can lead to increased cell membrane permeability, membrane damage, disruption of intercellular connections, and ultimately, cell loss. The detrimental effects depend not only on the concentration of bile acids during reflux but also on the duration of exposure of the mucous membrane to bile.

In the presence of pancreatic phospholipase A, bile acids, and trypsin, biliary lecithin undergoes a reaction to form lysolecithin. When bile acids and lysolecithin are present in the gastric mucous membrane, there is an increase in reverse hydrogen ion diffusion, along with elevated histamine and gastrin release. The negative effects of duodenogastroesophageal reflux on the esophageal mucous membrane are well-established. In this context, the esophagus is exposed to hydrochloric acid and pepsin, and the impact of bile acids depends on the pH. Conjugated bile acids have a negative effect in acidic pH conditions (pH 2-4), while unconjugated bile acids show their harmful effects at pH levels ranging from 5 to 8. In alkaline pH conditions, the negative effects are observed not only for unconjugated bile acids but also for trypsin [280].

The hydrophilic nature of UDCA and its choleretic effect play crucial roles in protecting the esophagus and stomach. UDCA does not harm cell membranes because UDCA micelles are nearly insoluble in them due to their hydrophilic properties. UDCA competes with toxic hydrophobic bile acids for receptor binding, effectively displacing them. Induction of cholerexis, where bile enriched with bicarbonate aids in the elimination of toxic bile acids through the intestines, is also important. In experiments and clinical studies, the cytoprotective properties of UDCA have been demonstrated in safeguarding the mucous membranes of the stomach and esophagus. UDCA achieves this by incorporating into the phospholipid layer of cell membranes, thereby contributing to their stabilization and increased resilience against damaging factors. Furthermore, the administration of UDCA medications reduces the subjective symptoms of gastric dyspepsia [270].

UDCA serves as a preventive measure against tumor development by counteracting the stimulatory effects of other bile acids, particularly deoxycholic acid (DCA). UDCA and DCA have contrasting impacts on the epidermal growth factor receptor (EGFR) and the expression of cyclooxygenase-2, both of which can play pivotal roles in colon tumorigenesis. While some clinical studies with theoretical foundations have suggested that UDCA can reduce the risk of colorectal cancer, it's important to note that there are limited high-quality trials available, and most of the evidence is retrospective in nature. UDCA exerts its tumor-preventing effect by negatively modulating the mitochondrial activation pathway, which involves inhibiting the translocation of Bax, the generation of active oxygen species, the release of cytochrome *C*, and the activation of caspase-3 [281].

Taurine is a vital component of bile acids, and conjugation is essential to maintain the solubility of bile acids in the watery environment of the intestinal contents. Sulfur's presence in tauroconjugates facilitates their ionization, enhancing their detergent action, solubility, and reabsorption. Bile acids play a crucial role in preserving intestinal barrier function and preventing the invasion of enterobacteria into tissues. Furthermore, bile acid tauroconjugates exhibit choleretic effects and prevent cholestasis, unlike glycine-conjugated bile acids [282]. *In vitro* studies have shown that

glycolithocholic acid is easily precipitated with calcium at physiological concentrations, unlike taurolithocholic acid. Therefore, taurine is essential for increasing bile turnover, enhancing bile acid production, and preventing cholestasis. The introduction of taurine is likely to result in decreased levels of cholesterol, triglycerides, low-density lipoprotein, and body weight. It also reduces cholesterol in the aorta wall, the quantity of lipid peroxidation products, while increasing glutathione levels. Studies have demonstrated that taurine administration inhibits cell proliferation by suppressing the expression of mitogen-activated protein kinase [87]. Additionally, taurine plays a crucial role in regulating cellular membranes, providing membrane protection and osmoregulation. It has a positive impact on the phospholipid composition of membranes and normalizes the electrolyte balance, retaining potassium and magnesium inside cells and sodium outside. Taurine is also involved in the movement of calcium ions through membranes [87].

Taurine plays a crucial role in regulating calcium levels in the heart, and its stabilizing effect on cell membranes is associated with a wide range of regulatory functions in the body. These functions include the normalization of protein, carbohydrate, and electrolyte metabolism, as well as the activity of various enzymes and hormones. Taurine also contributes to energy production and regenerative processes in the body, and it strengthens the immune system. Additionally, supplemental taurine has been shown to have a positive impact on antioxidant defense parameters and can reduce the manifestations of diabetic neuropathy, nephropathy, and retinopathy in experimental settings.

Taurine helps prevent a decrease in the activity of membrane-bound Na^+/K^+-ATPase and excessive calcium loss. It has been observed to improve glucose utilization, leading to beneficial effects on glycosylated hemoglobin levels and the intensity of lipid peroxidation processes in erythrocytes. This underscores the potential therapeutic value of taurine in diabetes [87].

Furthermore, taurine has another important function in diabetes management by helping maintain normal blood glucose levels through increased insulin receptor binding efficiency. The use of taurine in diabetes has been shown to normalize platelet

function and raise amino acid levels in the bloodstream. Experimental evidence suggests that taurine can improve glucose and lipid metabolism, reduce insulin resistance, and lower hypercholesterolemia. Additionally, taurine helps prevent the development of microangiopathy by reducing the degree of apoptosis in endothelial cells [87].

Taurine has been found to possess antioxidant properties, capable of binding active oxygen species. Its metabolic precursor, hypotaurine, also exhibits antioxidant characteristics. Preemptive taurine administration has been shown to mitigate acute bronchiolitis induced by NO_2 inhalation, possibly acting as a membrane stabilizer that regulates the flow of potassium, sodium, calcium, and magnesium ions [101].

In vitro studies have demonstrated that taurine, when forming taurochloramine, can bind to hypochlorous acid, a potent oxidant known to cause DNA damage. Taurochloramines may also play a regulatory role in inflammatory processes by inhibiting the production of interleukins 6 and 8, potentially through the downregulation of cytokine gene transcription [283]. The functional activity of macrophages is closely associated with the transport of taurine across cell membranes. Treatment of macrophages with lipopolysaccharide (at concentrations of 0.1 and 10 μg/ml) leads to a significant 60 % reduction in taurine transport ($P < 0.01$). However, simultaneous treatment with lipopolysaccharide and gamma-interferon (150 units/ml) restores taurine transport to control values after 24 hours. It has been demonstrated that inositol can reverse the suppression of taurine transport in macrophages under certain conditions [87].

Additionally, taurine conjugates with secondary bile acids, retinoids, and certain xenobiotics become more water-soluble after binding to taurine, which enhances their clearance. This suggests a potential role for taurine in detoxification processes. Taurine has shown effectiveness in conditions such as liver cirrhosis, depression, and male infertility [87]. It has also been reported to have beneficial effects on the gastric and intestinal mucosa. In cases of cystic fibrosis, taurine supplementation has been found to reduce the severity of steatorrhea. In Alzheimer's disease,

characterized by a decline in memory associated with a decrease in acetylcholine concentration, taurine may play a beneficial role.

In conclusion, taurine serves numerous physiological functions within tissues and effectively modulates them under various pathophysiological conditions, underscoring its importance in maintaining high concentrations within energy-dependent cells [283].

The pharmacological properties of artichoke extract can be attributed to the collective impact of various biologically active compounds present in artichoke leaves (*Cynara scolymus* L.). Notably, cynarine, in combination with phenolic acids and bioflavonoids found in artichoke, contributes to the choleretic, diuretic, and hepatoprotective effects of the extract. The potent diuretic effect of artichoke preparations promotes increased excretion of urea and the elimination of toxic substances, including “middle molecules”, through urine. The application of artichoke extract results in a significant detoxifying effect, particularly by reducing the intensity of endogenous or metabolic intoxication. The phenolic acids present in artichoke leaf extract, including coffee, chlorogenic, neochlorogenic, and caffeic acids, exhibit high biological activity and contribute to the pronounced immunoactive actions of artichoke preparations [284].

The beneficial effects of artichoke extract, particularly when used in combination with vitamin E (tocopherol acetate), in patients with conditions like non-alcoholic steatohepatitis (NASH) concurrent with chronic non-calculous cholecystitis (CNCC) and secondary immunodeficiency states (SIDSs), can be attributed to multiple factors. These include the normalization of lipid peroxidation indicators (LIPs), such as the reduction in lipoperoxidation products like diene conjugates (DC) and malondialdehyde (MDA) in the blood serum. Additionally, there was an improvement in clinical and biochemical parameters reflecting liver function, as well as a reduction in manifestations of SIDSs, such as T-lymphopenia and imbalances in T-lymphocyte subpopulations. This study demonstrated that the combination of artichoke extract and vitamin E exerts antioxidant and immunomodulatory effects [285].

Furthermore, the antioxidant activity of artichoke extract was examined in patients with NASH and CNCC who also had abdominal obesity. This investigation revealed a decrease in the levels of lipid peroxidation products, specifically MDA and DC, in the blood serum of patients with NASH and CNCC during periods of unstable remission or moderate exacerbation of chronic gallbladder inflammation in the context of obesity. The positive effects of artichoke extract, whether used alone or in combination with the immune-active preparation galavit, on lipid peroxidation indices were also observed in patients with NASH and osteoporosis [286].

It is firmly established that the activity of lipid peroxidation processes, coupled with a reduction in antioxidant defense, constitutes a common pathological mechanism in the progression of chronic liver and gallbladder disorders. Phytochemicals found in artichokes are widely acknowledged for their natural antioxidant properties, which have been substantiated through both experimental and clinical investigations. Hence, it can be reasonably deduced that the utilization of artichoke preparations may provide advantages in the treatment of diverse chronic liver and gallbladder pathologies [286].

The pharmacological characteristics of the *Angelica sinensis* extract stem from its influence on various systems within the patient's body, attributed to the comprehensive array of biologically active compounds it contains. Currently, the recognized pharmacological properties of this plant encompass effects on the cardiovascular and cerebrovascular systems, anti-inflammatory properties, antifibrotic effects, antispasmodic activities, antioxidant actions, neuroprotective qualities, immune support, and hematopoietic effects [287]. Considering the pharmacotherapeutic profile of the complex preparation under development, let us explore in detail its anti-inflammatory and antispasmodic attributes.

In 1986, Li et al. [287] discovered that sodium ferulate has the capability to regulate the prostacyclin (PGI2)/thromboxane A2 (TXA2) ratio by inhibiting TXA2 activity while leaving PGI2 unaffected. Ligustilide (LIG) exhibited a concentration-dependent anti-inflammatory effect when tested on lipopolysaccharides (LPS)-activated microglia without causing any cytotoxicity. When pretreated with LIG at concentrations of 2.5, 5, 10, and 20 μmol/l, it led to a reduction in LPS-induced NO

production by 75.9 %, 54.4 %, 43.1 %, and 47.6 %, respectively. Similarly, TNF-α content decreased to 86.2 %, 68.3 %, 40.1 %, and 39.9 %, Interleukin-1β (IL-1β) content to 31.5 %, 27.7 %, 0.6 %, and 0 ($P < 0.01$), and MCP-1 content to 84.4 %, 50.3 %, 45.1 %, and 42.2 %, respectively, compared to LPS treatment alone. LIG at a concentration of 10 μmol/l significantly inhibited LPS-stimulated immunoreactivity of activated nuclear factor κB (NF-κB), cyclooxygenase-2 (Prostaglandin-endoperoxide synthase 2), and inducible nitric oxide synthase (iNOS). This evidence suggests that LIG possesses strong anti-inflammatory properties against microglia by inhibiting the NF-κB pathway. These findings highlight the potential neuroprotective effects of LIG and its prospective application in treating neuroinflammatory diseases characterized by excessive microglial activation [287, 288].

Su et al., in their 2011 study [289], aimed to investigate the effects of Ligustilide (LIG) on inflammation induced by lipopolysaccharide (LPS) in RAW 264.7 macrophages. They observed that LIG significantly suppressed the production of nitric oxide (NO), prostaglandin E2 (PGE2), and tumor necrosis factor-alpha (TNF-α). The inhibition of NO production was accompanied by a decrease in both protein and mRNA levels of LPS-induced inducible nitric oxide synthase (iNOS). Moreover, LIG effectively inhibited the activation of activator protein-1 (AP-1) and nuclear factor-kappa B (NF-κB) in the nucleus, as well as the cytosolic degradation of IκBα. LIG also inhibited the phosphorylation of IκB kinase (IKK) and mitogen-activated protein kinases (MAPKs), including p38 MAPK, extracellular signal-regulated kinase (ERK1/2), and c-Jun N-terminal kinase (JNK). Additionally, the intracellular reactive oxygen species (iROS) level was significantly reduced. These findings suggest that LIG possesses anti-inflammatory properties by blocking the activation of MAPKs/IKK and downstream transcription factors AP-1 and NF-κB, possibly through the down-regulation of iROS production [288, 289].

Furthermore, in the context of ischemia-reperfusion (I/R) injury, LIG was found to have significant neuroprotective effects. It led to a reduction in neurological deficit score, infarct volume, and RTP801 expression while increasing erythropoietin (EPO) transcription in I/R rats. Additionally, LIG increased cell viability and EPO

levels while reducing lactate dehydrogenase (LDH) and RTP801 in I/R neurons. The positive effects of LIG on phosphorylated extracellular signal-regulated kinase (p-ERK), cell viability, and EPO were significantly blocked by PD98059 but not by LY294002 and SB203580. Transfection of SH-SY5Y cells with RTP801 plasmid DNA induced an increase in RTP801 levels and LDH release, and LIG effectively inhibited the effects of transfection on RTP801 expression while increasing cell viability. These findings suggest that LIG plays a significant neuroprotective role against I/R injury by promoting EPO transcription through the ERK signaling pathway and inhibiting RTP801 expression, making it a potential candidate for the prevention and treatment of ischemic disorders [287, 290].

Research into the antispasmodic activity of *Angelica sinensis* extract has spanned several decades. In one study, cell culture techniques were employed to investigate the mechanism by which *Angelica* polysaccharide (APS) inhibits the proliferation of HaCaT cells, which are spontaneously transformed aneuploid immortal keratinocyte cells derived from adult human skin. The study utilized trypan blue staining and flow cytometry to assess the impact of APS on HaCaT cell proliferation. The cell growth curve revealed that APS, within a dose range of 25-2500 mg/l, significantly inhibited HaCaT cell growth in a dose-dependent manner. Flow cytometry results indicated a reduction in the S phase and G2/M phase of HaCaT cells, along with a remarkable increase in the G0/G1 phase of HaCaT cells when treated with 250 mg/l APS. This study suggested that APS effectively inhibits HaCaT cell proliferation by disrupting the mechanism of DNA synthesis and preventing these cells from entering the S phase [287].

In another study, the effects of intrauterine hypoxia on the proliferation and differentiation of neural stem cells (NSCs) in neonatal rats were explored, along with the protective role of *Angelica* injection under hypoxic conditions. Immunohistochemistry and image processing systems were employed to analyze the expression of glial fibrillary acidic protein (GFAP) and neuron-specific enolase (NSE). The study yielded the following results: (1) Expression of GFAP-positive cells in the hippocampus of neonatal rats in the hypoxia group was higher than in the control

group; (2) Expression of NSE-positive cells was lower in the hypoxia group compared to the control group; (3) Expression of GFAP-positive cells in the hippocampus of neonatal rats was lower in the angelica group than in the hypoxia group, whereas expression of NSE-positive cells was higher in the angelica group than in the control group. These findings indicated that hypoxia stimulates the proliferation of NSCs in neonatal rats and promotes the differentiation of NSCs into glial cells. Simultaneously, the number of neurons in the hippocampal CA3 area decreased. Angelica injection attenuated the ability of NSCs to proliferate and differentiate into glial cells following hypoxia, and it also effectively mitigated the loss of neurons. Thus, it was suggested that angelica injection has a protective effect on the nervous system of neonatal rats exposed to intrauterine hypoxia [287].

The hepatoprotective effects of polysaccharides from various preparations of *Angelica sinensis* were the focus of a recent study. Polysaccharides are significant chemical compounds found in *Angelica sinensis*, known for their effectiveness in treating liver diseases and providing hepatoprotection. However, the specific molecular mechanisms of polysaccharides from different *Angelica sinensis* products have not been thoroughly investigated. This study aimed to evaluate the effects and potential mechanisms of polysaccharides from different Angelica sinensis products in countering carbon tetrachloride-induced liver injury in mice.

Liver injury was induced by intraperitoneal injection of carbon tetrachloride (CCl_4) in the mice. Gas chromatography-mass spectrometry (GC-MS) in combination with pattern recognition methods, such as principal component analysis (PCA) and partial least squares-discriminant analysis (PLS-DA), were employed to identify differentiating metabolites in both plasma and liver tissue.

The results showed that the PCA and PLS-DA score plots of the liver injury group clustered distinctly from the control group. However, the groups treated with polysaccharides from charred *Angelica sinensis* (ASTP), parched *Angelica sinensis* with soil (ASTUP), parched *Angelica sinensis* with wine (ASJP), and parched *Angelica sinensis* with sesame oil (ASYP) clustered closely with the control group. This suggests

that the metabolic profiles of the ASTP, ASTUP, ASJP, and ASYP groups closely resemble those of the control group.

Several potential metabolite biomarkers were identified, including citric acid, succinic acid, glycine, palmitelaidic acid, arachidonic acid, fumaric acid, malic acid, valine, alanine, and hexadecanoic acid (in both liver homogenates and plasma). Functional pathway analysis indicated that the alterations in these metabolites are associated with lipid, amino acid, and energy metabolism [291].

Notably, ASTP appeared to have a potential pharmacological effect by regulating multiple perturbed pathways back to their normal state. The study suggests that ASTP, ASTUP, ASJP, and ASYP intervene in the metabolic processes of liver injury in mice by affecting lipid and amino acid metabolism.

Metabonomics, as demonstrated in this study, is a robust and promising approach for identifying biomarkers and elucidating the mechanisms underlying diseases, which is highly relevant in the context of drug discovery [292].

Efficiency and safety studies

UDCA has emerged as a prominent treatment option for liver and bile duct disorders over the past three decades. Its application has significantly broadened to encompass a wide range of conditions, including PBC, PSC, chronic hepatitis with cholestatic features (especially in cases involving alcohol or medications), cystic fibrosis, biliary tract atresia, post-transplant cholestasis, cholestasis associated with parenteral nutrition, intrahepatic cholestasis during pregnancy, chronic viral hepatitis (either as a standalone therapy or in combination with antiviral treatment), and NASH. Additionally, UDCA is now utilized in the management of conditions like biliary reflux gastritis, reflux esophagitis, postcholecystectomy syndrome, disturbances in antroduodenal motility in various upper gastrointestinal tract diseases, and atherogenic dyslipidemia. Its formal indications include the dissolution of cholesterol gallstones in the gallbladder, treatment of gastritis resulting from bile reflux, and symptomatic management of PBC during the compensation phase. Beyond these established uses, UDCA is frequently employed in pharmacotherapy for various clinical conditions such

as acute and chronic hepatitis of diverse origins and cholestasis. Following reports of UDCA's effectiveness in dissolving gallstones, it has even served as a non-surgical alternative for cholelithiasis treatment. [60, 208, 274, 293]

Ukrainian clinicians conducted a study [293] to investigate the effectiveness and safety of UDCA drugs. One of their studies revealed that when UDCA was administered at a dosage of 15 mg/kg/day in 2-3 divided doses over the course of a month, complete dissolution of gallstones was observed in patients. It's worth noting that these gallstones were relatively small, ranging from fine dispersed particles to 7-8 mm in diameter, and they were often present in multiple numbers, ranging from 2 to 5.

In another clinical study, the efficacy and safety of UDCA drugs were evaluated in the oral treatment of 70 patients. Oral litholithic therapy represents a truly non-invasive approach for treating gallstone disease (GSD). This therapy offers several advantages, including the absence of significant side effects, a low risk of mortality, and the possibility of outpatient treatment. However, as demonstrated by clinical research [293], successful gallstone dissolution through this method is contingent on strict patient selection criteria:

1. Gallstone Size: Gallstones should not exceed 15 mm in size.
2. Composition: The gallstones should consist primarily of cholesterol and not produce shadows on X-rays or an “acoustic path” on ultrasound.
3. Gallbladder Function: The gallbladder must maintain normal function, and the bile ducts should be clear and unobstructed.
4. Gallbladder Filling: The gallbladder should contain gallstones occupying less than half of its volume.
5. Bile Duct Clearance: The bile ducts should be free of gallstones.

Patients were also cautioned not to take clofibrate, estrogens, cholestyramine, or antacids during the litholithic therapy period, as these substances contain compounds that can bind bile acids. Additionally, the regular and consistent intake of UDCA was crucial for the successful dissolution of gallstones. Patient selection was also influenced by the observation that the most favorable conditions for oral lithotripsy are

typically found in the early stages of the disease, when GSD is uncomplicated, bile duct episodes are infrequent, and there is only a moderate level of pain.

Seventy patients with GSD were monitored under supervision, comprising 18 men and 52 women. Abdominal ultrasound examinations were conducted using Toshiba-33 and Aloka-630 machines (Japan). It's noteworthy that cholelithiasis was incidentally discovered in these patients, as they were unaware of its presence. Their primary complaint was a feeling of heaviness in the right hypochondrium. This examination and treatment period spanned from 2002 to 2007. The average age for men was (56.4 ± 2.9) years, while for women, it was (48.7 ± 3.4) years.

Regarding body weight, 20 individuals weighed between 60-70 kg, 30 patients fell into the 71-80 kg range, and the remaining 20 patients weighed between 81-90 kg. Excess body weight was prevalent among half of the patients, with women being the majority. The majority of patients (65 out of 70) followed a diet predominantly composed of refined foods.

All patients had their liver's functional state assessed based on various biochemical parameters, including bilirubin levels, total protein, protein fractions, and the activities of alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma-glutamyltransferase (GGT), and alkaline phosphatase (ALP). Biochemical analyses of blood and ultrasound scans were conducted at intervals of 1, 3, 6, 9, and 12 months from the commencement of treatment.

In the biochemical analysis, a slight increase in the total bilirubin level was observed in 8 patients, while other indicators remained within the normal range. All patients were found to have cholesterol gallstones, which were typically round or oval in shape and were identified based on specific criteria [293]. The number of gallstones in the gallbladder ranged from 2 to 5. Among them, 30 patients had gallstones measuring up to 5 mm, another 30 had stones measuring between 6-8 mm, and the remaining 10 patients had stones measuring 10 mm or larger.

UDCA was administered at a dosage of 10 mg/kg/day, and the treatment duration varied from 1 month to 1 year. Patients were instructed to take UDCA capsules without chewing, typically before bedtime, ensuring a consistent release of the drug

throughout the day. Patients were provided with information about the pharmacological properties of UDCA, the expected duration of treatment, and anticipated results. Additionally, all patients were prescribed diet no. 5, also known as Pevzner's diet.

After 1 month of treatment, complete dissolution of gallstones was observed in 10 patients who had small, solitary gallstones. This number increased to 26 patients after 3 months, 21 patients after 6 months, 4 patients after 9 months, and 3 patients after 12 months of treatment. Among the 6 patients who initially had gallstones measuring up to 10 mm, their size was reduced to 2-5 mm. In one patient, calcinosis of gallstones with an acoustic shadow, as observed on ultrasound, developed, leading to the discontinuation of UDCA treatment. Patients with stones that initially appeared resistant to dissolution were subjected to an additional 2-month treatment course, after which their gallstones completely dissolved.

Globally, only one meta-analysis has been conducted to date, comprising 23 randomized controlled trials (RCTs) examining the efficacy of UDCA and CDCA in treating cholelithiasis [294]. This analysis involved 1949 patients who adhered to recommended medication doses for more than 6 months. The most favorable outcomes were achieved when UDCA was administered at doses exceeding 7 mg/kg/day, resulting in the dissolution of concretions in 37.3 % of the patients.

Despite the fact that gallstones primarily consist of cholesterol and should theoretically dissolve, a significant portion of gastroenterologists still hold reservations based on the results of the mentioned meta-analysis, and they consider the actual rate of gallstone dissolution to be around 10 %. Patients seeking UDCA treatment should have cholesterol-based (non-bilirubin) concretions without signs of weighing less than 20 mm in diameter and free-flowing bile ducts. Most clinicians view UDCA as optimally effective for cholelithiasis treatment at doses not exceeding 10 mg/kg/day, as increasing the dosage further does not significantly enhance the therapeutic effect. On average, gallstone diameter tends to reduce by about 1 mm per month during litholithic therapy. Concretions exceeding 20 mm in diameter have a negative impact on the speed of dissolution and overall success of litholithic therapy. The absence of

noticeable changes or minimal reduction in concretion size after 6-12 months of UDCA treatment indicates a less favorable outlook for dissolution [293]. The chance of reducing the diameter of large (over 20 mm) or multiple stones through litholithic therapy does not exceed 40-50 % after the first year of treatment [275].

In 2012, a meta-analysis examining the hepatoprotective properties of UDCA was conducted, based on the results of 3 randomized controlled trials (RCTs) involving microscopic evaluation of biopsy specimens. The analysis revealed a reduction in globular inflammation and serum gamma-glutamyltransferase (GGT) levels [295].

The most pronounced therapeutic effects of UDCA are observed in patients with PBC and PSC. Previous meta-analyses of RCTs showed that UDCA not only improved patients' well-being and biochemical parameters but also alleviated itching and had an impact on survival. However, a more recent systematic review did not confirm these findings [296]. This review encompassed 16 RCTs involving 1,447 patients and did not demonstrate significant benefits of adding UDCA to standard treatment regimens in terms of outcomes like disease-related mortality, overall mortality, or the need for liver transplantation. It also did not significantly improve the intensity of itching and fatigue. The inconsistency with previous results may be attributed to the inclusion of patients with severe and terminal stages of the disease, where UDCA treatment may not be effective.

A similar situation was observed in patients with PSC. In 2011, a group of Greek researchers, led by S. Triantos, conducted a meta-analysis of 8 placebo-controlled studies involving 567 patients to assess the treatment outcomes [297]. This study took place in the last decade and focused on the impact of high doses of UDCA (> 15 mg/kg) on the course of the disease. The findings indicated that the drug did not lead to a reduction in mortality, itching intensity, general weakness, or the risk of cholangiocarcinoma. Furthermore, there was no significant change in the histological picture. These results were corroborated the following year by a meta-analysis conducted by G. Poropat and colleagues. Despite potential improvements in biochemical parameters, this analysis did not show a reduction in the risk of death or improvement in the histological picture [298]. UDCA did not decrease the need for

transplantation and did not prevent the development of portal hypertension and encephalopathy.

A double-blind, double-simulated, randomized (1:1), cross-linked, multi-center clinical trial was conducted in eight centers in Germany and one in the Netherlands. This trial followed a two-stage, group-level, adaptive design and examined two different forms of UDCA administration: tablets and capsules [299]. A total of 65 patients participated in the trial and were randomized. The study found that UDCA tablets and UDCA capsules had an equal therapeutic effect on serum biochemical parameters, demonstrating therapeutic equivalence. In terms of safety, a total of 98 adverse events (AEs) were recorded, with AEs reported in 43 out of 64 (67.2 %) patients. The overall incidence of AEs during treatment with UDCA tablets was very similar to that observed with UDCA capsules: 28 out of 62 (45.2 %) patients and 29 out of 61 (47.5 %) patients, respectively. Most AEs were related to digestive disorders, such as upper abdominal pain, bloating, abdominal distension, nausea, and vomiting. No deaths were reported during the study, but two serious side effects unrelated to UDCA therapy led to hospitalizations—one patient required a nasal septum operation, and another underwent aortic valve replacement due to stenosis. Overall, both UDCA formulations were well-tolerated, and the safety analysis revealed a similar safety profile for UDCA tablets and capsules.

The study also assessed therapeutic efficacy using clinically relevant parameters, including ALT, AST, and GGT, and found that the maximum effect of UDCA on liver function parameters was generally observed after 8 weeks. Clinical efficacy of both UDCA capsules and tablets in patients with PBC was demonstrated, and this was further supported by the results of a pharmacokinetics study at a daily dose of 15 mg/kg. Both forms of UDCA were well-tolerated and had similar safety profiles. Notably, a significantly larger number of patients (45.3 %) preferred taking tablets compared to capsules (15.6 %).

According to evidence-based medicine, the hepatoprotective effect of UDCA remains somewhat uncertain. Traditional endpoints like death or organ transplant may not always be sufficient to demonstrate the effectiveness of hepatoprotective agents.

Many systematic reviews on UDCA pharmacotherapy emphasize the lack of high-quality randomized trials that are free from systematic errors, random errors, and biased results. Therefore, a conclusive determination of UDCA's hepatoprotective properties will likely require future studies designed with these considerations in mind.

Recently, a relatively new area of study for UDCA has emerged, focusing on biliary sludge, which represents an initial manifestation of gallstone disease (GD) [300]. This research has generated particular interest not only among gastroenterologists but also among general practitioners and family physicians. Biliary sludge is defined as a thick, viscous suspension in the gallbladder containing dense structures (0.05-1.2 mm crystals) and small concretions, measuring up to 2-3 mm in diameter. Another term for biliary sludge is biliary microlithiasis, which refers to the presence of thickened bile in the gallbladder that creates a distinct echo structure when observed by ultrasound and moves slowly when the patient changes position.

The clinical significance of biliary sludge has been a subject of debate because some patients experience its spontaneous disappearance (in about 18-70 % of cases). However, biliary sludge is rarely asymptomatic. Research indicates that in 25-70 % of patients, the presence of biliary sludge is associated with symptoms such as biliary dyspepsia, pain episodes (colic in 9-15 % of patients), and dysfunction of the sphincter Oddi. One of the most serious complications of biliary sludge, in addition to biliary colic attacks, includes episodes of cholecystitis, cholangitis (including purulent forms), the development of gallstone disease, and stenotic papillitis. Notably, biliary sludge is believed to be responsible for the development of acute idiopathic pancreatitis in 33-90 % of patients, according to various authors [300].

In accordance with the classification of gallstone disease (GSD) by the Russian Gastroenterologists Association in 2003 [301], biliary sludge is considered an early manifestation of the disease, typically occurring in its initial stage. Therefore, in many cases involving the presence of biliary sludge, medical intervention is not only possible but also advisable, often involving the use of UDCA preparations. Successful prophylaxis of gallstone disease can be achieved through drug therapy, particularly for individuals who are overweight and seeking to lose weight.

In 2007, Germany adopted recommendations for the diagnosis and treatment of GSD. According to these guidelines, administering UDCA at a daily dose of no less than 500 mg for 3-6 months significantly reduces the risk of developing gallstone disease, especially in individuals undergoing rapid weight loss (losing over 1.5 kg per week) [302].

UDCA preparations are intriguing not only for their hepatotropic (liver-focused) properties but also for their numerous extrahepatic clinical effects. One relatively recent indication for the use of UDCA is in cases of reflux gastritis and esophagitis resulting from alkaline biliary reflux. The hydrophilic nature of UDCA and its choleretic (bile-promoting) effect are important for protecting the esophagus and stomach. UDCA competes with toxic hydrophobic bile acids for receptors and promotes the induction of cholerexis, where bile rich in bicarbonate aids in eliminating harmful bile acids through the intestine.

It has been determined that alkaline gastroesophageal reflux occurs in 5-20 % of patients with gastroesophageal reflux disease (GERD). During this pathological reflux of duodenal contents, the mucin barrier of the esophageal mucosa is compromised, exposing it to aggressive pancreatic enzymes, particularly trypsin, and bile acids. This can complicate the course of GERD and potentially lead to the development of Barrett's esophagus and esophageal adenocarcinoma [300].

Recent studies have provided insights into the optimal dosage of UDCA (ursodeoxycholic acid) for bile reflux, indicating that 500 mg per day (250 mg in two doses) is effective. This dosage is preferred because it allows the bile acids present in the refluxate to interact with UDCA in a water-soluble form, causing less irritation to the stomach and esophageal mucosa. The treatment course for biliary reflux typically lasts for at least two months. In experiments and clinical studies, UDCA has demonstrated cytoprotective properties for safeguarding the mucous membranes of the stomach and esophagus. UDCA is believed to stabilize cell membranes by incorporating into the phospholipid layer, increasing their resistance to harmful factors. Additionally, the use of UDCA medications reduces subjective symptoms of gastric dyspepsia [302, 303].

UDCA also has extrahepatic effects related to inflammatory bowel disease and the primary and secondary prevention of colorectal cancer associated with inflammatory bowel disease. For example, UDCA has shown a prophylactic effect on colorectal cancer progression in patients with poorly differentiated dysplasia. In one study, patients who received UDCA for 2 years did not experience deterioration, whereas 22.2 % of patients who did not take UDCA progressed to dysplasia requiring colectomy [304].

In patients with PBC, clinical trials have explored the potential of tauroursodeoxycholic acid TUDCA as an alternative to UDCA for chronic cholestatic liver disease. However, direct comparisons between TUDCA and UDCA in PBC have been limited.

One study compared the effects of UDCA and TUDCA in 23 patients with PBC in a crossover clinical trial [305]. Both drugs were administered in a randomized manner at a daily dosage of 500 mg during two 6-month periods, with a 3-month washout period in between. Both UDCA and TUDCA consistently improved biochemical parameters in blood serum, including enzyme levels reflecting liver function and associated with cholestasis and cytolysis. However, no significant differences were found in the pharmacological effects of these two bile acids. Both preparations were well-tolerated, and none of the patients reported side effects. In the short term, TUDCA appeared to be safe and at least as effective as UDCA in the treatment of PBC [305].

Another group of researchers has shown interest in TUDCA, which, due to its high hydrophilicity, holds significant therapeutic potential for the treatment of chronic cholestatic liver disease. They conducted a study to explore the relationship between the pharmacological effect of TUDCA and its dosage in 24 patients with PBC who were randomly assigned to receive daily doses of 500 mg, 1000 mg, or 1500 mg of TUDCA for six months. The study found that the level of UDCA bile saturation ranged from 15 % to 48 % and was not related to the dose of the drug. Biochemical parameters in blood serum, including enzyme levels reflecting liver function and those associated with cholestasis and cytolysis, decreased significantly after the first month of treatment

with all three doses of TUDCA. At the interim control point, there were no significant differences between the three doses, but further reductions in biochemical parameters occurred in patients receiving 1000 mg and 1500 mg of TUDCA per day. There was a significant decrease in the levels of total cholesterol and cholesterol in high-density lipoprotein in patients who received the two higher doses. Diarrhea was the only reported side effect. Based on the final analysis of all the data from the clinical study, the optimal TUDCA dose for long-term use in patients with PBC was determined to be 10 mg/kg/day [306].

These studies have demonstrated that with bile acids not only enhances their hydrophilicity and solubility but also has a substantial impact on the solubility of cholesterol, leading to increased excretion. The introduction of taurine itself has also been shown to lower serum cholesterol levels in individuals. In a clinical blind placebo-controlled study involving 22 healthy male volunteers aged 18-29, participants were randomly assigned to two groups receiving a high-fat/high-cholesterol diet for three weeks to raise serum cholesterol levels. The experimental group received 6 grams of taurine daily. At the end of the trial period, the control group had significantly higher levels of total cholesterol and low-density lipoprotein cholesterol than the taurine group [307]. Taurine's ability to improve lipid profiles has also been studied by other researchers [308, 309].

Several clinical trials have explored the choleretic and hypolipidemic properties of artichoke extract and its effects in patients with gastrointestinal symptoms, including dyspepsia, functional bile duct disorders, constipation, and stomach irritation. One randomized, double-blind, placebo-controlled, cross-sectional study involved 20 volunteers and evaluated the choleretic effect of a single administration of artichoke extract at a dose of 1.92 grams. Bile secretion was monitored using multichannel probes starting 30 minutes after taking the drug and continuing for 4 hours. Both groups, with artichoke extract and placebo, showed increases in bile secretion. The group receiving artichoke extract experienced a maximum increase of 152 % in bile secretion 60 minutes after taking the drug, while the placebo group had a 40 % increase reached in 30 minutes. Statistically significant

differences between the artichoke extract and placebo groups were observed after 30, 60, and 90 minutes after taking the drug ($p < 0.01$), as well as 120 and 150 minutes after taking the drug ($p < 0.05$) [310]. The results of other clinical studies, both placebo-controlled and uncontrolled, on the choleretic effects of artichoke extract have been summarized in a review [215].

The effects of artichoke extract were also investigated in several studies involving patients with non-specific gastrointestinal complaints, including dyspepsia, functional bile duct disorders, constipation, and stomach irritation. Patients were given up to six capsules of artichoke extract daily for either six weeks (in the first study) or six months (in the second study). Each capsule contained 320 mg of standardized artichoke water extract. Both studies reported improvements in clinical symptoms and reductions in total cholesterol and triglyceride levels in patients' blood serum compared to baseline values. An analysis of a subgroup of 279 patients with at least three out of five symptoms of irritable bowel syndrome indicated a significant reduction in the severity of symptoms after taking artichoke extract [311].

The effectiveness of artichoke extract in patients with hyperlipoproteinemia was assessed in a randomized, double-blind, placebo-controlled, multicenter study involving 143 patients with initial total cholesterol concentrations greater than 7.3 mmol/L. Participants were given artichoke extract at a daily dose of 1800 mg, divided into two doses, or a placebo for six weeks. At the end of the study, the group receiving artichoke extract experienced an average reduction in total cholesterol concentration of 18.5 %, while the placebo group had an 8 % reduction ($p < 0.0001$) [312].

Another randomized, double-blind, placebo-controlled study of artichoke extract also showed a significant reduction in low-density lipoprotein cholesterol compared to placebo ($p = 0.0001$) [313]. The average baseline total cholesterol levels in participants in this study were low. Subgroup analysis revealed hypolipidemic effects of artichoke extract. However, the number of participants in this trial was small and insufficient.

Additionally, a series of three open, uncontrolled studies investigated the administration of concentrated artichoke juice (derived from fresh leaves and flower buds) at a dose of 10 ml three times a day for 12 weeks in 84 patients with secondary hyperlipidemia [314]. Six weeks after treatment, total cholesterol, low-density lipoprotein cholesterol, and triglyceride concentrations decreased, while high-density lipoprotein cholesterol tended to increase.

A number of clinical trials have explored the use of preparations based on Angelica sinensis extract in the treatment of gynecological and even oncological diseases, alongside their application in gastroenterology. In one retrospective observation, a group of 200 gynecological outpatients with dysmenorrhea and irregular menstruations, aged 16-46, were treated with the product 'Concentrated Danggui Wan' and another combination of *Angelica* and *Astragalus*.

The patients in this study had conditions that persisted from 6 months to 12 years, with an average duration of 5 years. Inclusion criteria were based on three groups of symptoms:

1. Dysmenorrhea: This included premenstrual and menstrual abdominal pain that significantly affected work and daily activities, and had shown an inadequate response to antispasmodic treatment.
2. Irregular menstrual cycle: Defined as a menstrual cycle shorter than 20 days or longer than 35 days, or menstruation lasting for more than 7 days in 2 consecutive months.
3. Reduced menstrual flow: This category included menstrual periods lasting less than 2 days or showing a progressive decline in menstrual flow.

The treatment group (148 cases) received daily doses of Concentrated Danggui Wan. Each dose unit “pill” contained 0.25 g of the drug, and patients took 10-20 pills twice a day with lukewarm water. Each treatment lasted for 4 weeks, and each patient received 2–3 treatments. During the treatment, the use of other medications for dysmenorrhea and irregular menstruation was prohibited.

The control group (52 cases) took large honey-based Angelica pills daily, which contained Angelica and Astragalus. They took 9 g of these pills twice a day, and

each treatment lasted for 4 weeks. Each patient normally received 2–3 treatments. Other medications for these conditions were also prohibited during the treatment.

The effects of the treatment and any side effects were measured and assessed based on a 3-step scale:

Significantly effective: Abdominal pain significantly reduced, no longer affecting daily activities and work; menstrual cycle largely normalized (less than 5 days early or late); menstrual flow increased by at least one third compared to before treatment; menstruation lasted for 5-7 days, and other symptoms were alleviated or disappeared.

Effective: Abdominal pain reduced, and with the help of painkillers, the patient could remain at work. Menstrual problem symptoms improved but to a lesser extent than in the "significantly effective" group.

Ineffective: No improvements in abdominal pain and other problems; no changes in menstrual cycle or flow.

The results of the treatment were as follows:

In the treatment group:

- Significantly effective in 59 / 148 patients (39 %)
- Effective in 81 / 148 patients (54 %)
- Ineffective in 7 / 148 patients (4 %)

In the control group:

- Significantly effective in 27/52 patients (52 %)
- Effective in 18 / 52 patients (34 %)
- Ineffective in 7 / 52 patients (13 %)

The author of the study stated that "there have been no clear side-effects in using Concentrated Danggui Wan for treating dysmenorrhea and irregular menstruation". In a few cases, patients developed mild nausea, but this symptom quickly disappeared when the treatment was halted. Unfortunately, more detailed data on side effects were not provided [254].

Another study, outlined in [315], aimed to investigate platelet dysfunction in patients with ulcerative colitis (UC) and assess the role of *Angelica sinensis* injection

(ASI) in this context. The study involved 39 patients with active UC, 25 with remissive UC, and 30 healthy individuals. Various parameters, including α-granule membrane protein (GMP-140) and thromboxane B2 (TXB2) measured using enzyme-linked immunosorbent assay (ELISA), 6-keto-PGF1a measured via radioimmunoassay, platelet count (PC), 1-minute platelet aggregation rate (1 min PAR) assessed with blood automatic testing, and von Willebrand factor related antigen (vWF:Ag) determined through monoclonal-ELISA, were analyzed.

The 64 UC patients were divided into two treatment groups. After receiving routine treatment and ASI in conjunction with routine treatment for three weeks, these parameters were reassessed. In patients with active UC, PC, 1 min PAR, and levels of GMP-140, TXB2, and vWF:Ag were significantly higher compared to those with remissive UC and healthy controls ($P < 0.05$-0.01). Moreover, in patients with remissive UC, 1 min PAR and levels of GMP-140, TXB2, and vWF:Ag remained significantly higher than those in healthy controls ($P < 0.05$). Conversely, the level of 6-keto-PGF1a was significantly lower in both active and remissive UC compared to normal controls ($P < 0.05$-0.01).

Following treatment, these parameters, except for 6-keto-PGF1a, showed significant improvements in the ASI therapy group ($P < 0.05$-0.01), while there were minimal changes in the routine therapy group ($P > 0.05$). The authors concluded that platelets could become significantly activated in UC, potentially due to vascular endothelial injury and an imbalance between TXB2 and 6-keto-PGF1a in the bloodstream. ASI was found to effectively inhibit platelet activation, alleviate vascular endothelial cell injury, and enhance microcirculation in UC.

Furthermore, there have been reports of the use of *Angelica sinensis* preparations for managing hemorrhoids, as discussed in [316]. Limited and modest evidence suggests that certain herbal formulations containing components such as *Radix Sanguisorbae*, *Radix Rehmanniae*, *Fructus Sophorae*, *Radix Angelicae Sinensis*, *Radix Scutellariae*, among others, may provide short-term relief for symptoms associated with hemorrhoids. These symptoms may include hematochezia (bloody stools), congestive hemorrhoidal cushions, and inflammation of the perianal mucosa.

Own clinical studies of the preparation

In recent years, there has been accumulating experience in the use of Choloplant-Tau capsules (containing artichoke leaf extract 200 mg, ursodeoxycholic acid 100 mg, taurine 100 mg, and *Angelica sinensis* extract 50 mg) for the treatment of biliary disorders [149, 150]. This particular preparation has been studied in combination therapy for patients with dysfunction of the sphincter Oddi [150].

The use of this complex of drug substances has proven effective in several aspects. It helps restore the normal drainage function of the biliary tract, enhances bile drainage, reduces the lithogenic (gallstone-forming) properties of bile, thus preventing the formation of gallstones. Additionally, it normalizes the motor function of the biliary tract, restores the tone of the sphincter Oddi, and increases the body's antioxidant protection.

A clinical examination was conducted on 23 patients who had experienced sphincter Oddi dysfunction following cholecystectomy. The duration of the condition in these patients ranged up to 2 years, and their ages ranged from 19 to 74 years (with a mean age of 54.05 ± 3.22 years). The diagnosis was confirmed through clinical, laboratory, and instrumental methods. Prior to treatment, 21 patients (91.3 %) reported experiencing moderate pain and a feeling of heaviness in the right upper abdomen, 14 (60.9 %) reported bitterness in the mouth, 18 (78.2 %) reported nausea, 16 (69.5 %) reported flatulence, and 12 (52.1 %) reported digestive disturbances.

Biochemical tests showed elevated cholestatic parameters before treatment, with bilirubin at 34.6 ± 2.7 μmol/l and cholesterol at 8.3 ± 0.6 mmol/l. Ultrasonography revealed an enlargement of the common bile duct in 22 (95.6 %) of the patients. Patients were administered the drug at a dosage of 2 capsules three times a day, and the effectiveness of the treatment was assessed on the 21st day.

After the treatment period, all patients who had reported pain and discomfort in the right upper abdomen experienced relief (100 %). Symptoms such as bitterness in the mouth and nausea improved in 16 (69.5 %) and 20 (86.9 %) patients, respectively. Flatulence was alleviated in 14 (60.8 %) patients, and 11 (47.8 %) patients reported normalization of their bowel movements. Biochemical parameters

demonstrated normalization in all patients, with a 2-fold reduction in total bilirubin levels to an average of 17.3 ± 2.7 μmol/l and a decrease in cholesterol levels to 5.8 ± 0.3 mmol/l ($p < 0.05$ compared to pre-treatment levels). Ultrasonography showed that choledoch dimensions had returned to normal in 19 (82.6 %) patients.

These findings suggest that the use of this drug is beneficial in the treatment of sphincter Oddi dysfunction following cholecystectomy. It demonstrates a significant therapeutic effect in this condition, reduces the lithogenic properties of bile, and helps prevent the development of gallstone formation.

Pharmaceutical quality profile for promised preparation

The quality specification for a new drug was established by taking into consideration various factors. These factors included the findings from prior studies conducted by the manufacturer and the experiences documented by other researchers regarding the analytical standardization of drugs based on UDCA and phytopharmaceutical products [317-322]. Additionally, the specification was developed in compliance with regulatory requirements, specifically those outlined in the European Pharmacopoeia (Ph. Eur.), 9th Edition [323].

To ensure the accuracy and comprehensiveness of the specification, established guidelines were followed. The "Note for Guidance Specifications: Test Procedures and Acceptance Criteria for New Drug Substances and New Drug Products: Chemical Substances" (CPRM/ICH/367/96) served as a valuable reference during the specification development process. Moreover, the manufacturer's own specifications for UDCA, taurine, and artichoke extract were taken into account.

This rigorous approach to specification development aligns with best practices in pharmaceutical manufacturing and regulatory compliance, ensuring that the new drug meets the necessary quality standards and requirements for its intended use.

Identification test. The specification includes additional tests for the identification of the individual components within the drug, in addition to determining the quantitative content of the main active substances. Here are the methods used for identification:

1. Artichoke Extract Identification: To identify the artichoke extract, the presence of hydroxycholic acids, which are the primary active components of dry artichoke leaf extract (*Cynara scolymus* L.), is determined. This identification is achieved through adsorption spectrophotometry in the ultraviolet region, following the guidelines of Ph. Eur., 2.2.25 [323], as specified in the assay test. The ultraviolet absorption spectrum of the test solution within the wavelength range of 250 nm to 400 nm should exhibit maximum absorption at approximately (327 ± 2) nm and a shoulder in the region of (300 ± 2) nm. This spectral pattern is characteristic of chlorogenic acid, which is the predominant hydroxycholic acid in dry artichoke leaf extract.

2. Ursodeoxycholic Acid Identification: For identifying ursodeoxycholic acid, liquid chromatography is employed, following the protocols outlined in Ph. Eur., 2.2.29 [323], concurrently with the assay test. The retention time of the primary peak corresponding to ursodeoxycholic acid in the chromatogram of the test solution should match the retention time of the ursodeoxycholic acid peak in the chromatogram of the comparison solution.

3. Taurine Identification: Identification of taurine is carried out using thin-layer chromatography in accordance with Ph. Eur., 2.2.27 [323]. This is performed simultaneously with the "Impurities of taurine" test. On the chromatogram of the test solution, the spot representing taurine should align with the location, shape, and color of the taurine spot in the comparison solution.

4. Polyphenolic Compounds Identification in *Angelica Sinensis* Extract: Identification of polyphenolic compounds within the Angelica sinensis extract involves a qualitative reaction with phosphoric-molybdenum-tungsten reagent P, which results in the appearance of a blue color.

These identification tests are essential to verify the presence and characteristics of key components within the drug, ensuring that it meets the specified quality and composition standards.

The ‘Uniformity of Mass” test ensures that the mass or weight of individual capsules in the drug product is consistent and falls within acceptable limits. In this case,

the specification states that no more than 2 individual capsule masses can deviate from the average mass by more than 7.5 %, and none of them should deviate by more than 15.0 %. These limits have been established based on experimental data and aim to guarantee that each capsule contains a consistent amount of the drug substance.

The "Related Impurities" test is designed to assess the presence of impurities in the drug product. The requirements for allowable impurity levels are determined in accordance with the "Note for Guidance on Impurities in New Drug Products" (CPMP/ICH/2738/99). This guidance likely provides thresholds and criteria for various impurities, ensuring that the drug product meets safety and quality standards by limiting the presence of potentially harmful or undesirable substances.

Both of these tests are crucial quality control measures to confirm that the drug product is manufactured consistently and meets established safety and efficacy standards. The specific limits and criteria are set based on scientific data and regulatory guidelines to ensure the product's safety and effectiveness for patients.

Related impurities of ursodeoxycholic acid include impurity A (xenodeoxycholic acid) and impurity C (lithocholic acid), which are specific to ursodeoxycholic acid. The tests for these impurities are conducted using two different methods:

For impurity A and unidentified impurities, as well as the total amount of impurities, the HPLC method is employed. This is carried out in accordance with the requirements specified in the European Pharmacopoeia (Ph. Eur.), 2.2.29 [323].

Specifically, when analyzing the chromatogram of the test solution, the peak area of impurity A (xenodeoxycholic acid) should not surpass 10 times the area of the main peak observed in the chromatogram of the comparison solution (equivalent to 1.0 %). Similarly, the area of any other impurity present should not exceed the area of the main peak in the chromatogram of the comparison solution (equivalent to 0.1 %). The cumulative sum of the areas of all impurity peaks should not exceed 15 times the area of the main peak observed in the chromatogram of the comparison solution (equivalent to 1.5 %).

Impurity C (lithocholic acid) can also be detected through the HPLC method, although its retention time on the chromatogram exceeds 90 minutes when determining other contaminant impurities of ursodeoxycholic acid. Therefore, the proposed approach for determining impurity C involves TLC in accordance with Ph. Eur., 2.2.27 [323]. This method uses a silica gel plate and a mobile phase consisting of ice acetic acid, acetone, and methylene chloride (in a ratio of 1 : 30 : 60). After treatment with a solution of phosphoric molybdic acid in a mixture of sulfuric acid and acetic acid, the stained areas develop a blue color. Importantly, the intensity and size of the stain produced by impurity C should not exceed that of the ursodeoxycholic acid spot on the comparison solution (less than 0.1%).

When assessing the related impurities of taurine, the TLC method specified in Ph. Eur., 2.2.27 is utilized. This is performed on Silicagel 60 F 254 plates using a mobile phase composed of butanol, ice acetic acid, and water (in a ratio of 60 : 20 : 20). After treatment with a ninhydrin solution and subsequent heating, solutions containing amino acids, polypeptides, peptones, and primary amines develop either a blue or violet color. In this test, it's essential that no stain from the impurity exceeds the size and intensity of the taurine spot on the comparison solution (less than 0.5 %).

These stringent testing methods are crucial to ensure the purity and quality of the pharmaceutical product. They help identify and quantify specific impurities in ursodeoxycholic acid and taurine, maintaining the product's safety and effectiveness.

The **dissolution test** for these capsules is conducted in six units following the guidelines outlined in Ph. Eur., 2.9.3, and employs a blade device. The quantity of ursodeoxycholic acid that has dissolved into the solution is determined using HPLC as per the requirements specified in Ph. Eur., 2.2.29 [323].

It is suggested to employ a single test point for assessing the release of active substances. This approach is applicable since it pertains to normal-release dosage forms containing rapidly soluble active ingredients. The pH levels of the dissolution medium, such as synthetic gastric juice (pH 8.0) and synthetic intestinal juice (pH 6.8) recommended in the United States Pharmacopeia monograph for ursodeoxycholic acid tablets, are not suitable for accurately determining ursodeoxycholic acid when it is

present alongside other active and concomitant substances. This limitation is due to the influence of the physicochemical properties of the substance and the auxiliary components. In these conditions, the chromatogram of the test solution does not exhibit a clear peak for ursodeoxycholic acid. However, taurine can be reliably determined under these circumstances.

To optimize the dissolution test methodology and simultaneously determine the presence of ursodeoxycholic acid and taurine in the capsules, it is proposed to use water P as the dissolving medium. In this setup, both ursodeoxycholic acid and taurine that have dissolved into the solution can be reliably detected, and this is supported by validation and experimental data obtained during pharmaceutical development. The dissolution tests are conducted in water for a duration of 45 minutes. The capsules are deemed compliant with the test if the amount of ursodeoxycholic acid and taurine dissolved into the solution within 45 minutes is at least 85 % (with a 5 % margin) of the nominal content. This approach is based on both experimental data and Ph. Eur., 2.9.3, for solid dosage forms with traditional release [323].

Disintegration test. The disintegration test for these capsules is conducted using the disc method in water, and the capsules are expected to fully disintegrate within 30 minutes. This specification is established based on experimental data, following the guidelines outlined in Ph. Eur., 2.9.1, test A [323].

Test "Uniformity of dose units". The determination of taurine involves the calculation and weighing method, as its content exceeds 25 mg and represents more than 25 % of the dosage form's weight. On the other hand, the direct method is used for determining the content of artichoke extract and ursodeoxycholic acid since their content is less than 25 % of the total weight of the medicinal product.

To ensure quality control, the acceptance value (AV) is calculated using the reference number M for case 1. Specifically, the AV should be less than or equal to L1 = 15 for the first 10 units, or the final AV calculated from 30 units should be less than or equal to L1 = 15. Additionally, no individual contents in the dosage unit should be less than 0.75 M and not more than 1.25 M. This criterion must align with the requirements specified in Ph. Eur., 2.9.40 [323]. It's important to note that this parameter

is monitored during the release of the product batch and is not considered a critical factor in stability studies.

Microbiological purity test. The eligibility criteria for the microbiological purity of the final non-sterile oral medicinal products are included in the specification in accordance with the general article Ph. Eur., 5.1.4. These criteria are tested according to the Ph. Eur., 2.6.12, and 2.6.13 requirements [323]. The established conditions are as follows: the total number of aerobic microorganisms (TAMS) should not exceed 10^3 CFU/g, the total number of yeast and mold (TYMS) should be no more than 10^2 CFU/g, and there should be an absence of *Escherichia coli* in 1 g of the product. These specifications ensure the microbiological quality of the oral medicinal product.

Assay test. The proposed specifications for the quantitative content of active substances involve a ± 5 % limit deviation from the declared quantity during release and a ± 10 % limit during the storage period.

The content of ursodeoxycholic acid is determined through HPLC according to Ph. Eur., 2.2.29 [323], using refractometric detection on a 5 μm octadecyl silicagel column, 4.6 × 150 mm, based on the average weight of the dosage form. The content of ursodeoxycholic acid should be within the range of 95.0 % to 105.0 % of the specified quantity at release and between 90.0 % and 110.0 % throughout the shelf life. This specification is based on both experimental data and Ph. Eur. requirements.

The quantity of artichoke extract is determined by measuring the hydroxycholic acid content, which is assessed based on the average weight of the dosage form using absorption spectrophotometry in the UV region at 327 ± 2 nm. This measurement relies on the optical absorption index of chlorogenic acid, and the hydroxycinnamic acids content should not be less than 5.0 mg in terms of chlorogenic acid per average weight. This specification is derived from experimental data as well (Ph. Eur., 2.2.25).

Taurine's quantitative content is determined through the acid-base titration method. Taurine, due to its amphoteric nature, cannot be directly titrated with an alkaline solution. The titration is possible when the amino group is blocked by formaldehyde. The formed compound can then be alkalimetrically titrated using phenolphthalein as an

indicator. The taurine content should fall within the range of 95.0 % to 105.0 % of the specified quantity at release and between 90.0 % and 110.0 % throughout the shelf life.

An extensive review of both personal research and existing literature data regarding a fixed combination of medicinal substances, including artichoke leaf extract (200 mg), ursodeoxycholic acid (100 mg), taurine (100 mg), and *Angelica sinensis* roots extract (50 mg), has been conducted. This review also includes a comprehensive scientific justification of the pharmaceutical quality profile for the corresponding finished solid dosage form.

This medication is established as a fixed combination of medicinal substances with a well-established track record in medical applications for the treatment of dyspeptic disorders associated with functional abnormalities of the biliary system, hypokinetic-type biliary dyskinesia, and gastritis with bile reflux. Each component within this fixed combination plays a crucial role in supporting the human hepatobiliary system.

Ursodeoxycholic acid's effect is attributed to its ability to partially replace lipophilic toxic bile acids, enhance hepatocyte secretory function, and regulate immune responses. These properties are particularly beneficial in the context of liver and cholestatic diseases. Taurine acts as a synergistic agent with ursodeoxycholic acid by forming biliary conjugates within the liver. The artichoke extract contributes choleretic, hepatoprotective, and diuretic effects, while the *Angelica sinensis* roots extract exhibits moderate spasmolytic and anti-inflammatory properties.

The fixed combination has demonstrated a favorable safety profile and has undergone clinical investigations, both as individual components and as a unified medication, in *in vivo* clinical settings. The pharmaceutical profile of the fixed combination adheres to general requirements for solid dosage forms and incorporates specific parameters and research methodologies that have been experimentally validated.

CHAPTER 6.
CURRENT STATE OF THE ANTIVIRAL HERBAL PREPARATIONS DEVELOPMENT

Viral infections kill millions of people every year. The most aggressive viral diseases are Ebola and Marburg hemorrhagic fevers, AIDS, influenza, acute respiratory syndromes MERS and SARS, and many others. And since 2019, a disease caused by the newly identified coronavirus, COVID-19, has been added to this list.

Viruses are a relatively simple type of organism that is a collection of genetic information, but they form complex mechanisms of influence, since their ultimate goal is their own replication. Despite the relative simplicity of viral organisms, vaccines and effective drugs have not been found for many of them (for example, hepatitis C, HIV, etc.). Consequently, there is an urgent need to discover new effective and affordable antiviral drugs to control diseases caused by viruses when vaccines, if developed, and standard therapies are ineffective. Additional difficulties in the search for potential drugs of chemical (synthetic) origin are the property of viruses to mutations and the rapid formation of mechanisms of "addiction" to such drugs [324, 325].

Phytoraw materials provide a huge resource for the development of medicines of all directions, including antiviral drugs. In numerous research laboratories of Ukraine and the world, research is being conducted on the creation of new and more effective medicines of both synthetic and plant origin [324-326]. Therefore, the analysis of the current state of development of antiviral phytopreparations in Ukraine and the world is relevant.

Phytopreparations are of particular interest to researchers, as compounds of natural origin can be used as inhibitors of various viral infections at various stages of their development and manifestation. And also, what is especially valuable, such herbal preparations can be used for a long time, do not cause addiction, as they act "gently" and safely.

Traditional medicine has always been a huge source of various plants for the treatment of viral diseases even before the advent of specific therapy and the invention

of antibiotics. A wide range of therapeutic properties of plants is due to the presence of a wide variety of chemical compound structures (alkaloids, glycosides, saponins, vitamins, tannins, essential oils, etc.), which have different pharmacological effects not only on the human body, but also on pathogens. One of the most promising groups of compounds of plant origin are the secondary metabolites of plants belonging to the class of polyphenolic compounds, steroids, alkaloids, terpenoids, lignins and other biologically active substances (BAS) [325-326]. Essential oil plants also occupy one of the leading places in the treatment and prevention of viral diseases [327].

Since medicinal plants have an infinite variety of chemical constituents, they can be used to inhibit the replication of both DNA and RNA viruses.

The basis of antiviral therapy is the effect on the virus or its constituent components at one or another stage of reproduction. In general, according to the mechanism of action, antiviral agents can be divided into several subgroups: blocking the penetration and release of the virus genome from the capsule into the host cell, inhibiting the assembly process of viral particles and their exit from the cell cytoplasm, blocking the synthesis of viral RNA or DNA, and the mechanism of inhibiting the assembly of virions.

Table 6.1 provides summarized information on the current state of research into antiviral drugs based on plant origin BAS (for 2018-22).

Table 6.1 – General information on the current state of research on antiviral drugs based on plant origin BAS

BAS name	Origin of the BAS	Target virus	Mechanism of action	Reference
Terpenes				
Glycyrrhizic acid	*Glycyrrhiza glabra*	Hepatitis B virus, human immunodeficiency viruses	Effect on surface antigen HBsAg, inhibition of HIV proliferation	[328]
β-Escin	*Aesculus hippocastanum*	Herpes simplex virus, vesicular stomatitis virus, dengue virus	Effect on virus replication	[329]
Tulsinol, cymarin,	*Ocimum sanctum*	H9N2 influenza virus	Masking/blocking of viral	[330]

BAS name	Origin of the BAS	Target virus	Mechanism of action	Reference
derivatives of ursolic acid			hemagglutination protein	
Oleanolic acid	*Rosmarinus officinalis*	Human immunodeficiency viruses, influenza virus, hepatitis B and C, herpes viruses	Effect on virus replication	[331-333]
Linalool, ursolic acid	*Ocimum basilicum, Prunella vulgaris*	DNA viruses (herpesviruses (HSV), adenoviruses (ADV), and hepatitis B virus) and RNA viruses (coxsackieviruses B1 (CVB1) and enterovirus 71 (EV71)		[334-336]
Iridoids				
Genipin, geniposide, gardenoside	*Gardenia jasminoides*	Influenza virus A	RNA suppression of viral replication	[337]
Adoxysidic acid-6-oleuropein ether, forsythioside	*Forsythia suspensa, Forsythia viridissima*	Influenza virus A (H5N1), coxsackievirus (B3), rhinovirus 1B (1B)	RNA suppression of viral replication	[338-340]
Phenol derivatives and their glycosides (flavonoids, phenolcarboxylic acids, lignins, tannins, etc.)				
Arctiin, arctigenin	*Arctium lappa, Forsythia viridissima*	Herpes simplex virus, Coxsackie virus (B3), rhinovirus 1B (1B)	Inhibition of viral DNA synthesis	[341-342]
Apigenin	*Apium graveolens, Ocimum basilicum, Petroselinum crispum*	Rabies virus, DNA viruses (herpesviruses (HSV), adenoviruses (ADV) and hepatitis B virus) and RNA (coxsackieviruses B1 (CVB1) and enterovirus 71 (EV71)	Suppression of the synthesis of viral DNA, mRNA and viral proteins	[343-344]

BAS name	Origin of the BAS	Target virus	Mechanism of action	Reference
Luteolin	*Arctium lappa, Salvia tomentosa, Linaria vulgaris*	Influenza A virus, SARS-CoV	Interferes with events occurring between hours three and nine of the HSV-1 replication cycle, which involves transcription and translation of viral proteins	[345-347]
Quercetin	*Sophora japonica*	Influenza A virus, SARS-CoV-2, herpes simplex virus, enterovirus 71	Inhibits the general synthesis of the viral protein	[348-351]
Xanthohumol	*Humulus lupulus*	Influenza virus, human immunodeficiency viruses	Inhibition of replication of various viral strains; inhibition of HIV-1 induced cytopathic effects, production of viral antigen p24 and reverse transcriptase in C8166 lymphocytes	[352]
Narcisin, rutin, isorhamnetin, calendoflazid	*Calendula officinalis*	SARS-CoV-2	Inhibition of viral protease *in silico*	[353-354]
Hyperoside, isoquercitrin, spiroside, quercitrin	*Filipendula vulgaris, Hypericum perforatum*	Hepatitis C virus	Inhibition of replication of various viral strains	[355-356]
Asperuloside, asperulin	*Galium verum, Faramea hyacinthina*	Dengue virus	Effect on virus replication	[357]
Linarin, linariin, acacetin	*Linaria vulgaris, Acacia arabica*	Influenza virus, SARS-CoV	Cytopathic effect	[358-360]
Alkaloids				
Derivatives of matrine, soforidine, flavesin, alopecurin	*Sophora japonica*	Hepatitis B virus, enterovirus 71	Effect on virus replication	[361-363]

Triterpene saponins have structural features that provide their ability to change the spatial structure of the cell membrane by binding to membrane cholesterol, and thus suppress virus adsorption. Polyphenolic compounds are able not only to change the charge of the cell surface, but also to prevent the specific sorption of the virus on the receptors and have an inhibitory effect on the replication of viruses. These two groups of BAS are the most promising for the development of antiviral phytopreparations.

Analogues of luteolin and quercetin suppress the reproduction of viruses at the stage of release of their internal component.

Medicines such as mangifering, gossypol, epigen are inhibitors of RNA polymerase and inhibitors of post-translational modifications of proteins, suppress the expression of the viral genome and assembly of vibrio [364].

In order to block the most difficult phase of virus reproduction – the release of viral offspring – molecules-inhibitors of the neuraminidase enzymatic activity of the virus can be used. Phenolcarboxylic acids (for example, caffeic, cinnamic, ferulic, rosmarinic and other acids, substances from the group of gallic acids) can imitate the structure of natural substrates of the catalytic site of neuraminidase and actually "distract" the virus [365].

Mixtures of different flavonoids or a combination of flavonoids with antiviral synthetic drugs provide an increase in their antiviral effect [351].

Recent drug delivery research strategies significantly contribute to overcoming the low bioavailability of flavonoids [366].

The antiviral mechanism of these phytopreparations can be of different orientations: antioxidant activity, inhibition of DNA, RNA synthesis, inhibition of virus penetration or inhibition of virus reproduction, etc. However, there are unexplained mechanisms of natural antiviral drugs. For a large number of molecules of plant origin that exhibit antiviral properties, such mechanisms of action should be investigated [367].

In this context, testing the antiviral activity of a “cocktail” of flavonoids would be beneficial for preventing viral infections and improving existing antiviral treatments [368].

The challenge of the global outbreak of COVID-19 caused a surge of interest in the role of flavonoids, as one of the most physiologically active and promising BAS in the treatment of coronavirus infection.

Over the past year, researchers have been actively reviewing the ability of well-known (eg, quercetin, baicalin, luteolin, hesperetin, gallocatechin gallate, epigallocatechin gallate) and less common (eg, scutellarein, amentoflavone, papyriflavonol A, etc.) flavonoids, secondary metabolites widely present in plant tissues with antioxidant and antimicrobial properties to inhibit key proteins involved in the damaging cycle of the coronavirus, such as proteases PL^{pro}, $3CL^{pro}$, NTPases / helicases.

The vast majority of modern publications are devoted to *in silico* and *in vitro* studies of flavonoid and other natural structures using the capabilities of molecular docking [346, 369].

In addition to screening natural compounds, several Ukrainian groups have successfully designed and synthesized new analogues with promising antiviral activity. The main purpose of structural modification is to reduce toxicity and increase bioavailability compared to the original molecule. Thus, Ukrainian scientists conduct a number of studies on the study of changes in the natural qualities of plants using genetic engineering methods, which allow for controlled influence on the genome of plants, in particular, genetic transformation with the help of the phytopathogenic *Agrobacterium rhizogenes*.

The analysis of the current state of development of antiviral drugs based on phytoraw materials in Ukraine and the world shows the perspective and feasibility of studying the antiviral activity of flavonoids and flavonoid-containing raw materials with the aim of selecting the most promising substances of this class and the possibility of synthesizing substances based on them that could find application in clinical practice for treatment of coronavirus and other widespread viral infections. Taking into account

the phytochemical composition and biological activity of the relevant biologically active substances, it can be considered promising for studying the antiviral activity of a complex phytopreparation consisting of the following medicinal plant raw materials: *Apium graveolens, Arctium lappa, Calendula officinalis, Filipendula vulgaris, Galium verum, Humulus lupulus, Linaria vulgaris, Petroselinum crispum, Sophora japonica.*

Acknowledging Funding

This research was supported by the National Research Fund of Ukraine grant No. 2020.01/0226 “Study of the effectiveness and safety of the use of new phytochemical and bacterial drugs for the treatment of coronavirus and other common infections”.

CHAPTER 7.
BIOLOGICAL ASSESSMENT OF PLANT EXTRACTS BASED MEDICAL DEVICES IN THE FORM OF RECTAL USE SUPPOSITORIES

Today, it's inconceivable to envision the establishment of a contemporary, forward-thinking healthcare system without the advancement and integration of cutting-edge medical technologies across all medical domains, encompassing both preventative and clinical aspects. The widespread utilization of medical devices (MD) plays a pivotal role in efficiently addressing challenges in medical diagnosis, prevention, and treatment. High-tech and innovative MDs have become an indispensable mechanism for ensuring the repeatability, scalability, and predictability of clinical and diagnostic outcomes [370].

The distinctive characteristics of pharmaceuticals within the healthcare system primarily stem from their extensive variability in design, origins, manufacturing methods, and usage [371, 372]. These circumstances significantly impede the formulation of comprehensive (universal) standards for MD standardization, particularly with regard to their quality and safety. In contrast to pharmaceutical products, which have detailed directives concerning quality and safety (such as Pharmacopoeias, the Guidelines of the International Council on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use, and national regulatory authorities), equivalent comprehensive international and national regulatory documents are lacking for medical devices. This characteristic of MDs is also reflected in the access systems for MDs in different national markets, which frequently involve compliance assessment procedures with the participation of a wide array of authorized entities [373, 374].

The regulatory bodies of developed nations, industry associations, and international organizations play significant roles in the global standardization of medical devices. The International Organization for Standardization has developed Guidelines for Biological Assessment of Medical Devices, which are currently adopted by the majority of countries, including Ukraine. The ISO 10993 series of standards (Biological evaluation of medical devices) has enabled the standardization of requirements for

various types of MDs based on their method of administration, interaction with the body, and duration of contact. The assessment concept outlined in ISO 10993-1:2018 serves as the foundation for the development of assessment programs for medical devices, although it is not binding due to the extensive range of peculiarities associated with MDs.

The diversity of MDs is compounded by the fact that many of them have manufacturing processes that closely resemble those of pharmaceutical products (e.g., eye drops, vaginal and rectal suppositories, skin solutions, patches, etc.). In such cases, the primary challenge lies in accurately categorizing the product into a specific class of medical products [375]. The outcomes of class-specific risk assessment and the biological evaluation of MDs are crucial pieces of information for the assessment and risk management of MDs, which are integral components of the overall quality assurance system for any medical device manufacturer [376-378].

The objective of this study was to present the results of the development of a classification algorithm for medical devices in the form of rectal suppositories and their biological evaluation in accordance with the ISO 10993 series of standards.

Methodical aspects:

Medical device. We employed the following samples for our testing procedure, specifically focusing on the medical device referred to as “Pravenor”, which are rectal suppositories.

To prepare the suppositories for testing, we utilized centrifuge tubes and a mixture of ether (3 ml) and physiological solution (3 ml) in a 1:1 volume-to-volume ratio. The suppository was carefully placed in a sealed tube and left undisturbed for one hour to ensure complete dissolution. In the subsequent step, the lower phase of the solution was carefully transferred into another tube, which was left uncovered for 30 minutes to allow for the evaporation of the ether. The resulting extract, with a pH ranging from 6.8 to 7.2, served as the basis for the biological evaluation of the medical device.

The calculated concentration of active components within the obtained extract was as follows: 50 mg/ml of dwarf palm berries extract (*Saw palmetto*), 16.67 mg/ml of lovage root extract (*Levisticum officinale*), and 16.67 mg/ml of calendula flowers extract (*Calendula officinalis*).

Cell culture and its culturing. Vero cell culture, which is derived from green monkey kidney cells and originally obtained from the cell bank of the D.I. Ivanovsky Institute of Virology at RAMS (Moscow, Russian Federation), was used in this study. The cell culture was maintained at the research facility known as the "L.V. Gromashevsky Institute of Epidemiology and Infectious Diseases of the NAMS of Ukraine" located in Kyiv, Ukraine.

To sustain the cells in culture, a standard method was employed. A complex medium was utilized, comprising 90 % RPMI-1640 medium (Sigma, USA) supplemented with 10 % inactivated fetal bovine serum (FBS) from Sigma, USA, and the antibiotic Kanamycin (50 IU/ml).

The cell cultures were grown in either 50- or 100-ml glass or plastic containers from Nunc in Denmark. These cultures were maintained at a temperature of 37 °C in an atmosphere containing 5 % CO_2. Every 3-4 days, the live cells were quantified by staining them with trypan blue and then seeded at the initial cell concentration per 1 ml.

For passaging, the cells were extracted from the surface of the culture containers using Gibco® Versene Solution, which consists of 0.2 g of EDTA per liter of phosphate-buffered saline and is produced by Thermo Fisher Scientific in the USA. After extraction, the cells were centrifuged and resuspended in 1 ml of medium for precipitation. They were then pipetted and counted using Goryaev's hemocytometer. Subsequently, the cells were seeded into well plates designed for cell culture from Sigma in the USA, with an estimated density of 200,000 cells per 1 ml of medium.

Determination of the medical devices' cytotoxic concentration (CC_{50}). To determine the CC_{50} (cytotoxic concentration at which 50 % of cells are affected) of the medical devices, we conducted experiments using at least ten rows of wells in culture plates for each dilution of the product. These culture plates containing cell cultures were then incubated at 37°C in an environment with 5 % CO_2 for a period of 5 days.

On a daily basis, we closely observed both the test and control cultures to detect the presence or absence of cytopathogenic effects (CPE). The degree of CPE was assessed by evaluating changes in cell morphology, which included rounding, wrinkling

of cells, and detachment from the surface of the culture well. We utilized the following rating system to quantify degenerative changes:

- "-" indicated the complete absence of cell degeneration.
- "+" indicated that no more than 25 % of the cell monolayer was affected.
- "++" indicated that no more than 50 % of the cell monolayer was affected.
- "+++" indicated that no more than 75 % of the cell monolayer was affected.
- "++++" indicated complete degeneration of the cell monolayer.

The CC_{50} of the medical device was determined as the highest concentration that did not cause degeneration of 50 % of the cells in the culture. The control group consisted of a cell monolayer without the addition of extracts from the medical devices.

MTT assay. This method relies on the activity of the dehydrogenase system within intact cell mitochondria, which converts 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) into formazan. The resulting reaction product can be quantitatively measured using a spectrophotometer. The conversion of MTT into formazan decreases when cells die, especially when exposed to toxic substances.

To perform this assay, cells at a concentration of $5 \times 10^{\wedge 5}$ cells per milliliter were cultured in 96-well plates using RPMI-1640 medium supplemented with 10 % FBS. This medium contained test substances at various concentrations. Control samples consisted of cells that were not exposed to the test product. Each concentration was tested in triplicate. The plates containing the cells were then incubated at 37°C in an environment with 5 % CO_2 for 48 hours.

MTT substrate (provided by Sigma, USA) was dissolved in sterile phosphate-buffered saline solution (PBS) with a pH of 7.2. This solution was prepared at room temperature and had a concentration of 5 mg/ml. To each well, 25 µl of filtered MTT solution was added to 100 µl of the cell suspension, and the mixture was incubated for 3 hours at 37 °C in the presence of 5 % CO_2. After incubation, the plates were centrifuged at 1500 rpm for 10 minutes to precipitate the cells, and the supernatant was discarded. To dissolve the crystalline formazan, 100 µl of 96 % ethanol was added to the cell pellet. After thorough shaking for 10 minutes at 37 °C, the optical density of the solutions was measured using a spectrophotometer at a wavelength of 540 nm.

The percentage of inhibition of cell viability caused by exposure to the test products was determined by comparing the optical density of the test samples to that of the control cells, which were set at 100 %. To facilitate the assessment of cytotoxicity results obtained through the MTT assay, a corresponding plot was used to indicate the value corresponding to half of the control cell viability ($CC_{50\%}$).

Animals. In our study, we employed random-bred laboratory Guinea pigs that were between 3 to 4 months old and had a weight range of 300 to 400 grams. The care and handling of these animals adhered to the guidelines outlined in the international standard ISO 10993-2:2006, titled "Biological evaluation of medical devices – Part 2: Animal welfare requirements".

Sensitization and skin irritation effects study. In the study, hair was trimmed from 2x3 cm areas on the backs of the animals. Melted suppositories were then applied directly to the skin and secured in place with a gauze bandage. This application was left in position for a duration of 4 hours. Subsequently, the skin was monitored for signs of erythema (redness) and edema (swelling) at intervals of 12, 24, 48, and 72 hours following the removal of the bandage. Photographic documentation of the results was conducted. Each test group comprised 6 animals.

Bioethics norms. All procedures involving animals were conducted in full compliance with the Law of Ukraine "On Protection of Animals from Abuse", the European Convention for the Protection of Vertebrate Animals, and the guidelines outlined in the "Guide for the Care and Use of Laboratory Animals" (8th edition). At the conclusion of the study, the animals were humanely euthanized using methods designed to minimize both physical and psychological suffering.

Justification of biological testing program:

Functional characteristics of the medical device "Pravenor". To comprehend the functional characteristics of this product, it is essential to analyze its composition and evaluate the roles of its ingredients in the male urogenital system's operation. The berries of dwarf palm, known as *Saw palmetto*, contain various biologically active substances crucial for the proper functioning of the prostate, including phytosterols

(precursors of hormones naturally synthesized in the human body), fatty acids such as palmitic, linoleic, and linolenic acids, as well as lipase, which aids in the digestion of fats, fatty acids, and fat-soluble vitamins A, D, and E [379]. Lovage roots (*Levisticum officinale*) are rich in essential oils like terpineol, cineole, acetic, isovaleric, and benzoic acids, which support healthy urination [380]. Calendula flowers (*Calendula officinalis*) contain essential oils, carotenoids, and flavonoids that promote tissue regeneration processes [381].

Therefore, the phytocomponents present in this medical device constitute a complex of biologically active substances necessary for and conducive to the normal functioning of the male urogenital system, particularly the prostate. It's worth noting that these same phytocomponents are also included in various medicinal products and dietary supplements, primarily in oral forms. The majority of medicinal products containing extracts or tinctures of dwarf palm, lovage, and calendula fall into the category of traditional medicinal products, which have been developed and marketed in Ukraine for over two decades. Presently, new oral products with similar components are introduced to the Ukrainian market as dietary supplements, emphasizing their functional principle, which is the provision of nutrients to enhance overall bodily function. These products not only act preventively but also contribute to the amelioration of specific diseases.

Hence, the impact of the medical device "Pravenor" in the form of rectal suppositories on the human body cannot be classified as pharmacological, immunological, or metabolism-altering. As such, this product may be categorized as a medical device according to the Technical Regulations for Medical Devices, as approved by Cabinet of Ministers of Ukraine Decree No. 753, dated October 2, 2013, and Regulation (EU) 2017/745 of the European Parliament and the Council dated April 5, 2017, on medical devices.

The medical device "Pravenor" is categorized as a short-term device since its intended use involves a period of less than 30 days. Given that it is administered rectally and is partially absorbed by the body, Rule 21 from Regulation (EU) 2017/745 of the European Parliament and the Council, dated April 5, 2017, on medical devices may be applicable to this medical device. Rule 21 pertains to devices composed of substances

or combinations of substances that are designed for introduction into the human body via a body orifice or skin application, and which are either absorbed or locally dispersed within the human body. This rule applies to devices classified as class III and those that achieve their intended purpose in the stomach or lower gastrointestinal tract, or whose metabolic products are systemically absorbed by the human body.

Biological Testing Program. In designing the biological testing program for this medical device, several factors were considered. These factors include the recommendations outlined in the standard ISO 10993-1:2018, titled "Biological Evaluation of Medical Devices – Part 1: Evaluation and Testing within a Risk Management Process." The device falls into the category of surface devices with contact primarily with mucosal membranes, and the duration of contact ranges from 24 hours to 30 days.

Considering the well-established safety profile of the substances present in the device, the biological testing program focused on three key parameters:

1. Cytotoxicity: This assessment evaluates the potential of the device to cause harm or toxicity to cells, particularly those in contact with the mucosal membranes.
2. Sensitizing Effects: This component of the testing program assesses whether the device has the potential to induce sensitization, which is an allergic or hypersensitive response upon contact with the skin or mucosal membranes.
3. Skin-Irritating Effects: The testing program also includes an evaluation of whether the device may cause skin irritation when in contact with the mucosal membranes.

By assessing these parameters in accordance with ISO 10993-1:2018, the aim is to comprehensively evaluate the biological safety and potential risks associated with the medical device "Pravenor." This approach ensures that the device meets the necessary safety standards for its intended use.

Biological evaluation of medical device:

Determination of the device cytotoxic concentration (CC_{50}). In the determination of the CC_{50} (cytotoxic concentration at which 50 % of cells are affected)

of the products, Vero cell cultures were employed. For each dilution of the device in the culture medium, a minimum of ten rows of wells in culture plates were utilized in the experiments. Both the test and control cultures were observed on a daily basis to assess the presence or absence of cytopathogenic effects (CPE). The findings of this study are presented in Table 7.1.

Table 7.1 – The study results of the device cytotoxic concentration in Vero cell culture

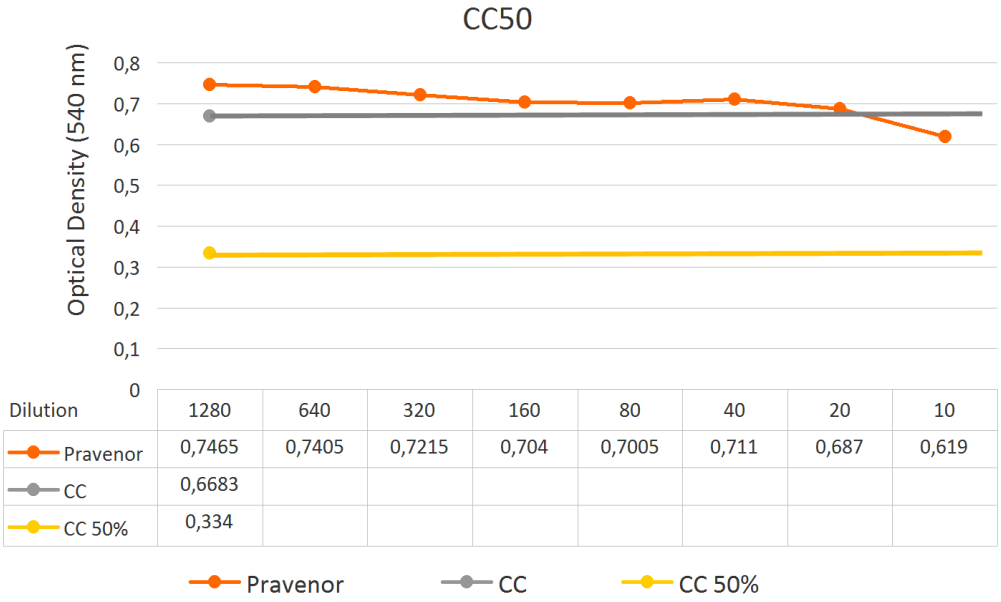
Dilution	Pravenor Medical Device
1:10	0/10
1:20	0/10
1:40	0/10
1:80	0/10
1:160	0/10
1:320	0/10
1:640	0/10
1:1280	0/10
CC_{50}	Non-toxic

Note: *numerator – number of wells with CPE, denominator – total well number in the experiment.

Pravenor was determined to be entirely non-toxic when tested in Vero cell culture.

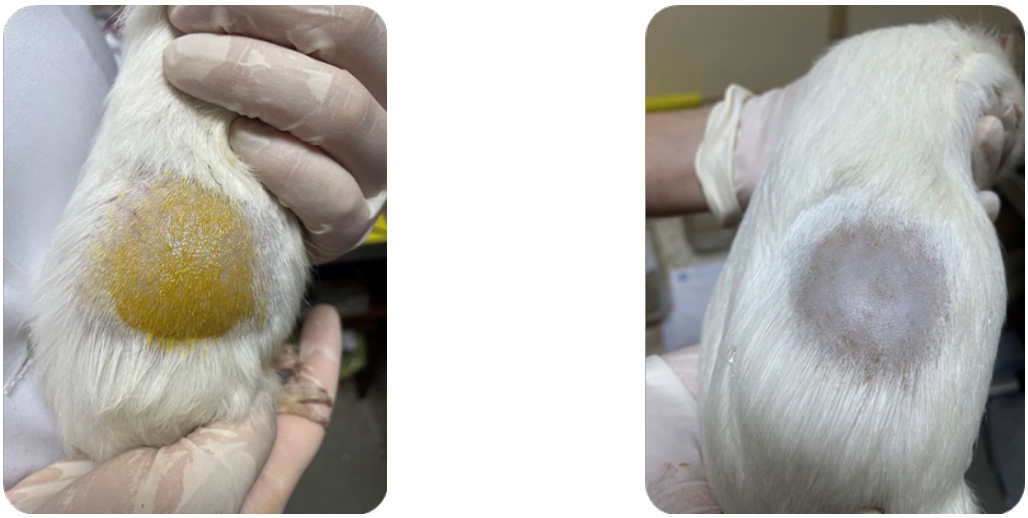
As per the MTT assay results, the medical device “Pravenor” showed no signs of toxicity, as evidenced by the optical density ratio of the test product compared to the control cell culture (Fig. 7.1). The obtained data indicate that the CC_{50} (concentration that causes a 50 % decrease in cell viability) for this product exceeds 50 mg/ml for dwarf palm berried extract (Saw palmetto), 16.67 mg/ml for lovage root extract (*Levisticum officinale*), and 16.67 mg/ml for calendula flowers extract (*Calendula officinalis*).

Studies of sensitization and skin irritation effects. The monitoring of experimental animals demonstrated that both medical devices did not induce any skin irritation and were deemed safe for use. These observations were meticulously documented in photographs, as illustrated in Figure 7.2.



Dilution	1280	640	320	160	80	40	20	10
Pravenor	0,7465	0,7405	0,7215	0,704	0,7005	0,711	0,687	0,619
CC	0,6683							
CC 50%	0,334							

Figure 7.1 – Results of cytotoxicity assessment by MTT assay



A – guinea pig with the skin zone, on which “Pravenor” was applied

B – guinea pig skin after “Pravenor” testing

Figure 7.2 – Results of determining of the devices’ sensitizing and skin-irritating effects

It's important to note that the regulatory status of a medical device carries significant implications for the strategic planning of a scientific research project. There exists a notable distinction between studies focused on the safety and efficacy of medications as compared to medical devices. Some healthcare products may assume similar or even identical forms (e.g., solutions for injection, skin solutions, nasal sprays, vaginal and rectal suppositories, etc.), yet they may fall into different regulatory

categories. Beyond the aforementioned aspects of preclinical and clinical investigations of medical products, their regulatory status can sometimes impact the pharmacoeconomic parameters of the respective projects [382].

In the context of facilitating access for developed medical products, especially medical devices, to various segments of the global pharmaceutical market, the issue of harmonizing regulatory requirements across different countries and supranational entities, such as the EU, is of paramount importance. This harmonization is crucial for the effective management of quality, safety, and efficacy standards for such products [383, 1384]. Manufacturers of medical devices and other medical products frequently engage in discussions concerning the interplay between scientific and technical aspects within this field, particularly with regard to regulatory requirements [385-388].

There is a wide range of products designed for vaginal and rectal use that are available in developed countries' markets, and many of these products are classified as medical devices. Typically, intra-vaginal medical devices in traditional pharmaceutical forms such as suppositories, tablets, and capsules are developed for purposes such as correcting or restoring vaginal microbiota [389-391]. Other indications for such devices include promoting fertilization [392] and delivering anti-HIV drugs [393], among others. Medical devices in the form of rectal suppositories, creams, and gels are used for the treatment and prevention of proctological and urological diseases [394, 395]. In light of this, our medical-scientific justification for classifying the studied products as medical devices aligns with the practices of regulatory bodies, specifically Conformity Assessment Bodies, in European Union countries.

It's worth noting that the current Ukrainian Technical Regulations on Medical Devices and Medical Device Regulations, which have been adopted in the European Union [388, 396], may not completely match in terms of establishing risk classes for certain medical devices, including invasive ones. However, such regulatory disparities between the Ukrainian and EU markets do not significantly impact the determination of the strategy for biological evaluation of these devices.

The phytocombination of medicinal herbal extracts present in the medical device “Pravenor” is unique. Therefore, the study of the cytotoxic effect of this product on cell

cultures was not only scientifically relevant but also important from a regulatory perspective. Existing literature data [397-402] on the cytotoxic effects of extracts from *Saw palmetto, Levisticum officinale,* and *Calendula officinalis*, as well as their biologically active substances, typically focus on individual studies (as single preparations) or are centered on assessing anti-cancer activities. Consequently, the data obtained regarding the absence of a cytotoxic effect of the medical device “Pravenor” constitute an important prerequisite for its safe use as a prostate protector.

A scientific-medical and regulatory justification has been conducted to classify Prodexyn and Pravenor as invasive medical devices for prolonged use. The studies conducted to assess cytotoxicity, sensitization, and skin irritation are deemed sufficient for the biological evaluation of such me'dical devices.

In the case of the medical device “Pravenor”, it was found to be completely non-toxic in the Vero cell culture study. Similar results were obtained in the MTT assay, which indicated that the CC_{50} (cytotoxic concentration at which 50 % of cells are affected) for this product exceeded 50 mg/ml for the extract of dwarf palm berries (*Saw palmetto*), 16.67 mg/ml for lovage root extract (*Levisticum officinale*), and 16.67 mg/ml for calendula flowers extract (*Calendula officinalis*). The MTT assay, which assesses the condition of the dehydrogenase system within mitochondrial cells, was found to be five times more sensitive compared to the approach of detecting cytopathic effects on Vero cell culture. Consequently, the MTT assay can be considered a more informative method for evaluating the cytotoxicity of medical devices.

Furthermore, the medical device did not demonstrate sensitizing or skin-irritating effects after application to the skin. These findings collectively provide substantial evidence of the safety and biocompatibility of the medical device “Pravenor” for its intended use.

Acknowledging Funding

This research was supported by the Univesral Agency “Pro-Pharma” LLC, Kiyv, Ukraine.

CHAPTER 8.
PHARMACOLOGICAL SUBSTANTIATION OF THE NEW DRUGS ELABORATION ON THE BASE OF RESVERATROL

The number of the older age group women significantly increased due to demographic changes that occurred in the second half of the XX century. Each year the rate of women that enter menopause increases because of a rise of life expectancy and the early occurrence of menopause.

Whereas menopause is not an illness, but an age-related changes manifestation, it may cause the endocrine balance disturbance in the body, and this phenomenon leads to the advancement of symptoms such as hot flashes, irritability, insomnia, genitourinal disorders, and also provokes the risk of metabolic, cardiovascular, neurological diseases, osteoporosis. At the onset of natural menopause, the level of estrogen decreases gradually, over several years, and the body of women adapts to life conditions in state of estrogen lack. The suggested data underlines the need of the implementation of medical and social measures in order to maintain health, enhance efficiency and proper quality of women's life in the peri-and postmenopausal periods, and the elaboration of effective and safe medicines for menopausal disorders personalized prevention and treatment remains a crucial and not fully solved task of modern pharmacology and pharmacy.

According to the widespread opinion the menopausal therapy, that uses monocomponent or combined drugs of sex hormones remains the “gold standard” of pharmacological correction of symptoms of pathological menopause, but unfortunately only 30-40 % of women in developed countries and even only 2-3 % of women in Ukraine receive menopausal hormonal therapy [403]. Such a trend is connected with contraindications, adverse reactions identification, “hormone phobia” or insufficient knowledge about the benefits of menopausal hormonal therapy for a significant proportion of those who need it. Thus, an urgent need for alternative drugs remains the same, in particular for medicinal agents of non-hormonal origin for the timely correction of menopausal disorders.

The curative properties of grapes are well established for a long time. There are the drugs from the dry leaves, seeds of the grapes. Now the drug resveratrol is obtained from the grapes' peel and other plants. Resveratrol is a phytoalexin of the plant polyphenol group origin. Resveratrol has a wide spectrum of pharmacological activity and is used in case of cardiovascular, nervous and metabolic diseases. From the first experiments the resveratrol antioxidant properties have been found [403]. Resveratrol has cardioprotective properties in the model of ischemia-reparation. It was demonstrated comparative protective effect as distant preconditioning. The mechanisms of antiischemic effect are related to resveratrol antioxidant properties. Resveratrol protects heart and vessels by antiaggregant, hypocholesterinergic actions. Resveratrol reduces cholesterol level, inhibits angiotensin II-induced proliferation of fibroblasts. Resveratrol has been shown to inhibit NF-KB transcription and offers cardioprotective benefits through ischemic preconditioning via nitric oxide synthase. Additionally, resveratrol exhibits a hypoglycemic effect by enhancing glucose transport in C_2C_{12} myotubes through the activation of AMP-activated protein kinase [404]. Resveratrol exerts a neuroprotective effect by blocking amyloid synthesis and through its antioxidant properties. During the screening research it was found that the daily studied gels with resveratrol and hyaluronic acid were effective in the terms of intravaginal 28-day administration to ovariectomized female rats, since they restrained the increase in body weight that occured because of estrogen deficiency, normalized body temperature at the root of the tail (preventing manifestations of "hot flashes"), contributed to the restoration of vaginal biotope and acidic environment of the vaginal secretion, prevented the advancement of atrophic changes in the vaginal mucosa. According to the results of the integral assessment of the effectiveness of the therapeutic effect of vaginal gels, it was found that the optimal resveratrol content in the medicinal form is 0.5 %, since according to the vast majority of indicators in the model of the experimental hypoestrogen state in animals, it received the highest number of points, that is, it turned out to be the most effective. The experimental study data show not only local, but also weak systemic action of the vaginal dosage form of the combined composition with resveratrol and hyaluronic acid, which necessitates

further in-depth study of the suggested composition. The results of this stage of the study became an experimental justification for the development and comprehensive study of the vaginal gel with phytoestrogen resveratrol and hyaluronic acid as an alternative to hormone-containing drugs of the group of drugs for the therapy of pathological hypoestrogen states of different genesis, primarily genitourinal manifestations of climacteric syndrome [405].

The next step served the safety profile research of the vaginal gel of a combined composition with resveratrol and hyaluronic acid, in particular, the study of local tolerability, acute toxicity with single intravaginal and intragastric administration and chronic toxicity of the gel with multiple (90 day) intravaginal administered to rats.

It was determined that the vaginal gel with resveratrol and hyaluronic acid does not provoke local irritation during single and prolonged vaginal use. The abovementioned gel is particularly a non-toxic substance.

The pharmacodynamics evaluation of the vaginal gel with resveratrol and hyaluronic acid was conducted on two models of hypoestrogen state, such as bilateral ovarioectomy and hypoestrogenemia by 4-vinylcyclohexene diepoxide.

The pharmacological action of the vaginal gel towards local (genitourinal) and systemic manifestations of hypoestrogenia was observed in a model of bilateral ovariectomy (surgical castration).

It was found that under the influence of treatment with gel with resveratol and hyaluronic acid, the pathological manifestations of hypoestrogenemia from the vaginal mucosa most clearly declined. According to the total indicator of macroscopic manifestations, the gel with phytoestrogen decreased the signs of hypoestrogen atrophy from 7 points, which was recognised in animals of the group of control, to 2 points, not inferior in effectiveness to the pharmacological activity of the cream with estrogen. This was associated with a notable positive impact of the vaginal gel on both the structural and functional condition of the vaginal mucosa, which was confirmed by histological studies, in particular, reflected by an increase in the thickness of the epithelial plate, the appearance of signs of a typical estrogen-like effect, an increase in

regenerative processes, mucous formation and the restoration of the pH level of vaginal secretion to the level of physiological values (4.4-4.5).

Morphological examination of the heart muscle and bone system state revealed the protective effect of vaginal gel with resveratrol and hyaluronic acid and its ability to restrain the advancemect of degenerative changes in the myocardium, articular cartilage and subchondral trabecular bone that occurred in untreated female rats with estrogen deficiency.

In the model of pharmacological castration of female rats, possible mechanisms of neuroprotective action of the gel with resveratrol and hyaluroniic acid and its influence on behavioral reactions characterizing the functional state of the CNS were investigated.

The expression pattern of major endogenous markers of neuroprotection was determined by real-time polymerase chain reaction. It was demonstrated that the expression of HIF-1α mRNA in the group of control group was 92.2% lower compared to the intact group group. There was also a 92.5 % decline in HSP70 mRNA expression and an 80.6 % reduction in c-fos mRNA expression. Enzyme immunoassay revealed a decrease in the concentration of heat shock proteins in HSP70 mitochondria by 61 % and the cytosol of the brain of animals with hypoestrogenemia.

During the treatment of animals with an acquired hypoestrogenic condition as a result of pharmacological castration, vaginal gel with resveratrol and hyaluronic acid showed an increase of 150 % in the expression of mRNA HIF-1α mRNA HSP70 33 % and 200 % - c-fos mRNA in the hippocampal SA1 zone.

Modeling of VCD hypoestrogenemia led to a significant deficiency of progesterone, to a decrease in estradiol against the background of a expressed increase in FSH, to a violation of mRNA expression, and, in particular, mRNA encoding the superoxide dismutase and glutathioreductase gene, and a decline in the level of restored glutathione and an increase in the expression of iRNA NOS, activation of oxidative stress in the brain of animals.

HSP70 has the capacity to reduce the accumulation of denatured proteins, attenuate the activation of free radical processes, enhance the activity of antioxidant

enzymes, mitigate the adverse effects of calcium overload by binding to the calcium receptor calmodulin, and also restrict the expression of iNOS [8]. The ability of HSP70 to prolong the life of the main adaptation factor, HIF-1, has been shown, which under extreme conditions initiates the start of compensatory mechanisms for energy production [8]. Thus, experimental hypoestrogenemia leads to a expressed deprivation of the mechanisms of endogenous neuroprotection associated with HSP70. The neuroprotective properties of resveratrol, particularly when used in combination with its various dosage forms such as vaginal gel and tablets, can be attributed to the ability of phytoestrogens to mimic the expression of key transcription factors that play a role in the synthesis of HSP proteins. Activation of the modulation of β-estrogen receptors of the brain causes detachment from the last HSP70 - proteins, which ensures the entry of these proteins into the cell and the realization of their biological function. The mechanism of this interaction is associated with the role of HSP70 in maintaining estrogen receptors in an inactive state that are not associated with estrogens [406].

The flavonoid resveratrol can increase the activity of estrogen receptors decreased by oxidative stress due to active oxygen form / SH regulation. Estrogen receptor modulators can enhance HSP70 expression by stimulating the protein transcription factor - heat shock factor (FHS). Estrogen modulators can inhibit neuroapoptosis by reducing caspase-3 activity and decline the activity of two transcription factors nuclear factor- κ B (p65/RelA and p50) by regulating the expression of the c-fos early response gene. Resveratrol can also function as a direct antioxidant due to its phenolic group, allowing it to capture reactive oxygen and nitrogen species and thereby regulate the JNK (ROS sensitive) cascade. JNK induces the activation of DNA-binding proteins cJun, c-Fos and AR-1, and the binding of these proteins to palindromic DNA sequences induces apoptosis [407-410].

In the conditions of transmitter autocoidosis in neurons (cerebellum, hippocampus, cortex), activation of neuronal synthase NO (nNOS) and an increase in NO production are observed, which is involved in the initiation of neuroapoptosis, the opening of the mitochondrial pore and the formation of mitochondrial dysfunction, nitrosation of SH-containing signal molecules and their loss of function, as well as a

decrease in the activity of Zn-Cu superoxidase [411]. The most ominous role in neuronal damage belongs to the inducible form of NOS, the expression of which by glial cells leads to hyperproduction not so much of nitrogen oxide NO, as its numerous cytotoxic forms from peroxynitrite to the nitrosonium ion. In the regulation of iNOS expression and activation of nitrosative stress, an important role is played by a low level of reduced intermediates of the thiol-disulfide system and an increase in the concentration of anti-inflammatory cytokines, especially (protein encoded by the eponymous gene) IL-1b. Thus, IL-1b activate AP-1 and universal transcription factor (NF-kB), which under conditions of ischemia change the cellular signality and enhance the expression of other anti-inflammatory factors, stimulate the expression of iNOS by astrocytes [412]. Excess IL-1b can adversely affect the transport of reduced glutathione to reduce its synthesis. Intracellular glutathione deficiency, which is involved in NO transport mechanisms and its bioavailability, enhances the formation of ONOO- [410]. The role of IL-1b in modulating the expression of heat shock proteins of HSP70, in particular from increase to suppression depending on its concentration [410], is known.

Expression modulation of various isoforms of NOS, aimed at their normalization and inhibition of nitrosative stress, under the influence of resveratrol in different routes of its administration in different dosage forms can be explained as next phenomenon. Resveratrol, due to its chemical structure, can regulate the activity of two transcription factors of nuclear factor- κ B (p65/RelA and p50). Resveratrol can also work as a direct antioxidant, due to the phenolic group to bind reactive oxygen species, and thereby inhibit the ROS-dependent mechanism of IL-1b and iNOS activation. Also, the antioxidant effect of resveratrol can be explained by the fact that an increase in the concentration of estradiol under its action leads to a Ye2- dependent activation of the expression of mitochondrial Mp-SOD. Increased expression of Mp-SOD significantly reduces the neurotoxicity flows of the superoxide produced by mitochondria.

A rise in the concentration of the oxidized form of glutathione leads to an increase in the production of anti-inflammatory cytokines TNF- α, IL-1 β, IL-6 and IL-8, which, in turn, enhances deficiency of glutathione reduced by disrupting its transport into the cell and strengthens the mechanisms of neurodestructure [412]. The increased

concentration of oxidized intermediates of the thiol-disulfide system inhibits the expression of eNOS (see Above) and enhance the production (active form of oxygen) of ROS due to the release of arachidonic acid from platelets, suppresses glutathione peroxidase and glutathione reductase, and also stimulates the intracellular signaling pathway, including neuroapoptosis. Course treatment with vaginal cream with promestrin did not significantly affect the indicators of the thiol-disulfide system.

The course introduction of the gel in the group with resveratrol and hyaluronic acid to ovariectomized female rats contributed to the restoration of the functional state of the CNS: tentative research activity and a decrease in the manifestations of vegetative-emotional reactions, the indicators of which almost reached the level of the group of intact animals. Combination therapy with gel and resveratrol tablets demonstrated the most expressed psychotropic effect, which completely eliminated the manifestations of depression in animals that arose due to estrogen deficiency. The combined administration of gel and tablets with resveratrol was significantly superior to monotherapy with gel with resveratrol and hyaluronic acid and the effect of the reference drug with synthetic estrogen promestrin in terms of the degree of influence on all test parameters of the CNS state.

New data have been obtained that helped establish the rationale for developing an innovative locally administered drug containing resveratrol and hyaluronic acid, determine the optimal composition of the dosage form, and demonstrate the effectiveness of the therapeutic effect on various experimental models of hypoestrogen conditions and to find out the individual mechanisms of its neuroprotective action.

Resveratrol has anti-inflammation effect. It is also characterized by antioxidant properties with the blockade cyclooxygenase and lipoxygenase, phosphatase activity. Resveratrol exhibits anti-inflammatory and gerontological effects by activating sirtuin-1, a histone deacetylase gene known as SIRT1. It also has radioprotective properties, playing a role in DNA repair. Resveratrol has estrogenic effects and is relevant in addressing osteoporosis. Additionally, it demonstrates antimicrobial, antiviral, antifungal, and antineoplastic actions. Resveratrol activates fatty acid oxidation, mitochondrial oxidative processes, respiration, and gluconeogenesis. Given the

adverse effects associated with hormonal drugs prescribed for menopause, the development of new medications containing resveratrol for the treatment of mucosal atrophic changes in gynecology and proctology could be highly beneficial.

Our research focused on conducting a pharmacological examination of a novel vaginal gel composed of resveratrol and hyaluronic acid. This gel is designed for addressing hypoestrogenic conditions, specifically genitourinary issues prevalent in women due to natural (menopausal) or pathological (post-castration) causes. The study provides both theoretical and experimental evidence supporting the feasibility of developing a locally applied medicinal product. This innovative formulation includes active pharmaceutical components like resveratrol, a phytoestrogen analogous to natural sex hormones and an estrogen receptor agonist, and hyaluronic acid, an exogenous analogue of glucosamine glycan found in vaginal mucus. Hyaluronic acid serves as a physiological moisturizer, lubricant, and a vital component of the genital tract's protective barrier. In conjunction with additional excipients such as lactic acid, this composition is unique within the Ukrainian pharmaceutical market.

As women enter menopause, they experience genetically predetermined age-related changes marked by a gradual decline in ovarian function. However, in cases of premature ovarian “exclusion”, such as after surgical or pharmacological castration, typically as a result of ovarian depletion syndrome following assisted reproductive technologies, younger women can undergo sudden hormonal shifts. Various etiologies of menopausal disorders emerge as a consequence of estrogen deficiency, leading to the development of pathological symptoms collectively known as menopausal syndrome. These symptoms significantly impact the overall quality of life for affected women. Menopause is responsible for the onset of various health issues among female patients, including osteoporosis, cardiovascular complications, cognitive impairment, and metabolic disorders.

Hormone replacement therapy (HRT), also known as menopausal hormone therapy (MHT), currently stands as the most effective treatment for addressing the symptoms of menopausal syndrome. Nevertheless, certain women either cannot use hormonal medications due to contraindications or discontinue their use due to side

effects. In Ukraine, a substantial portion of women doesn't seek MHT primarily due to hormonal phobia or a lack of awareness about this treatment. It's crucial to note that the effectiveness and safety of MHT depend on initiating therapy at the right time when the “therapeutic window” is open. Consequently, there is a need to provide alternative treatments for menopausal symptoms, encompassing both systemic and topical phytoestrogen therapy.

Ukraine boasts a considerable number of drugs and dietary supplements containing phytoestrogens in oral dosage forms. However, there is a noticeable absence of topical drugs designed to complement the systemic effects or primarily address genitourinary symptoms like vaginal dryness, itching, irritation, dyspareunia, and urinary incontinence. In summary, the substantial demand for topical drugs remains unmet by the limited variety of available options.

An encouraging approach to addressing the contemporary scientific challenge within modern pharmacology involves the creation of multifunctional vaginal medications containing phytoestrogens. These medications are produced using state-of-the-art pharmaceutical techniques and exhibit substantial pharmacological efficacy in conditions characterized by reduced estrogen levels. They can effectively and safely alleviate pathological symptoms, including those related to the genitourinary system. Notably, there is a scarcity of Ukrainian medications with these capabilities, with the majority of the available options being foreign-made [406-408].

Technology at the National University of Pharmacy in Kharkiv has successfully developed vaginal gels incorporating resveratrol and hyaluronic acid. These gels consist of resveratrol, a polyphenol and phytoestrogen that does not necessitate prior metabolism for its pharmacological effects, hyaluronic acid, and additional excipients, including lactic acid. The resveratrol component contains 50 % trans-resveratrol of plant origin, sourced from *Polygonum cuspidatum*.

For comparison purposes, two other medications were selected. The first, Ginodek gel, shares two common components (hyaluronan and lactic acid) with the experimental gels and serves similar therapeutic indications. The second comparison drug, Colpotrophin cream, was used in more extensive pharmacological investigations

as the benchmark and is employed for hormone replacement therapy. It contains promestriene, a synthetic estrogen.

Performing the research, a comprehensive approach was used with the involvement of physiological, histological, biochemical, pharmacological, toxicological, enzyme-linked immunosorbent assays, molecular genetic, and statistical research methods.

To establish the ideal formulation for the vaginal gel containing resveratrol and hyaluronic acid, preliminary screening studies were conducted on four test samples of vaginal gels. These samples varied in the concentration of the primary active ingredient, resveratrol, with percentages of 0.5 %, 1 %, 2 %, and 3 %. The quantities of all other ingredients in the gels remained consistent across these test variations.

Screening studies were conducted using a model of surgical castration in female white non-linear rats, aged 3 to 6 months. The experimental test samples were administered 35 days after the onset of the pathology, following a treatment regimen of 1 ml of gel applied intravaginally once a day for 28 days. For comparison, Ginodek vaginal gel was chosen as a reference drug.

Control groups were established, including intact animals (Intact Control Group - ICG) and operated but untreated animals (Controlled Pathology Group - CPG). These control groups received isotonic sodium chloride solution intravaginally in the same quantity as the test samples. Following the experiment, various assessments were made, including macroscopic examination of the vaginal mucosa and lower genital tract, evaluation of physical parameters such as body weight changes and tail root temperature, measurement of vaginal secretion pH, analysis of the vaginal microbial environment, and histological examination of the vaginal mucosa.

The outcomes of the screening study revealed the effectiveness of the assessed gels containing resveratrol and hyaluronic acid when administered intravaginally to ovariectomized female rats over a 28-day period. These gels effectively mitigated the increase in body weight induced by estrogen deficiency, normalized body temperature at the base of the tail (commonly associated with “hot flushes”), facilitated the

restoration of the vaginal microbiome and the acidic environment of vaginal secretions, and prevented the development of atrophic changes in the vaginal mucosa.

Upon conducting a comprehensive assessment of the therapeutic impact of the vaginal gels, it was determined that the optimal concentration of resveratrol in the formulation is 0.5 %. This concentration yielded the highest scores for the majority of parameters in the experimental model of hypoestrogenic conditions in the animals.

The experimental study data suggest the presence of not only localized effects but also a mild systemic influence from the vaginal dosage form with the combined composition of resveratrol and hyaluronic acid. This underscores the need for further in-depth research concerning this proposed formulation. The findings from this phase of the study provide an empirical basis for the development and comprehensive investigation of a vaginal gel containing phytoestrogen resveratrol and hyaluronic acid as an alternative to hormone-based medications for managing pathological hypoestrogenic conditions of various origins, particularly addressing menopausal genitourinary symptoms.

The subsequent step involved evaluating the safety profile of the vaginal gel with the combined composition of resveratrol and hyaluronic acid. This included the assessment of local tolerability, acute toxicity following a single intravaginal (i/v) and intragastric (i/g) administration, as well as chronic toxicity associated with multiple (90-day) intravaginal (i/v) applications.

The research revealed that the vaginal gel containing resveratrol and hyaluronic acid does not induce local irritation when used both in single and extended vaginal applications. The study on the acute toxicity of this gel classifies it as a non-toxic substance, specifically falling into the V toxicity class, which denotes it as an almost non-toxic substance according to the classification obtained [409]. Moreover, data from prolonged intravaginal administration indicate the absence of general toxic effects on animals and any local irritation of the mucous membrane in the lower genital tract.

A comprehensive pharmacological investigation was carried out to assess the pharmacodynamics of the vaginal gel containing resveratrol and hyaluronic acid. This

study utilized two models closely resembling the clinical conditions associated with hypoestrogenic states.

The pharmacological impact of the vaginal gel was examined in terms of its effects on both local (genitourinary) and systemic manifestations of hypoestrogenism. This evaluation was conducted using a surgical castration model, specifically bilateral ovariectomy, which emulated hypoestrogenic conditions as closely as possible.

The research demonstrated that the 28-day administration of the gel containing resveratrol and hyaluronic acid had a therapeutic effect by slowing down the rate of weight gain in animals. In contrast to untreated females in the CPG group, whose body weight gain reached 23 %, the animals treated with the vaginal gel for 4 weeks experienced a much lower body weight gain, not exceeding 5.96 %. For animals treated with the comparison drug, a cream containing synthetic estrogen promestriene, the weight gain was slightly higher at 6.83 %.

Furthermore, there was evidence of a systemic therapeutic effect of the gel with resveratrol and hyaluronic acid, as indicated by a significantly less pronounced symptom of tail root fever, corresponding to menopausal “hot flashes” in women. In the CPG group, this symptom increased by 10 % compared to intact females, while it only increased by 1.0 % under the influence of the phytoestrogen gel and by 1.6 % under the comparison drug's influence. The therapeutic effectiveness of the vaginal gel positively influenced the balance of sex hormones, with the level of estradiol being 1.5 times and progesterone being 1.4 times higher than in untreated animals. The therapeutic effect of the gel was almost as effective as the topical comparison drug promestriene.

The treatment with the gel containing resveratrol and hyaluronic acid demonstrated a substantial reduction in pathological manifestations of hypoestrogenism, particularly in relation to the vaginal mucosa. In terms of overall macroscopic indicators, the phytoestrogen-containing gel significantly diminished signs of hypoestrogenic atrophy, reducing the score from 7 points, as observed in animals of the CPG, to 2 points. This efficacy was on par with the pharmacological activity of the estrogen-containing cream.

These findings were in alignment with a noticeable positive impact of the vaginal gel on the structural and functional condition of the vaginal mucosa, as evidenced by histological examinations. This was exemplified by an increase in epithelial thickness, the presence of indicators of typical estrogen-like effects, and the promotion of regenerative processes, as reflected in a pH range of 4.4-4.5.

A morphological examination of the heart muscle and skeletal system revealed that the vaginal gel containing resveratrol and hyaluronic acid exhibited a protective effect. It had the ability to inhibit the development of degenerative changes in the myocardium, articular cartilage, and subchondral trabecular bone, which typically occurred in untreated estrogen-deficient rats. In terms of its capacity to alleviate genitourinary symptoms of hypoestrogenism, along with its cardio- and chondroprotective effects, the gel with resveratrol and hyaluronic acid demonstrated similar efficacy to the topical comparison drug, promestriene.

Furthermore, in an investigation utilizing the model of pharmacological castration in female rats, the study explored potential mechanisms underlying the neuroprotective action of the gel containing resveratrol and hyaluronic acid. It also assessed the impact of the gel on behavioral reactions that characterize the functional state of the CNS.

female rats' brains. These markers included HIF-1α mRNA, HSP70 mRNA, c-fos mRNA, and the concentration of HSP70 in both the cytoplasm and mitochondria. A real-time polymerase chain reaction was used to assess these markers.

The results showed a significant reduction in the expression of HIF-1α mRNA in the group of animals with hypoestrogenic conditions (CPG) compared to the intact group (ICG). Specifically, there was a 92.2 % decrease in HIF-1α mRNA expression. Similarly, there were substantial decreases in the expression of HSP70 mRNA (92.5 % reduction) and c-fos mRNA (80.6 % reduction) in the CPG group.

Enzyme-linked immunosorbent assay (ELISA) findings also indicated a 61 % decrease in the concentration of HSP70 heat shock proteins in both the mitochondria and brain cytosol of animals in a hypoestrogenic state.

When animals with induced hypoestrogenic conditions due to pharmacological castration were treated, the vaginal gel containing resveratrol and hyaluronic acid led to significant increases in the expression of certain neuroprotective markers in the CA1 hippocampal region. Specifically, HIF-1α mRNA expression increased by 150 %, HSP70 mRNA by 33 %, and c-fos mRNA by 200 %.

A more pronounced neuroprotective effect was observed when the animals received combination therapy, involving the use of the vaginal gel containing resveratrol and hyaluronic acid along with intragastric administration of resveratrol at a dose of 100 mg/kg. This combination therapy appeared to have a greater impact on enhancing neuroprotection in this context.

The combination of topical application of resveratrol through the vaginal gel and systemic exposure via tablets led to a remarkable increase in the expression of neuroprotective markers in the CA1 hippocampus of animals with hypoestrogenic conditions. Specifically, HIF-1α mRNA expression increased by 23 times, HSP70 mRNA by 30.7 times, and c-fos mRNA by 6.7 times.

Furthermore, the expression levels of these markers in the CA1 hippocampus of animals with hypoestrogenic status were restored to levels similar to or even higher than those observed in the ICG. The concentration of HSP70 in both the cytosolic and mitochondrial fractions of the brain also significantly increased by 118 % and 177 %, respectively, when the combination therapy was administered.

The prolonged administration of the gel to ovariectomized females, containing resveratrol and hyaluronic acid, led to the restoration of the normal functional state of the central nervous system. This was evident through improved exploratory behavior and a reduction in autonomic-emotional reactions, with these indicators reaching levels comparable to those observed in intact animals. When combined with resveratrol tablets, the therapy demonstrated a more pronounced psychotropic effect, effectively eliminating depressive symptoms associated with estrogen deficiency.

Notably, the combined administration of the gel and tablets with resveratrol had a more significant and comprehensive impact on all aspects of central nervous system indicators, surpassing the therapeutic effects of monotherapy with the gel containing

resveratrol and hyaluronic acid, as well as active drugs, including synthetic estrogenic compounds.

In conclusion, these recent scientific findings provide robust support for the development of an innovative topical medication containing resveratrol and hyaluronic acid. They underscore the importance of determining the optimal composition of the dosage form and demonstrate its effectiveness in addressing various experimental models of hypoestrogenic conditions while shedding light on specific mechanisms underlying its neuroprotective action.

As a result of bilateral ovariectomy in female rats, the number of opportunistic pathogens, aerobic bacteria, *Escherichia coli*, staphylococci, and clostridia increased statistically significantly in cultures of vaginal secretions. A significant decrease in the number of lactobacilli was established. Therefore, significant changes occurred, indicating significant changes in the vaginal microflora (Table 8.1).

Under the influence of test samples of vaginal gels with resveratrol concentrations of 0.5 %, 1 %, 2 %, 3 %, a moderate improvement of the studied indicators of the vaginal biotope was observed: the growth of opportunistic pathogens decreased compared to animals of the CPG, the number of *Lactobacilli* was almost at the level of the indicator of the group ICG, and a certain effect on the number of anaerobic microorganisms – clostridia – was also observed.

After the use of the comparative drug “Ginodek” in female rats, the number of opportunistic pathogens, *Escherichia coli, staphylococci, clostridia* decreased and the number of *Lactobacilli* increased.

The obtained results indicate that the studied test samples in general showed a less expressed antimicrobial activity than the comparative drug “Ginodek”, which normalized the vaginal microbiocenosis of female rats with hypoestrogen.

The findings from the pharmacological study of the novel vaginal gel containing phytoestrogen resveratrol and hyaluronic acid have been integrated into the research activities of associated departments within Ukrainian higher education institutions specializing in medical and pharmaceutical fields.

Table 8.1 – Indicators of the state of biocenosis of the lower part of the genital tract of ovariectomized female rats treated with vaginal gels with resveratrol and hyaluronic acid, (colony forming units / ml, x ± Sx)

Microorganisms	Experimental group, n = 6						
	ICG	CPG	CPG + gel with resveratrol 0,5 %	CPG + gel with resveratrol 1 %	CPG + gel with resveratrol 2 %	CPG + gel with resveratrol 3 %	CPG + gel «Ginodek»
Anaerobic bacillus	9,06±0,08	9,92±0,29*	9,17±0,05*#^	9,18±0,04*#^^	9,23±0,04*#^	9,42±0,12*#^	9,06±0,03*#^
Clostridia	0	6,39±0,015*	4,72±0,12*#^	4,82±0,12*#^	4,74±0,17*#^	5,74±0,3*#^	4,57±0,11*#^
Escherichia coli	6,87± 0,11	9,86±0,14*	7,07±0,20*#^	7,19±0,25*#^	7,24±0,26*#^	7,13±0,33*#^	6,77±0,14*#^
Other aerobic Gram negative bacillus	6,15±0,07	8,17±0,06*	6,97±0,15*#^	7,14±0,14*#^	7,11±0,30*#^	7,22±0,19*#^	6,15±0,08*#^
Staphylococcus	3,20±0,05	5,20±0,07*	3,68±0,28*#^	4,06±0,20*#^	4,01±0,13*#^	4,13±0,23*#^	3,21±0,04*#^
Lactobacteria	10,05±0,05	7,76±0,28*	9,89±0,14*#^	9,68±0,21*#^	9,76±0,18*#^	9,53±0,14*#^	10,07±0,07*#^

Note: *p <0.05 compared to the group of ICG animals, #p <0.05 compared to the group of CPG animals, ^p <0.05 compared to the group of animals that received the comparison drug, n is the number of animals in the experimental group.

The proposed gels with resveratrol and hyaluronic acid proved to be effective under the conditions of intravaginal 28-day administration to ovariectomized female rats, as they restrained the increase in body weight that occurs against the background of estrogen deficiency, normalized the body temperature at the base of the tail (preventing the manifestations of “hot flushes”), contributed to the restoration of vaginal biotope and the acidic environment of the vaginal secretion, prevented the development of atrophic changes in the vaginal mucosa.

Based on the results of an integral assessment of the effectiveness of the therapeutic effect of vaginal gels, it was established that the optimal content of resveratrol in the dosage form is 0.5 %, since according to the vast majority of indicators on the model of the experimental hypoestrogenic state in animals, it turned out to be the most effective.

The obtained results indicate not only a local, but also a possible systemic effect of the new vaginal dosage form of the combined composition with resveratrol and hyaluronic acid, which justifies the need for further in-depth research.

The results of the study of the acute toxicity of the gel with resveratrol and hyaluronic acid allow it to be assigned to the V class of toxicity (practically non-toxic substance). Data on long-term 90-day IV administration indicate the absence of a general toxic effect on animals and a local irritant effect on the mucous membrane of the lower part of the genital tract.

Experimental research data indicate the feasibility of developing and using vaginal dosage forms with phytoestrogen resveratrol and hyaluronic acid as an alternative to hormone-containing drugs for the treatment of pathological hypoestrogenic conditions.

CHAPTER 9.
POSSIBLE MECHANISMS OF RESVERATROL INFLUENCE ON THE REPRODUCTIVE SYSTEM IN OVARIECTOMIZED RATS

Menopause causes osteoporosis, cardiovascular complications, cognitive impairment, and metabolic disorders among women [413-415]. The onset of menopause is a genetically programmed, age-related phenomenon marked by a gradual decline in ovarian function. However, the premature cessation of ovarian function, which can occur as a result of surgical procedures or pharmacological interventions such as ovarian depletion syndrome following assisted reproductive technologies, can lead to abrupt hormonal changes in younger women.

Various etiologies of menopausal disorders can manifest due to estrogen deficiency and result in pathological symptoms and what is commonly referred to as the “menopausal syndrome”. This syndrome often diminishes the quality of life for affected women.

Menopausal symptoms, as a result from ovarian failure, commonly cause menopausal women to consult their physicians (hot flashes, bone mass loss, urinary complaints, vaginal dryness, and dyspareunia caused by vaginal atrophy) [413, 416]. This period is associated with the decrease of hormonal function of the ovaries that dramatically affects the female reproductive system, inducing changes in uterus, vagina, and vulva structure, microbiome, local immunity, and functioning [417]. With increased life expectancy, the influence of vulvovaginal atrophy (hereafter – VVA) on the quality of life, sexual function, and pelvic floor health is becoming more evident in the practice of medicine nowadays [414, 418]. In the hypoestrogenic state, the vaginal epithelium becomes thinner, glycogen production declines, and, as a result, lactic acid production is reduced, increasing vaginal pH. The change in pH encourages the overgrowth of nonacidophilic coliforms [415].

Estrogen plays a crucial role in addressing VVA caused by estrogen deficiency, and as a first-line treatment, estrogen therapy is recommended for this condition [414, 419]. Systemic administration of estrogen, often as part of post-

menopausal hormone therapy, can effectively alleviate urogenital atrophy. However, following the increased risk of venous thromboembolism, stroke, and breast cancer as determined by the Women's Health Initiative (WHI) trial [420], many women have become hesitant to pursue such therapy [421, 422]. In order to mitigate potential adverse effects and risks associated with systemic estrogen use, vaginal estrogen therapy is generally recommended unless other menopausal symptoms, such as hot flashes, are also present [422].

Nevertheless, concerns persist that vaginal estrogen treatment may be absorbed into the bloodstream, possibly leading to adverse systemic estrogen-related effects [423]. Despite promising findings, the labeling for commercially available vaginally applied estrogens, in terms of contraindications and warnings, often mirrors that of systemically administered estrogens due to a practice known as “class-labeling”, which is commonly mandated by health authorities. This raises the question of whether the side effects and risks are dependent on the type of estrogens used vaginally and whether the potential for systemic effects can be reduced through the use of low-dose or “ultra-low dose” estrogen products [422].

The symptoms of vaginal dryness and VVA tend to worsen over time, and various treatments are available, including different over-the-counter options such as vaginal moisturizers and lubricants [424]. Research conducted in the United States has indicated that vaginal dryness can also affect younger women, often due to the use of antiestrogen medications [425]. Consequently, the use of vaginal lubricants can be effective in alleviating post-menopausal atrophic vaginitis. Studies have shown that vaginal moisturizers and lubricants provide rapid relief from vaginal dryness and dyspareunia (painful intercourse) and can help reduce discomfort associated with such conditions [426, 427].

Currently, there is a notable absence of topical medications that can complement systemic treatments or primarily address genitourinary symptoms, such as vaginal dryness, itching, mucosal irritation, dyspareunia, and urinary incontinence. An intriguing approach to address this ongoing scientific challenge in

modern pharmacology is the development of vaginal medications with multimodal effects incorporating phytoestrogens and hyaluronic acid.

Phytoestrogen resveratrol serves as an analogue of natural sex hormones and acts as an agonist for estrogen receptors [428]. Resveratrol, a plant polyphenol found in grapes, berries, nuts, and primarily red wines, is produced by plants in response to stress, injury, or exposure to UV radiation. It assists the plant in adapting to changing environmental conditions [429]. Resveratrol possesses diverse properties, including activity against glycation, oxidative stress, inflammation, neurodegeneration, several types of cancer, and the aging process [430, 431]. Nevertheless, the bioavailability and pharmacokinetics of resveratrol remain challenging when administered orally. To address these issues, experimental vaginal gel formulations containing resveratrol and hyaluronic acid (R-HA gel) have been developed and tested for their *in vivo* effects. Hyaluronic acid is an exogenous glycosaminoglycan analogue and is a natural component of vaginal mucus, serving as a physiological moisturizer, lubricant, and playing a vital role in the protective barrier of the genital tract [424, 425]. When combined with excipients, such as lactic acid, this unique composition currently lacks analogs in the pharmaceutical market.

The study's objective was to evaluate the effects of vaginal R-HA gel on the reproductive system of ovariectomized rats and to understand the potential mechanisms underlying its action.

An increase in body weight in females following the removal of their ovaries indirectly confirms the validity of the hypoestrogenemia model. This weight gain is indicative of disruptions in lipid metabolism, a characteristic feature of the menopausal syndrome [432]. These changes may significantly contribute to the development of metabolic syndrome, type 2 diabetes, and obesity, all of which can be attributed to the hormonal imbalances in the bloodstream that occur during menopause [433].

At 28-day administration, the therapeutic effect of the R-HA gel was manifested by a slowing of the rate of weight gain (Table 9.1). In contrast to

untreated OVX group, in which body weight gain was 23 %, in the R-HA group after 4 weeks of treatment, body weight gain was not more than 5.96 %.

Table 9.1 – Weight and tail temperature dynamics before and after treatment

Parameters	Intact Rats (n = 7)	OVX Group (n = 7)	R-HA Group (n = 7)
Weight before ovariectomy, g	208.67 ± 1.82 [a]	211.33 ± 1.15 [a]	218.00 ± 1.63 [a]
Weight on week 5 after ovariectomy, g	214.17 ± 2.18 [a]	246.33 ± 1.19 [b]	245.33 ± 3.16 [b]
Weight after 4 weeks of treatment, g	217.83 ± 2.51 [a]	260.17 ± 1.92 [b]	231.00 ± 2.46 [c]
Tail t° before ovariectomy, °C	32.83 ± 0.14 [a]	32.9 ± 0.12 [a]	32.8 ± 0.8 [a]
Tail t° on week 5 after ovariectomy, °C	32.92 ± 0.05 [a]	36.18 ± 0.05 [b]	35.67 ± 0.05 [b]
Tail t° after 4 weeks of treatment, °C	32.94 ± 0.11 [a]	36.15 ± 0.03 [b]	33.12 ± 0.12 [a]

Data are presented as the M ± SEM. One-way ANOVA with post hoc Tukey's test for multiple comparisons were performed for data analysis. a, b, c Values at the same row with different superscript letters show significant differences at $p < 0.05$.

The systemic therapeutic effect of the R-HA gel was evident through a notably reduced occurrence of tail base temperature elevation, which corresponds to the menopausal symptom of hot flashes in women. In ovariectomized (OVX) rats, the tail temperature increased by 10 % compared to intact females. However, when treated with topical R-HA gel, the increase was only 1.0 % (Table 9.1). This reduction in tail temperature increase demonstrates the efficacy of the gel in alleviating the symptom of hot flashes, a common occurrence in menopausal women.

R-HA topical treatment for 4 weeks was associated with an insignificant increase of estradiol (35.6 ± 2.32 *vs*. 28.54 ± 1.6 pg/mL, $p = 0.072$) and progesterone (15.47 ± 1.72 *vs*. 10.64 ± 1.07 pg/mL, $p = 0.064$) levels as compared to OVX rats. On the other hand, hormone levels did not reach the value of intact rats (Figure 9.1).

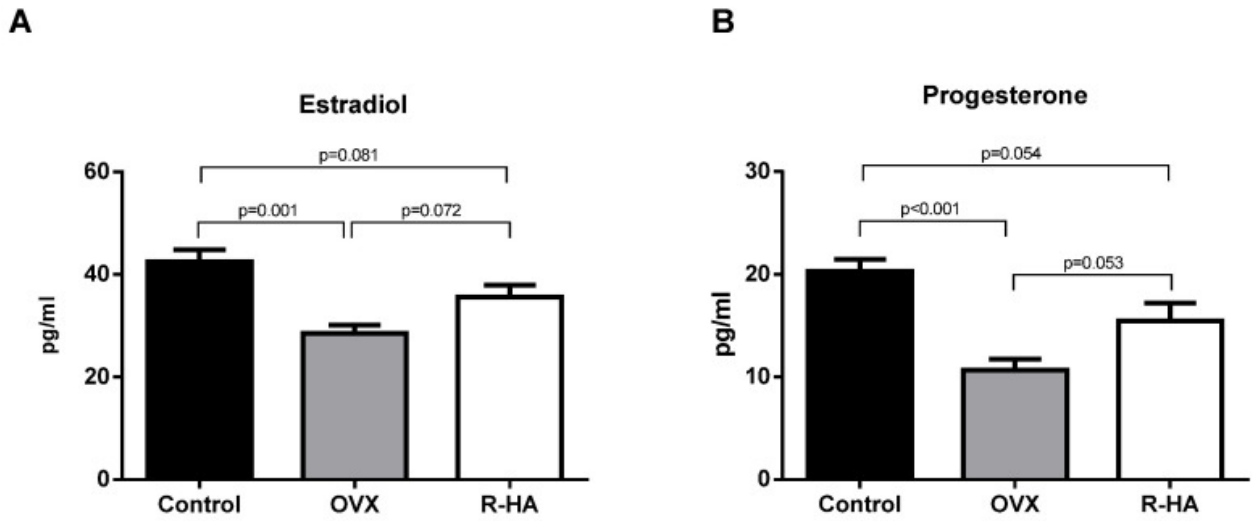


Figure 9.1 – Serum sex hormones levels in control, OVX, and after 4 weeks of R-HA administration ((A)—estradiol; (B)—progesterone). Data are presented as the M ± SEM. One-way ANOVA with post hoc Tukey's test for multiple comparisons was performed for data analysis

Although the R-HA treatment did not result in reaching the primary levels, the trend of elevation of sex hormones was detected. As a result, R-HA treatment provided a limited effect on systemic levels of estradiol and progesterone in OVX rats.

In ovariectomized (OVX) rats, significant reductions were observed in both endometrial and vaginal mucosa thickness, with endometrial thickness decreasing by up to three times and vaginal mucosa thickness decreasing by approximately two times (as shown in Figure 9.2). The thinning of the endometrium was associated with complex changes that affected both the luminal epithelium and the glands. The epithelial lining became thinner with a higher nuclear-to-cytoplasmic ratio. Additionally, there was a substantial reduction in the number of glands in the lamina propria, with only a few small glands remaining in the endometrium of ovariectomized animals. These changes are indicative of the impact of ovariectomy on the reproductive tissues.

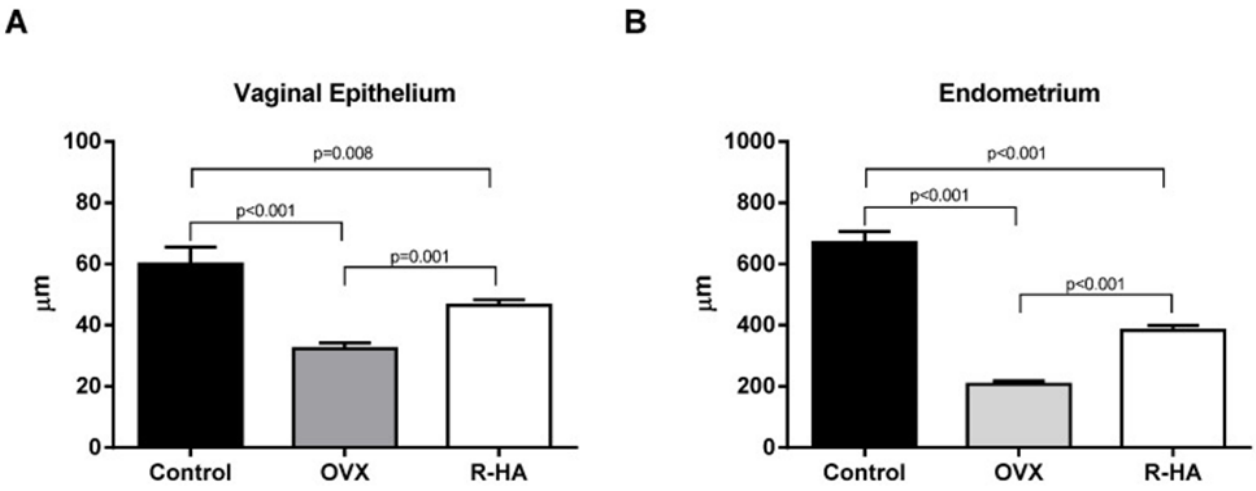


Figure 9.2 – Effect of ovariectomy and R-HA gel administration on the thickness of vaginal epithelium (A) and endometrium (B). Data are presented as the M ± SEM. One-way ANOVA with post hoc Tukey's test for multiple comparisons was performed for data analysis

Vaginal mucosa in OVX group also demonstrated a decrease in thickness due to thinning of squamous stratified epithelium with altered cell differentiation. There were features of inflammatory infiltration of lamina propria by polymorphonuclear leucocytes (hereafter – PMN) as compared with the control group (Figure 9.3). These changes were associated with the increased COX-2 expression in both stratified squamous epithelium and lamina propria infiltrated by highly immunopositive cells.

R-HA gel administration lessened the atrophy of the endometrium (382.79 ± 17.18 *vs*. 207.46 ± 10.7 µm, $p < 0.001$) and thinning of the vaginal epithelium (46.56 ± 1.76 *vs*. 32.33 ± 1.87 µm, $p = 0.001$) as compared with the OVX group, though the endometrial structure still varied from the control rats (Figure 9.2).

Endometrium showed higher luminal epithelium and density of endometrial glands, which determined the increase of endometrial thickness. Glands were surrounded by numerous small vessels reflecting the ameliorating effect of R-HA administration. Evaluation of vaginal mucosa revealed the anti-inflammatory action of R-HA treatment as compared with OVX. This conclusion was confirmed by the decrease in COX-2 expression both in vaginal epithelium and stromal cells (Figure 9.3).

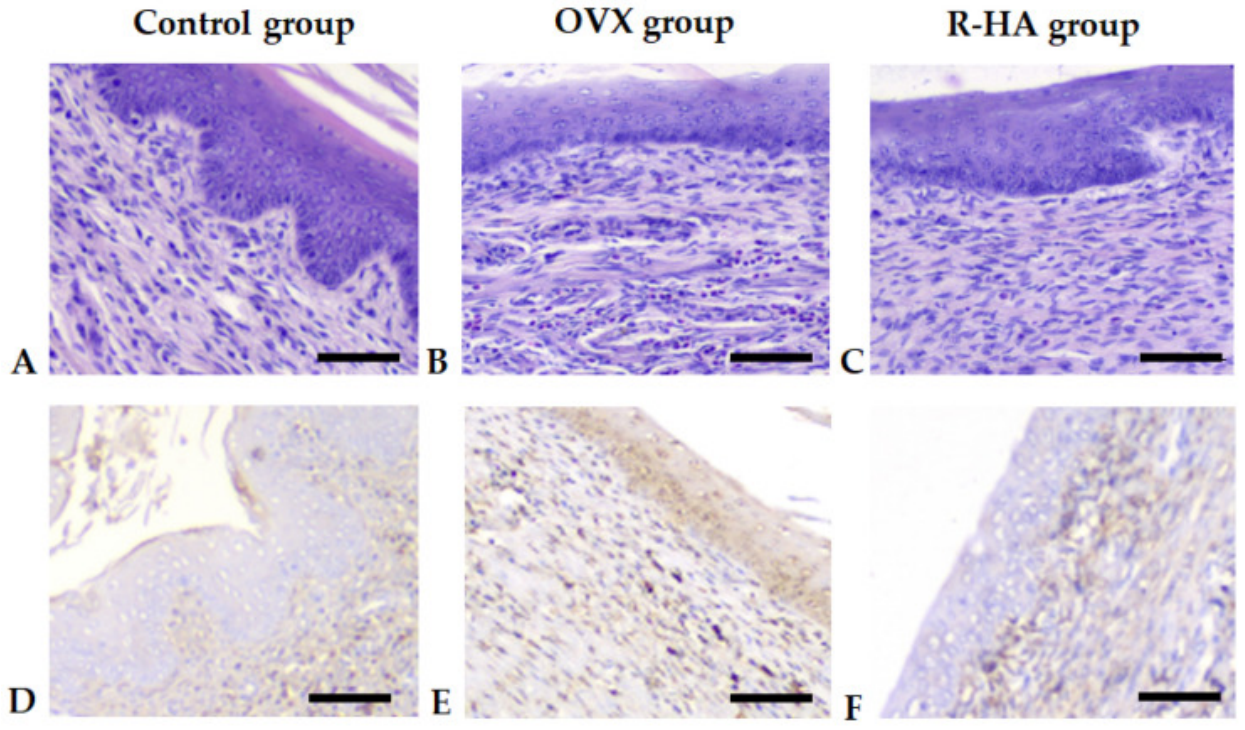


Figure 9.3 – Impact of ovariectomy and R-HA treatment on vaginal mucosa. (A–C)—H&E staining. Scale bars 50 µm. (D–F) — Immunohistochemistry demonstrating COX-2 expression. Scale bars 50 µm. (A) —The vaginal mucosa of the control group. (B) —The vaginal mucosa of the rats after ovariectomy demonstrates thinning of the stratified squamous nonkeratinized epithelium and inflammatory infiltration of the lamina propria by polymorphonuclear leukocytes. (C) — R-HA treatment of ovariectomized rats attenuated inflammatory infiltration and prevented covering epithelium atrophy. (D) — A few COX-2 positive cells in vaginal mucosa of the control group animals. (E) — Increased number of COX-2 positive cells in the vaginal mucosa. (F) — Decline of COX-2 expression in vaginal mucosa after R-HA treatment

Under the treatment of the R-HA gel, notable improvements were observed in the pathological manifestations associated with hypoestrogenism, particularly concerning the condition of the vaginal mucosa. These positive outcomes were supported by histological examinations, which revealed several indicators of estrogen-like effects, such as increased epithelial thickness and accelerated regenerative processes. The restoration of vaginal pH to a range of 4.4-4.5 also indicated the positive impact of the treatment, reflecting a healthier structural and functional state of the vaginal mucosa (Figure 9.4).

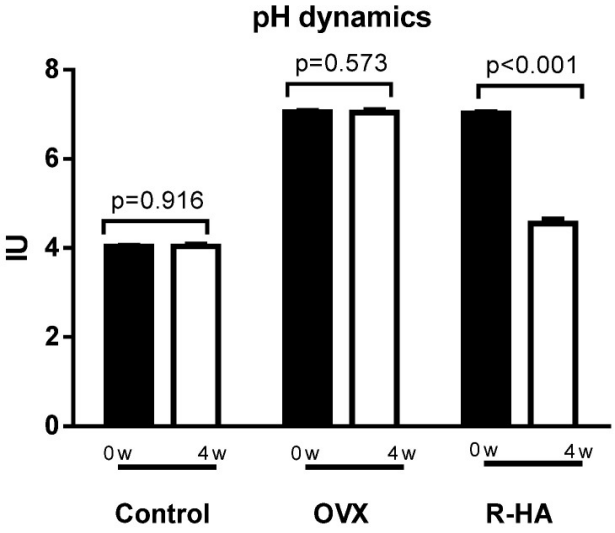


Figure 9.4 – The value of the vaginal pH in control, OVX animals, and in female rats before and after R-HA topical treatment.
Data expressed as mean ± SEM. For comparison, paired sample t-tests were used

Analyzing the mechanisms of topical treatment with the R-HA gel, we examined the biomarkers involved in regulating apoptosis. Ovariectomy led to an increase in Casp-3 expression and a decrease in Bcl-2 immunoreactivity in the endometrium, indicating proapoptotic changes. However, R-HA treatment counteracted these proapoptotic stimuli and increased Bcl-2 expression, ultimately providing an antiapoptotic effect in the endometrium.

The ovariectomy procedure led to an increase in Casp-3 immunoreactivity in the lining epithelium, endometrial stroma, and glands of the OVX animals ($p < 0.001$). However, when the R-HA gel was administered, there was a significant reduction in Casp-3 expression in all cell types, with the most noticeable effect observed in the glands when compared to the OVX group ($p < 0.001$) (Figure 9.5).

When assessing the impact of R-HA on Bcl-2 expression, we observed specific patterns in different tissues. In normal endometrium, there was mild Bcl-2 expression in the epithelium, high expression in stromal cells, and moderate to high expression in glandular cells. However, ovariectomy primarily affected Bcl-2 expression in glandular cells. In luminal epithelium, there were changes in the pattern of Bcl-2 expression, transitioning from a mild, diffuse pattern under normal conditions to an intercalated distribution of cells with varying immunostaining

intensities in ovariectomized rats, although the total score remained unchanged ($p = 0.561$) (Figure 9.6).

While R-HA administration did not significantly alter the Bcl-2 scores in the luminal epithelium and stroma of the endometrium, the most significant effect of R-HA treatment was observed in the glands when compared to the OVX group ($p < 0.001$). This effect would provide protection against apoptosis and endometrial atrophy.

Ovariectomy resulted in increased Casp-3 expression but decreased Bcl-2 expression, particularly in endometrial gland cells. The antiapoptotic effect of R-HA administration was linked to increased VEGF expression. This signifies that R-HA treatment helped counteract the proapoptotic changes induced by ovariectomy through the upregulation of VEGF, which plays a crucial role in promoting angiogenesis and cell survival.

The anti-apoptotic effect of R-HA was correlated with an increased expression of VEGF in endometrial glands ($p < 0.001$) and stromal cells ($p = 0.007$). Likewise, elevated VEGF expression was observed in both the stratified squamous non-keratinized epithelium and the vaginal lamina propria. This suggests that R-HA treatment has a positive impact on the expression of VEGF, which is associated with angiogenesis and cell survival, in various tissue types, including the endometrium and vaginal tissues (Figure 9.6).

Therefore, R-HA treatment attenuates atrophic changes in the genital tract by antiapoptotic pathways in the endometrium and stimulating angiogenesis in the endometrium and vagina.

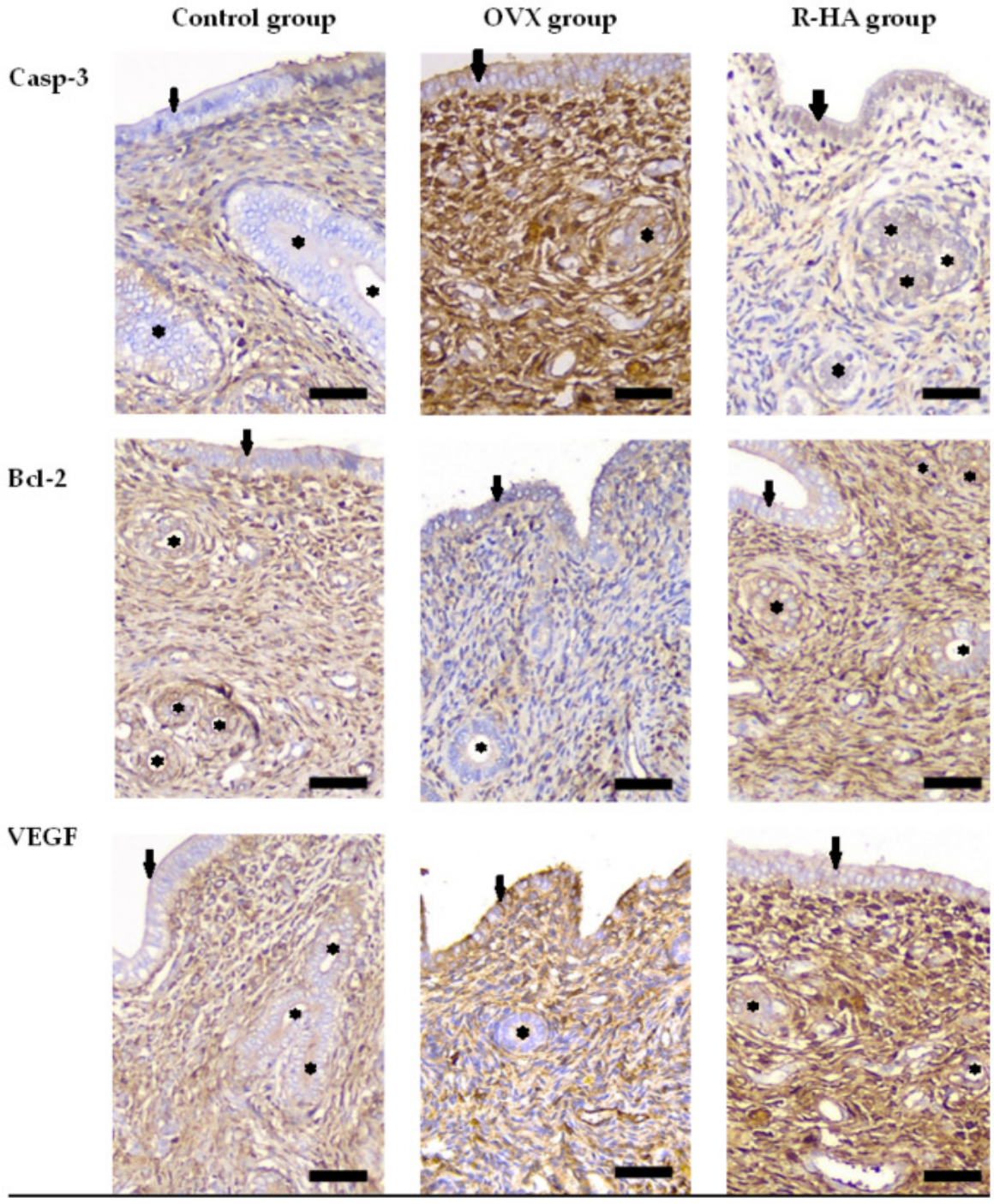


Figure 9.5 – Changes in Casp-3, Bcl-2, and VEGF expression in experimental groups' immunohistochemistry. Scale bars 50 μm. Ovariectomy resulted in thinning of endometrial lining epithelium (arrows), declining number of endometrial glands (asterisks), and changes in biomarkers expression. While Casp-3 expression increased in epithelium lining, endometrial stroma, and glands after ovariectomy, the levels of Bcl-2 reduced significantly in glandular cells as compared with the control group, shifting the balance between Casp-3/Bcal-2 towards pro-apoptogenic regulation. R-HA treatment attenuated the activation of pro-apoptogenic pathways through elevation and Bcl-2 expression and reduction of Casp-3 levels especially in glands. This effect was associated with enhancement of VEGF expression in both endometrial stroma and glands

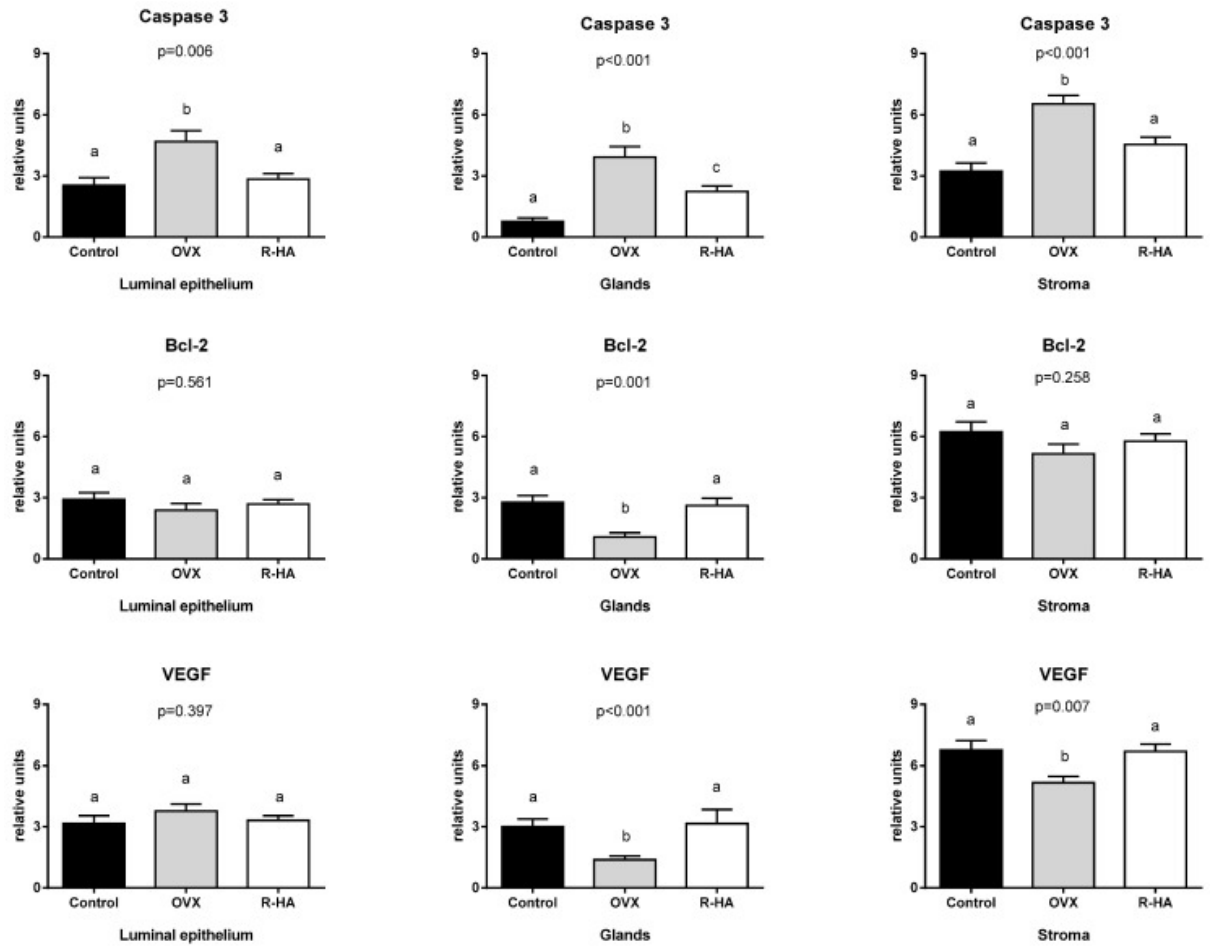


Figure 9.6 – R-HA treatment attenuates proapoptotic changes in endometrium and recovers Bcl-2 and VEGF expression. Data are presented as the M ± SEM. One-way ANOVA with post hoc Tukey's test for multiple comparisons was performed for data analysis. a, b, c values on the same row with different superscript letters show significant differences in $p < 0.05$

Since menopause is not an illness but a manifestation of age-related changes, it provokes an endocrine imbalance in the body, which leads to the onset of symptoms such as hot flashes, irritability, sleep disturbances, genitourinary disorders, and increases the risk of metabolic, cardiovascular–vascular, neurological diseases, and osteoporosis [434, 435]. During natural menopause, estrogen levels gradually decline over several years, allowing women's bodies to adapt to life with reduced estrogen. However, in the case of surgical menopause, where the ovaries are removed, a significant number of women (75–90 %) experience post-ovariectomy syndrome shortly after the surgery, leading to a rapid onset of

symptoms associated with estrogen deficiency [436]. This underscores the importance of implementing both medical and social measures to safeguard women's health, enhance their productivity, and maintain their overall quality of life during the peri- and post-menopausal periods. It also emphasizes the critical need for the development of effective and safe medications tailored for the personalized prevention and treatment of menopausal disorders.

Classical estrogen replacement therapy has been associated with an elevated risk of cancer and other health issues [413-415, 420, 422, 455]. Consequently, there is a pressing need for safer and more effective treatment approaches. A recent clinical investigation sought to compare the therapeutic effectiveness of alternative vaginal medications, such as promestriene (a low-potency estrogen agonist) and sodium hyaluronate (NaH), a nonhormonal, water-based agent. Notably, both the low-potency estrogen agonist promestriene and nonhormonal vaginal NaH applications were found to be equally effective in treating menopausal vaginal atrophy [437].

Moreover, a recent review explored the relationship between resveratrol and reproductive health, delving into the unique aspects of phytoestrogen resveratrol, its effects on embryogenesis, spermatogenesis, pregnancy-related complications, umbilical blood vessels, and women's reproductive health in general [428, 438]. Research has also shown that three months of peroral resveratrol therapy can improve endothelial function and reduce blood pressure in ovariectomized rats [439]. Additionally, a 24-month randomized, controlled, crossover trial demonstrated that resveratrol supplementation could enhance aspects of well-being, including the management of chronic pain, a common issue among post-menopausal women [440].

Nonetheless, the bioavailability and pharmacokinetics of resveratrol remain challenging when administered orally [428]. To address this, the research study utilized an experimental vaginal gel containing resveratrol and hyaluronic acid. Consequently, this investigation aimed to examine the pharmacological effects of the vaginal gel with resveratrol and hyaluronic acid on both local (genitourinary)

and systemic manifestations of hypoestrogenism using a model of bilateral ovariectomy (surgical castration).

The results of our study suggest that a 28-day treatment with R-HA vaginal gel can effectively alleviate the typical symptoms of menopause. The therapy's effectiveness was demonstrated by a slowdown in weight gain in the treated animals, a reduction in vaginal pH, and a decrease in the indicators of inflammatory infiltration and atrophy observed in OVX rats. Importantly, we did not observe a significant increase in sex hormone levels with R-HA treatment [456, 457].

To address the question of potential systemic effects of R-HA treatment, it's important to recognize that R possesses various effects on estrogen-estrogen receptor signaling. First, R, as a phytoestrogen, can enhance the expression of native estrogen-regulated genes. Second, R can directly activate estrogen receptors (ER) as an agonist, thereby triggering mitogen-activated protein kinase (MAPK) and subsequent signaling pathways [441, 458]. Finally, the genomic impact of R on ER transcription has been identified, indicating that R can function as an activator and sensitize ER in OVX animals. These mechanisms might explain how R-HA gel treatment can effectively address menopausal symptoms without significantly affecting sex hormone levels.

In addition to its estrogen receptor ER-stimulating effects, R has a broad range of beneficial impacts on various biological processes within cells. For instance, R has been shown to decrease cell proliferation, induce apoptosis in cancer cells, and promote smooth muscle relaxation [428, 442, 443]. This polyphenol also possesses anti-inflammatory and antioxidant properties, as well as cytoprotective action [444-446]. R's antioxidant effect is achieved through the upregulation of various antioxidant enzymes and the reduction of superoxide production by enhancing the activity of the enzyme GTP cyclohydrolase I, which plays a role in the synthesis of tetrahydrobiopterin and has an impact on endothelial nitric oxide synthase (eNOS). This mechanism contributes to the protection of the cardiovascular system [447]. R is also associated with other favorable effects, including anti-lipid, anti-aging, anti-bacterial, anti-viral, and neuroprotective actions [447]. Additionally,

polyphenols like R have been linked to weight management [447, 448]. Furthermore, the genomic effects of R involve the modulation of sirtuin 1 and the nuclear factor E2-related factor-2, leading to the epigenetic regulation of various gene transcription processes [448].

In our study, we observed a significant positive effect of local R-HA gel on the health and structure of the vaginal mucosa. The reduction in vaginal pH is attributed to lactic acid present in the vaginal mucous [449]. This decrease in pH is influenced by both the maturation of squamous epithelial cells, which is dependent on sex hormones, and the microbial flora in the vagina. Ovariectomy resulted in an increase in vaginal pH and inflammatory infiltration of the vaginal wall, but R-HA treatment improved vaginal homeostasis by reducing inflammation and pH levels. Furthermore, our data clearly showed that local administration of R-HA prevented atrophic changes in the vaginal epithelium and exhibited anti-inflammatory effects. This could be attributed to the normalization of the vaginal microflora as well as the direct anti-inflammatory actions of R. The local activity of the gel may also be attributed to the distribution and action of hyaluronic acid. Research has suggested that hyaluronic acid is effective, safe, and well-tolerated, making it a potential alternative for women who cannot use hormonal therapy to address symptoms of vaginal atrophy [450-451].

To understand the mechanisms of the R-HA gel's effects on the endometrium and vagina, we conducted an immunohistochemical study using specific biomarkers, including COX2, Casp-3, Bcl-2, and VEGF. Previous research has demonstrated that estrogens play a vital role in the survival of reproductive system cells and possess potent antioxidative and anti-inflammatory properties. Since the endometrium and vagina are highly sensitive to sex hormone levels, a deficiency of estrogens can disrupt the balance between cell proliferation, survival, and apoptosis [451]. As a result, ovariectomy (OVX) leads to elevated levels of oxidized glutathione, lipid peroxidation, and mitochondrial DNA damage, all of which can induce oxidative stress and inflammation [452]. Notably, OVX rats exhibited a significant increase in the expression of Casp-3, a key player in the execution phase

of apoptosis. Conversely, topical treatment with R-HA attenuated the proapoptotic activation by increasing Bcl-2, a mitochondrial protein contributing to antiapoptotic signals. Essentially, R-HA administration reversed the changes induced by ovariectomy. The beneficial effects of the R-HA gel can be attributed to the combined influence of both resveratrol and hyaluronic acid. HA is abundant in mucosal connective tissue and contributes to moisture retention and nutrient transport. Additionally, HA supports the proliferative phenotype of fibroblasts and protects them from apoptosis. Resveratrol has several positive effects, including its impact on estrogen receptor alpha, stimulating their expression and signaling [453]. It also facilitates angiogenesis by enhancing the production of VEGF, which is essential for maintaining proper blood supply and preventing sclerotic changes [454, 459]. Lastly, by harmonizing oxidative stress events and modulating pro- and anti-apoptotic signaling, the R-HA gel helps maintain tissue homeostasis and reduce inflammation.

In this study, the treatment with R-HA vaginal gels effectively improved women's reproductive health, offering a promising alternative, especially for patients who cannot use hormonal therapies. Therefore, further preclinical and clinical studies are necessary to validate the positive results of R-HA gel. Larger randomized studies are needed to confirm these findings.

In this chapter, we have gathered new scientific data that support the potential development of an innovative topical medication containing R and HA. The 28-day application of this gel has demonstrated its therapeutic effectiveness in an experimental model of hypoestrogenic conditions. Moreover, it has shed light on specific mechanisms responsible for its anti-inflammatory effects. More precisely, our research has uncovered a novel anti-inflammatory mechanism, dependent on COX-2, that leads to increased Bcl-2 expression, resulting in an antiapoptotic effect in both the endometrium and vagina. This effect is closely linked to the gel's ability to stimulate angiogenesis through an increase in VEGF expression in endometrial glands and stromal cells.

The limitations of the study that there are only a few experimental animals included and the absence of molecular biology methods such as Western blotting, etc. for results interpretation.

The obtained data proved the pharmacological efficiency of using the R-HA vaginal gel to reduce the symptoms of menopause and constitute an experimental rationale for further preclinical and clinical studies.

CHAPTER 10.
DIFFERENCES IN NO/SH-MECHANISMS OF NEURON DAMAGE IN EXPERIMENTAL VCD-HYPOESTROGENEMIA UNDER RESVERATROL IMPACT

In menopause, despite its physiological nature, persistent molecular and biochemical changes occur, leading not only to the extinction of the reproductive function of a woman but to a deterioration of her quality of life [460]. During menopause, against the background of hypoestrogenemia, against the background of autonomic dysfunction, increased anxiety, cognitive impairment, the growth of cardiovascular pathologies, and adverse outcomes increase [461]. Through this period, as shown by experimental and clinical studies, the formation of endothelial dysfunction, disturbance in the NO system, changes in lipid and carbohydrate metabolism, and increased platelet aggregation are observed.

There are experimental data that hypoestrogenemia against the background of modeling peri- and postmenopause is accompanied by an increase in the mitochondrial BAX/Bclx1 ratio, release of cytochrome C into the cytoplasm, increased caspase-3 expression, kinase activation, and initiation of apoptosis [462]. On this point, there is no doubt about the importance of adequate complex treatment of menopausal problems that occur in the peri- and postmenopausal period in women. Hormone replacement therapy is a pathogenetic method for correcting menopausal disorders, which is prescribed to relieve menopausal symptoms, and urogenital diseases, prevent bone loss, the occurrence of colorectal cancer, and the development of metabolic and other disorders [463]. However, under the conditions of hormone replacement therapy in women with hypoestrogenemia, the risk of developing thromboembolism, coronary artery disease, myocardial infarction, and stroke increases. Lately, it has been determined that hormone replacement therapy does not have the desired effect on cognitive impairment in perimenopausal and menopausal women and is associated with the risk of developing major side effects [464].

Usage of recently synthesized phytoestrogens leads to decreasing side effects [465]. However, the prescription of vaginal forms of phytoestrogen preparations does not lead to the mitigation of cognitive disorders, which requires the administration of additional neuroprotective drugs into the complex treatment of menopause [466]. Considering the identified molecular-biochemical disorders in early menopause, the attention of pharmacologists and physicians is drawn to drugs that reduce the intensity of oxidative stress, prevent the formation of mitochondrial dysfunction, initiate neuroapoptosis, and normalize thiol-disulfide balance [467]. These drugs include resveratrol, which has antioxidant and estrogen-like properties [468].

Analyzing the data presented in Table 10.1, characterizing the expression of mRNA eNOS, mRNA nNOS, and iNOS in the CA1-zone of the hippocampus of the brain of females with VCD hypoestrogenemia, the following was found out.

Table 10.1 – Expression pattern of eNOS mRNA, nNOS mRNA, and iNOS in the brain of female rats with VCD-hypoestrogenemia and against the background of pharmacological correction on day 29 after treatment (M ± m)

Experimental groups	eNOS mRNA, c.u.	mRNA nNOS, c.u.	mRNA iNOS, c.u.
Intact control (IC)	1000±0.008	1.000±0.0032	1.000±0.0112
Control pathology (CP)	0.0918±0.0001	0.549±0.0178	5.194±0.0922
CP + gel with R	0.214±0.008*	0.710±0.0057*	5.8765±0.0875*
CP + gel with R and tablets with R	2.1506±0.0033*[1,2]	1.155±0.011*[1.2]	2.3008±0.0764*[1,2]
CP + “Colpotrophine” cream	0.093±0.001	0,.6011±0.012	7.0543±0.0765*

Note: * – $p < 0.05$ in relation the control pathology (CP)
[1] – $p < 0.05$ in relation to the group CP + “Colpotrophine” cream
[2] – $p < 0.05$ in relation to the group CP + gel with Resveratrol

The expression of eNOS mRNA in the group of untreated rats (control) was 90.8 % lower than in the group of intact animals. A decrease in nNOS mRNA expression by 45.1 % and a significant decrease in iNOS mRNA by 5.2 times were also observed. Enzyme immunoassay revealed an increase in the concentration of nitrotyrosine in mitochondria (3.94 times) and in the cytosol of the brain (3.48 times) with VCD-hypoestrogenemia compared with healthy females of the same age. The revealed facts indicate significant disorders of the nitroxidergic system of the brain and the activation of nitrosating and oxidative stress.

A significant role in the mechanisms of neuron death in various neurodegenerative diseases belongs to NO-mediated mechanisms, which are realized by increasing the expression and activity of various NOS isoforms [469]. Under conditions of transmitter autocoidosis in neurons (cerebellum, hippocampus, cortex), activation of neuronal NO synthase (nNOS) and an increase in NO production are observed, which is involved in the initiation of neuroapoptosis, opening of the pore of the mitochondrion, and the formation of mitochondrial dysfunction, in the nitrosylation of SH-containing signaling molecules, and their loss functions, as well as suppression of Zn-Cu-SOD activity [470].

The most sinister role in neuronal damage belongs to the inducible form of NOS, the expression of which by glial cells leads to hyperproduction, not so much of NO as of its numerous cytotoxic forms – from peroxynitrite to nitrosonium ion.

The low level of reduced intermediates of the thioldisulfide system and an increase in the concentration of pro-inflammatory cytokines, especially IL-1b, play an important role in regulating iNOS expression and activating nitrosating stress. IL-1b activates AP-1 and NF-kB, which change the cell signal under ischemia and increase the expression of other pro-inflammatory factors while stimulating iNOS expression by astrocytes [470, 476].

Elevated levels of IL-1b can have adverse effects on the transport of reduced glutathione, leading to a reduction in its synthesis. A deficiency in intracellular glutathione, which plays a role in nitric oxide (NO) transport and bioavailability, can increase the production of $ONOO^-$ (as described in [471]). The impact of IL-1b on

the regulation of HSP70 expression is complex and can vary from stimulation to inhibition depending on its concentration (as discussed in [470]).

Course treatment (Table 10.2) with vaginal cream Colpotrophin did not have a distinct effect on the expression of eNOS mRNA, nNOS mRNA, iNOS, and mRNA, as well as on the concentration of nitrotyrosine and markers of oxidative modification of the protein in the brain of females with VCD – hypoestrogenemia.

Table 10.2 – The concentration of nitrotyrosine in the brain of female rats with VCD-hypoestrogenemia and against the background of pharmacological correction on the 29th day after treatment (M ± m)

Experimental group	Nitrotyrosine, cytosol pg/ml	Nitrotyrosine, mitochondrial pg/ml
Intact control (IC)	6.000±0.572	2.43±0.23
Control pathology (CP)	20.9±0.638	9.59±0.44
CP + gel with R	16.8±0.72*	8.60 ±0.36*
CP + gel with R and tablets with R	10.4±0.70*[1,2]	5.96 ±0.29 *[1.2]
CP + “Colpotrophine” cream	18.7 ± 0.41*	9.19±0.39

Note: * – p <0.05 in relation the control pathology (CP)

[1] – p <0.05 in relation to the group CP + “Colpotrophine” cream

[2] – p <0.05 in relation to the group CP + gel with Resveratrol

The course administration of vaginal gel with resveratrol to females with VCD-hypoestrogenemia led to a significant increase (by 133 %) in the expression of eNOS mRNA and nNOS mRNA (by 29.3 %). At the same time, the introduction of resveratrol did not significantly affect the expression of iNOS mRNA. Also, the introduction of resveratrol gel led to a significant decrease level of nitrotyrosine by 10.3 % in mitochondria and by 19.6 % in the cytosol of the brain of females with hypoestrogenemia.

The course combined use of the gel and tablets of resveratrol led to a significant increase in the expression of eNOS mRNA by 23 times and nNOS mRNA by 2.1 times, as well as to a decrease in iNOS mRNA expression by 55.7 %. In the

CA1 hippocampus of females with hypoestrogenemia. The combined administration of resveratrol gel and tablets to females with VCD hypoestrogenemia significantly reduced the level of nitrotyrosine by 37.8 % in mitochondria and by 50.2 % in the cytosol of the brain of females with hypoestrogenemia (Table 10.3). In terms of the degree of influence on the studied parameters, the combined administration of resveratrol gel and tablets significantly exceeded monotherapy with resveratrol gel and colpotrophine gel. Modulation of the expression of various NOS isoforms, aimed at their normalization and inhibition of nitrosating stress, under the influence of resveratrol at various routes of its administration in various dosage forms can be explained by the following fact. Due to its chemical structure, resveratrol can regulate the activity of two nuclear factor-κB transcription factors (p65 / RelA and p50) [403].

Table 10.3 – Indicators of the non-enzymatic link of the thiol-disulfide system in the brain of female rats with VCD-hypoestrogenemia and against the background of pharmacological correction on the 29th day after treatment (M ± m)

Experimental group	SH-groups, μm/g protein	SS-groups, μm/g protein	Glutathione restored, μg/protein	Glutathione oxidized, μg/protein
Intact control (IC)	20.9±1.59	2.99 ±0.33	4.57±0.49	0.24±0.032
Control pathology (CP)	8.9±0.82	5.58±0.56	1.57±0.18	0.73±0.08
CP + gel with R	11.1±1.66*	5.02±0.59	2.01±0.18*	0.59±0.05*
CP + gel with R and tablets with R	18.1±2.9*[1.2]	3.56±0.28 *[1.2]	3.3±0.22*[1.2]	0.37±0.03*[1.2]
CP + “Colpotrophine” cream	9.95±1.13	4.98±0.81	1.72±0.26	0.67±0.08

Note: * – $p < 0.05$ in relation the control pathology (CP)
[1] – $p < 0.05$ in relation to the group CP + “Colpotrophine” cream
[2] – $p < 0.05$ in relation to the group CP + gel with Resveratrol

Resveratrol can also work as a direct antioxidant – due to the phenol group, it binds reactive oxygen species, and thereby inhibits the ROS-dependent activation mechanisms of IL-1b and iNOS.

Also, the antioxidant effect of resveratrol can be explained by the fact that an increased concentration of estradiol under its action leads to E2-dependent activation of the expression of mitochondrial Mn-SOD [472].

Increased expression of Mn-SOD significantly reduces the fluxes of neurotoxic superoxide produced by mitochondria [471] and thereby inhibits ROS-dependent activation mechanisms of IL-1b and iNOS [472].

Modeling of VCD hypoestrogenemia led to significant disturbances in the thiol-disulfide system of the brain of female rats, especially its glutathione link – a decrease in the pool of its reduced forms and a decrease in the activity of GPR and GR cytosolic fraction compared with intact animals. Glutathione is a crucial component of neuron protection, increases its resistance to hypoxia, limits NMDA hyperexcitability, acts as a reserve of cysteine in the cell, and regulates synthesis and stability of HSP70, involved in NO- and IL-1b-dependent mechanisms of apoptosis [469].

Table 10.4 – Parameters of the enzymatic link of the thiol-disulfide system in the brain of female rats with VCD-hypoestrogenemia and against the background of pharmacological correction on the 29th day after treatment (M ± m)

Experimental group	GR, μm/g protein/min	GPR, μm/g protein/min
Intact control (IC)	29.3±3.7	61.4±4.0
Control pathology (CP)	8.31±0.64	39.5±3.7
CP + gel with R	12.2±2.7*	41.2±4.4
CP + gel with R and tablets with R	22.8±1.9*[1.2]	51.2±6.6 *[1]
CP + «Colpotrophine» cream	9.48±1.4	39.1±3.1

Note: * – $p < 0.05$ in relation the control pathology (CP)

[1] – $p < 0.05$ in relation to the group CP + “Colpotrophine” cream
[2] – $p < 0.05$ in relation to the group CP + gel with Resveratrol

In experiments *in vitro* (Table 10.4), conducted under the guidance of professor I.F. Belenichev, were determined that deprivation of the GSH level in neurons leads to a drop level of HSP70 as well under conditions of brain ischemia, as well as under the influence of toxic concentrations of steroids, nitrosamines, neurotransmitters, a correlation was found between the severity of neurological disorders and GSH deficiency and HSP70 in the brain of animals [473]. It has been shown that an increase in the concentration of the oxidized form of glutathione leads to increased production of pro-inflammatory cytokines TNF-α, IL-1β, IL-6, and IL-8, which, in its turn, can increase the deficiency of reduced glutathione due to disruption of its transport into the cell and enhance the mechanisms of neurodestruction [468].

Increased concentration of oxidized intermediates of the thiol-disulfide system suppresses the expression of eNOS [470] and increases the production of ROS due to the release of arachidonic acid from platelets, inhibits GPR and GR, and also stimulates many pathways of intracellular signaling, including neuroapoptosis [474]. Course treatment with vaginal cream Colpotrophine did not have an expressed effect on the performance of the thiol-disulfide system. Course treatment with vaginal gel with resveratrol led to a significant increase in reduced thiol groups by 24.7 % against the background of an increase in the reduced form of glutathione by 27.3 % and a decrease in its oxidized form by 19.1 % in the cytosol of the brain of premenopausal females.

In groups of animals with experimental hypoestrogenemia during treatment with a gel with resveratrol, a significant increase in GR activity by 46.8 % in the cytosolic fraction of the brain was observed compared with untreated animals. The administration of the gel with resveratrol did not affect the activity of GPR [473]. The course combined use of the gel with resveratrol and resveratrol tablets led to a significant increase in GR activity by 174 % and GP by 29.6 % against the background of an increase in the level of reduced glutathione by 110.2% and a decrease in its oxidized form by 49.3 %, as well as an increase in the content of reduced thiols by 103.4 % and a decrease in oxidized SS-groups by 36.2 % in the

cytosol of the brain of female rats with VCD-hypoestrogenemia compared with the group of untreated animals [471].

Increasing the functionality of the glutathione system, as well as increasing the activity of GSH enzymes, not only leads to increased protection of the brain from neurotoxic products of oxidative and nitrosative stress (reduction of nitrotyrosine in mitochondria and cytosol) but can also cause GSH-dependent mechanisms of endogenous neuroprotection due to increased expression of HSP70 [404].

Increasing the functionality of the thiol-disulfide system in VCD hypoestrogenemia and increasing its recovery of intermediates, under the influence of vaginal and intraventricular resveratrol, can contribute to an increase in the bioavailability of NO due to the formation of nitrosotiols and an increase in the expression of mRNA eNOS, as well as a decrease in the conversion of NO into cytotoxic peroxynitolol. In previous studies, we have shown the possible effects of resveratrol on HSP70-dependent mechanisms of the endogenous neuroprotector [413].

It is possible that resveratrol, modulating the expression of the subtotal transcription factor NFkappa-B, can increase the concentration of HSP70 and GSH in the brain and inhibit neuroapoptosis through the Fas/Apo-1 trigger mechanism [475].

Resveratrol, due to its biochemical properties and features of its chemical structure, can suppress ROS/ IL-1b-dependent mechanisms of iNOS expression, as well as the accumulation of nitrotyrosine and markers of oxidative protein modification, thereby reducing the change in the thiol-disulfide system of the brain towards its proapoptotic oxidative forms [475].

We have established for the first time that the modeling of hypoestrogenemia in female rats by 15 day administration of VCD leads to disruption in the conjugated systems of the brain – thiol-disulfide and nitroxidergic

Evaluation of the neuroprotective and antioxidant effects of a course of 28-day administration of vaginal gel Colpotrophine, vaginal gel with resveratrol, as well as a combination of gel and resveratrol tablets in female rats with

hypoestrogenemia revealed the presence of the above effects only when resveratrol was administered in dosage forms. The most expressed effect was observed with the combined use of tablets and gel with Resveratrol.

In the mechanism of the neuroprotective and antioxidant action of resveratrol is its ability to normalize the nitroxidergic system – to reduce the expression of iNOS mRNA and increase the expression of eNOS mRNA, inhibiting nitrosating stress, and also to increase the activity of the glutathione unit of the thiol-disulfide system (the level of reduced glutathione and GR activity) in the brain

The obtained results confirm the expediency of elaboration of a new vaginal gel with a combined composition of resveratrol and hyaluronic acid as an alternative to hormone-containing drugs for the prevention and treatment of pathological hypoestrogenic conditions that rise against the background of estrogen deficiency.

GENERAL CONCLUSIONS

For many years, medicinal plants known as phytodrugs have been used in nontraditional medicine practices worldwide. Nowadays, there is a growing interest in natural medicinal ingredients, making these plants even more popular. These plants contain a rich source of bioactive natural compounds that have gained worldwide attention. One of the medical issues without a proven treatment is premenstrual syndrome. Calendula drugs have anti-inflammatory, bactericidal, hypotensive, sedative, and cardiotonic effects, making them useful in gynecology as vaginal douche, infusion, and tincture. They are also used in oral cavity and throat diseases. Common toadflax seeds, roots, and leaves are used in practical medicine for gastroenterological, urological, and gynecological conditions. The crushed seeds are also used in cases of alopecia and pediculosis. Yellow bedstraw has diuretic, anti-inflammatory, analgesic, hemostatic, depurative, wound-healing, and sedative effects. Garden parsley has roots and leaves used in medical practice, with its medicinal agents used for treating urology, gastroenterology, gynecology, alopecia, and pediculosis.

Meadowsweet can positively affect gastrointestinal diseases by reducing spasms and promoting healing. It also has astringent properties and can be used for hemostatic, diaphoretic, and diuretic activities. Celery's roots, herbs, and seeds can enhance physical and intellectual work capacity and increase overall body tone. Its medicinal properties include antiflatulent, diuretic, laxative, and antiallergic effects. A Chaste tree's leaves, flowers, fruits, and branches are used for medicinal purposes. Its flavonoids have pharmacological benefits, including antioxidant, anti-inflammatory, and antihypoglycemic effects. *Vitex agnus-castus* mainly consists of fruits, leaves, and bark, and its extract has progesterone-like effects. Its drugs can be used for menstrual irregularities and mastopathy.

Plants have multiple therapeutic effects, including sustainable compositions like Tazalok oral drops and other drugs. Medicinal agents can improve hormone replacement therapy, while diseases of the hepatobiliary system are widespread

worldwide. A combination of ursodeoxycholic acid, artichoke leaf extract, and taurine has gained popularity in the preliminary stages, and Chinese authors have since added *Angelica sinensis* extract to the mix. Ursodeoxycholic acid is present in small amounts in human bile and exhibits cytoprotective action on hepatocytes and cholangiocytes. Its high molecule molarity allows it to form nontoxic mixed micelles with apolar toxic bile acids, reducing gastric reflux damage to cells. Additionally, taurine binds with bile acids in the liver to emulsify fats and has hepatoprotective, cardiotonic, and hypotensive properties. It also affects chronic heart failure and type 2 diabetes mellitus.

The third ingredient in this drug is an extract from the leaves of the *Cynara scolymus* plant, commonly known as an artichoke. It has several beneficial effects on the body, such as promoting bile secretion, increasing urine production, protecting the liver, and reducing lipid levels. The fourth active ingredient is an extract from *Angelica sinensis*, a plant used for medicinal and culinary purposes for centuries. It has pain-relieving properties, promotes bowel movement, and helps regulate menstrual cycles.

The monograph contains data about plant-based medicines used to treat viral and respiratory infections. Unfortunately, current treatments for viral infections are mainly focused on relieving symptoms. However, various authors have conducted studies on the pathogenesis of influenza and acute respiratory viral diseases. Homeopathic treatments have been expanded due to the development of complex remedies created in labs. These remedies usually combine homeopathic remedies with biogenic and mineral components to treat colds and the flu. *Aconitum napellus* and *Atropa belladonna* are commonly used in various complex homeopathic remedies to treat upper respiratory tract infections at different dilution levels. There has also been a study on the effects of homeopathic drugs of aconite on leucocyte cells. Other homeopathic remedies of the group A include *Aconitum napellus*, *Apis mellifica*, *Belladonna*, *Capsicum*, *Chamomilla* and others. For the treatment of tonsillitis, it is recommended to use homeopathic remedies such as *Ignatia amara*, *Lycopodium clavatum*, *Natrium muriaticum*, etc. Some homeopathic drugs, such as

Bryonia and *Apis mellifica*, have anti-inflammatory and analgesic effects. The current state of antiviral herbal drugs was also discussed.

One of the chapters of the monograph focuses on a complex herbal medicine agent with sedative and cardioprotective properties. The phytodrug Carvelis contains a component that influences psycho-emotional function and may normalize vegetative-vascular dystonia. Motherwort has activating effects on the GABA, making it an effective mild anti-anxiety and hypnotic agent. Valeriana, on the other hand, has a sedative effect and can restore emotional balance, reduce headaches and feelings of anxiety. Melissa is a psycho-emotional stabilizer and a cognitive activator. The complex of phytodrugs may help treat functional disorders, chronic fatigue syndrome among youth, age-related cardiovascular changes, and decreased exercise tolerance in elderly patients. The authors also emphasized the use of plant extracts in the form of rectal suppositories for more rapid action. They recommended the use of Pravenor in suppositories to decrease its toxicity. Finally, the monograph discusses the drug resveratrol found in grapes. This medicinal agent has many protective effects on all systems and organs. It was found that, during menopause, there were changes not only in the urogenital system but also in the activity of the cardiovascular and nervous systems. The administration of a gel with resveratrol tablets normalized hypoestrogenemia in female rats and had a favorable impact on the nervous and cardiovascular systems. In conclusion, the monograph emphasizes the popularity of phytodrugs in developed countries due to their effectiveness and lower toxicity than traditional drugs.

REFERENCE LIST

1. Altemus M. Neuroendocrine networks and functionality. Medical Clinics of North America [Internet]. 2019 Jul 1;103(4):601–12. Available from: https://doi.org/10.1016/j.mcna.2019.03.003

2. Syan SK, Minuzzi L, Costescu D, Smith M, Allega OR, Coote M, et al. Influence of Endogenous Estradiol, Progesterone, Allopregnanolone, and Dehydroepiandrosterone Sulfate on Brain Resting State Functional Connectivity Across the Menstrual Cycle. Fertility and Sterility [Internet]. 2017 May 1;107(5):1246-1255.e4. Available from: https://doi.org/10.1016/j.fertnstert.2017.03.021

3. Yu J. Endocrine disorders and the neurologic manifestations. Annals of Pediatric Endocrinology & Metabolism [Internet]. 2014 Jan 1;19(4):184. Available from: https://doi.org/10.6065/apem.2014.19.4.184

4. Rezaei M, Javadmoosavi SY, Mansouri B, Azadi NA, Mehrpour O, Nakhaee S. Thyroid dysfunction: how concentration of toxic and essential elements contribute to risk of hypothyroidism, hyperthyroidism, and thyroid cancer. Environmental Science and Pollution Research [Internet]. 2019 Nov 8;26(35):35787–96. Available from: https://doi.org/10.1007/s11356-019-06632-7

5. Gorchakova N, Bondarenko L, Galkin A. Obgruntuvannia bezpechnosti kombinovanoho zastosuvannia ursodezoksykholevoi kysloty, taurynu ta ekstraktu artyshoku pry zakhvoriuvanniakh hepatobiliarnoi systemy. [Grounds of safety use of ursodezoxyholic acid, taurine and extractum folii artishoke combination in hepatobiliary system diseases (Literature review)]. Phitoterapia [Fitoterapia]. 2017;2:7–12. Available from: http://www.phytotherapy.vernadskyjournals.in.ua/journal/2017/2/4.pdf

6. Gorchakova N, Gots T, Galkin A. Farmakoterapevtychne obgruntuvannia zastosuvannia likarskykh roslyn v endokrynnii hinekolohii (Ohliad literatury). ["Pharmacotherapeutic substantiation of medical plants in endocrine ginecology (Literature review)."]. Phitoterapia [Fitoterapia]. 2017;3:6–14. Available from: hhttp://www.phytotherapy.vernadskyjournals.in.ua/journal/2017/3/4.pdf

7. Givol O, Kornhaber R, Visentin D, Cleary M, Haik J, Harats M. A systematic review of Calendula officinalis extract for wound healing. Wound Repair and Regeneration [Internet]. 2019 Jun 20;27(5):548–61. Available from: https://doi.org/10.1111/wrr.12737

8. Simões FV, Santos VO, Da Silva RN, Da Silva RC. Effectiveness of skin protectors and calendula officinalis for prevention and treatment of radiodermatitis: an integrative review. Revista Brasileira De Enfermagem [Internet]. 2020 Jan 1;73(suppl 5). Available from: https://doi.org/10.1590/0034-7167-2019-0815

9. Siddiquee S, McGee MA, Vincent A, Giles E, Clothier R, Carruthers S, et al. Efficacy of topical Calendula officinalis on prevalence of radiation-induced dermatitis: A randomised controlled trial. Australasian Journal of Dermatology [Internet]. 2020 Sep 23;62(1). Available from: https://doi.org/10.1111/ajd.13434

10. Wachtel-Galor S, Benzie IFF. Herbal medicine: an introduction to its history, usage, regulation, current trends and research needs. In: CRC Press, Taylor and Francis Group eBooks [Internet]. 2011. Available from: https://europepmc.org/article/MED/22593939

11. Qu L, Zhou W, Wang Y, Wang M. European regulation model for herbal medicine: The assessment of the EU monograph and the safety and efficacy evaluation in marketing authorization or registration in Member States. Phytomedicine [Internet]. 2018 Mar 1;42:219–25. Available from: https://doi.org/10.1016/j.phymed.2018.03.048

12. Kuang W, Zhang X, Lan Z. Flavonoids extracted from Linaria vulgaris protect against hyperlipidemia and hepatic steatosis induced by western-type diet in mice. Archives of Pharmacal Research [Internet]. 2017 Aug 2;41(12):1190–8. Available from: https://doi.org/10.1007/s12272-017-0941-y

13. Zorić A, Morina F, Toševski I, Tosti T, Jović J, Krstić O, et al. Resource allocation in response to herbivory and gall formation in Linaria vulgaris. Plant Physiology and Biochemistry [Internet]. 2019 Feb 1;135:224–32. Available from: https://doi.org/10.1016/j.plaphy.2018.11.032

14. Falzon CC, Balabanova A. Phytotherapy. Primary Care: Clinics in Office Practice [Internet]. 2017 Jun 1;44(2):217–27. Available from: https://doi.org/10.1016/j.pop.2017.02.001

15. Jachuła J, Konarska A. Micromorphological and histochemical attributes of flowers and floral reward in Linaria vulgaris (Plantaginaceae). Protoplasma [Internet]. 2018 Jun 3;255(6):1763–76. Available from: https://doi.org/10.1007/s00709-018-1269-2

16. Salmerón-Manzano E, Garrido-Cardenas JA, Manzano-Agugliaro F. Worldwide Research Trends on Medicinal Plants. Int J Environ Res Public Health. 2020 May 12;17(10):3376. Available from: https://doi.org/10.3390/ijerph17103376

17. Arsenijevic N, Selaković D, Stanković JSK, Mihailović V, Mitrović S, Milenkovic J, et al. Variable neuroprotective role of Filipendula ulmaria extract in rat hippocampus. Journal of Integrative Neuroscience [Internet]. 2021 Dec 30;20(4):871–83. Available from: https://doi.org/10.31083/j.jin2004089

18. Šćepanović R, Selaković D, Stanković JSK, Arsenijevic N, Andjelkovic M, Milenkovic J, et al. The Antioxidant Supplementation with Filipendula ulmaria Extract Attenuates the Systemic Adverse Effects of Nanosized Calcium Phosphates in Rats. Oxidative Medicine and Cellular Longevity [Internet]. 2021 Aug 17;2021:1–16. Available from: https://doi.org/10.1155/2021/8207283

19. Sukhikh S, Ivanova S, Skrypnik L, Bakhtiyarova A, Larina V, Krol OV, et al. Study of the Antioxidant Properties of Filipendula ulmaria and Alnus glutinosa. Plants [Internet]. 2022 Sep 16;11(18):2415. Available from: https://doi.org/10.3390/plants11182415

20. Bone K, Mills S. Principles and Practice of Phytotherapy: Modern Herbal Medicine, 2nd edition. Churchill Livingstone/Elsevier, [Internet]. 2013. Available from: Borum MML, Fromm H. Expression of HLA molecules on hepatocytes: Does ursodeoxycholic acid prevent immunologic injurY? Gastroenterology [Internet]. 1990. Available from: https://www.sciencedirect.com/book/9780443069925/principles-and-practice-of-phytotherapy#book-info

21. Bradić J, Živković V, Srejović I, Jakovljević V, Petković A, Turnic TN, et al. Protective Effects ofGalium verum L.Extract against Cardiac Ischemia/Reperfusion Injury in Spontaneously Hypertensive Rats. Oxidative Medicine and Cellular Longevity [Internet]. 2019 Feb 4;2019:1–11. Available from: https://doi.org/10.1155/2019/4235405

22. Tava A, Biazzi E, Ronga D, Avato P. Identification of the Volatile Components of Galium verum L. and Cruciata leavipes Opiz from the Western Italian Alps. Molecules [Internet]. 2020 May 16;25(10):2333. Available from: https://doi.org/10.3390/molecules25102333

23. Vuletic M, Jakovljević V, Živanović S, Papić M, Papic M, Mladenović R, et al. The evaluation of healing properties of Galium verum-Based oral gel in aphthous stomatitis in rats. Molecules [Internet]. 2022 Jul 22;27(15):4680. Available from: https://doi.org/10.3390/molecules27154680

24. De Kruse Villas Bôas G, Gadelha CAG. Oportunidades na indústria de medicamentos e a lógica do desenvolvimento local baseado nos biomas brasileiros: bases para a discussão de uma política nacional. Cadernos De Saude Publica

[Internet]. 2007 Jun 1;23(6):1463–71. Available from: https://doi.org/10.1590/s0102-311x2007000600021

25. Harnyk TP, Kniazevych VM, Tumanov VA Osnovy farmakohnozii i fitoterapii [Basics of pharmacognosy and phytotherapy.]. Zhytomyr: Ruta, 2015, 250 p.

26. Khalil NS a. M, Abou-Elhamd AS, Wasfy SIA, Mileegy IMHE, Hamed MY, Ageely H. Antidiabetic and Antioxidant Impacts of Desert Date (Balanites aegyptiaca) and Parsley (Petroselinum sativum) Aqueous Extracts: Lessons from Experimental Rats. Journal of Diabetes Research [Internet]. 2016 Jan 1;2016:1–10. Available from: https://doi.org/10.1155/2016/8408326

26. Kotb MA. Molecular Mechanisms of Ursodeoxycholic Acid Toxicity & Side Effects: Ursodeoxycholic Acid Freezes Regeneration & Induces Hibernation Mode. International Journal of Molecular Sciences [Internet]. 2012 Jul 17;13(7):8882–914. Available from: https://doi.org/10.3390/ijms13078882

27. Goulas V, Banegas-Luna AJ, Constantinou A, Pérez-Sánchez H, Barbouti A. Computation Screening of Multi-Target antidiabetic properties of phytochemicals in common edible Mediterranean plants. Plants [Internet]. 2022 Jun 21;11(13):1637. Available from: https://doi.org/10.3390/plants11131637

28. Wong P, Kitts DD. Studies on the dual antioxidant and antibacterial properties of parsley (Petroselinum crispum) and cilantro (Coriandrum sativum) extracts. Food Chemistry [Internet]. 2006 Aug 1;97(3):505–15. Available from: https://doi.org/10.1016/j.foodchem.2005.05.031

29. Foudah AI, Alqarni MH, Alam A, Salkini MA, Alam P, Alkholifi FK, et al. Determination of Chemical Composition, *In Vitro* and *In Silico* Evaluation of Essential Oil from Leaves of Apium graveolens Grown in Saudi Arabia. Molecules [Internet]. 2021 Dec 4;26(23):7372. Available from: https://doi.org/10.3390/molecules26237372

30. Jambor T, Árvay J, Tvrdá E, Kováčik A, Greifová H, Lukáč N. The effect of Apium Graveolens L., Levisticum Officinale and Calendula Officinalis L. on cell viability, membrane integrity, steroidogenesis, and intercellular communication in mice Leydig cells *in vitro*. Physiological Research [Internet]. 2021 Aug 31;615–25. Available from: https://doi.org/10.33549/physiolres.934675

31. Kooti W, Daraei N. A Review of the Antioxidant Activity of Celery (Apium graveolens L). Journal of Evidence-Based Complementary & Alternative Medicine [Internet]. 2017 Jul 13;22(4):1029–34. Available from: https://doi.org/10.1177/2156587217717415

32. Chen SN, Friesen JB, Webster DE, Nikolić D, Van Breemen RB, Wang ZJ, et al. Phytoconstituents from Vitex agnus-castus fruits. Fitoterapia [Internet]. 2011 Jun 1;82(4):528–33. Available from: https://doi.org/10.1016/j.fitote.2010.12.003

33. Qi Y, Zhang Q, Zheng C, Wang Y, Qin LP. Casticin, a flavonoid isolated from Vitex rotundifolia, inhibits prolactin release *in vivo* and *in vitro*. Acta Pharmacologica Sinica [Internet]. 2010 Nov 1;31(12):1564–8. Available from: https://doi.org/10.1038/aps.2010.178

34. Zamani M, Neghab N, Torabian S. Therapeutic effect of Vitex agnus castus in patients with premenstrual syndrome. PubMed [Internet]. 2012 Jan 1;50(2):101–6. Available from: https://pubmed.ncbi.nlm.nih.gov/22359078

35. Heskes AM, Sundram TCM, Boughton BA, Jensen NB, Hansen NL, Crocoll C, et al. Biosynthesis of bioactive diterpenoids in the medicinal plant Vitex agnus-castus. Plant Journal [Internet]. 2018 Feb 14;93(5):943–58. Available from: https://doi.org/10.1111/tpj.13822

36. Naseri R, Farnia V, Yazdchi K, Alikhani M, Basanj B, Salemi S. Comparison of Vitex agnus-castus Extracts with Placebo in Reducing Menopausal Symptoms: A Randomized Double-Blind Study. Korean Journal of Family Medicine [Internet]. 2019 Nov 20;40(6):362–7. Available from: https://doi.org/10.4082/kjfm.18.0067

37. Whocc. WHOCC - ATC/DDD Index. www.whocc.no/atc_ddd_index.

38. Tarasenko, Tetiana, et.al. Doslidzhennia antyestrohennoi aktyvnosti fitopreparatu Tazalok [Study of the anti-estrogenic activity of the herbal preparation Tazalok]. Farmakolohiia ta likarska toksykolohiia [Pharmacology and medicinal toxicology], 2011;6(25): 30-35.

39. Yonkers KA, O'Brien PS, Eriksson E. Premenstrual syndrome. The Lancet [Internet]. 2008 Apr 1;371(9619):1200–10. Available from: https://doi.org/10.1016/s0140-6736(08)60527-9

40. Nees LK, Heublein S, Steinmacher S, Juhasz-Böss I, Brucker S, Tempfer C, et al. Endometrial hyperplasia as a risk factor of endometrial cancer. Archives of Gynecology and Obstetrics [Internet]. 2022 Jan 10;306(2):407–21. Available from: https://doi.org/10.1007/s00404-021-06380-5

41. Franchuk AA. Likuvannia peredmenstrualnoho syndromu z vykorystanniam fitopreparatu Tazalok [Treatment of premenstrual syndrome with the use of phytopreparation Tazalok]. Medychna khimiia [Medical Chemistry]. 2012;14(4):130-33. Available from: http://nbuv.gov.ua/UJRN/Med_chim_2012_14_4_30

42. Tatarchuk TF, Kaluhyna LV, Efymenko OA Opyt lechenyia mastalhyy u patsyentok s dyshormonalnymy zabolevanyiamy molochnoi zhelezy [Experience of mastalgia treatment in patients with dyshormonal breast diseases.] Reproductive Endocrinology 2012 July;5:63, Available from: https://doi.org/10.18370/2309-4117.2012.5.63-68.

43. Kyshakevych, IT, Konar RS. Correction of dismetabolic manifestations in perimenopausal and early postmenopausal women with fibrocystic disease. Reproductive Endocrinology [Internet]. 2016 Sep 30;0(30):82. Available from: https://doi.org/10.18370/2309-4117.2016.30.82-86

44. Tatarchuk TF, Ganzhiy IYu, Pedachenko NYu, Kapshuk IM . Modern nonhormonal possibilities of treatment of polycystic ovary syndrome in obese women. Reproductive Endocrinology [Internet]. 2013 Nov 17;0(13):14. Available from: https://doi.org/10.18370/2309-4117.2013.13.14-21

45. Lee SS, Kim DH, Nam GE, Nam HY, Kim YE, Lee SH, et al. Association between Metabolic Syndrome and Menstrual Irregularity in Middle-Aged Korean Women. Korean Journal of Family Medicine [Internet]. 2016 Jan 1;37(1):31. Available from: https://doi.org/10.4082/kjfm.2016.37.1.31

46. Badawy A, Elnashar A. Treatment options for polycystic ovary syndrome. International Journal of Women's Health [Internet]. 2011 Feb 1;25. Available from: https://doi.org/10.2147/ijwh.s11304

47. Gainder S, Sharma B. Update on management of polycystic ovarian syndrome for dermatologists. Indian Dermatology Online Journal [Internet]. 2019 Jan 1;10(2):97. Available from: https://doi.org/10.4103/idoj.idoj_249_17

48. Pankiv VI. The influence of thyroid disorders upon the female reproductive function. modern possibilities of phytotherapy. Reproductive Endocrinology [Internet]. 2016 Apr 5;0(27):52. Available from: https://doi.org/10.18370/2309-4117.2016.27.52-57

49. Gerasimova T, Gopchuk EN. Korrektsiya nedostatochnosti lyuteinovoy fazy u zhenshchin reproduktivnogo vozrasta na fone gipotireoza [Correction of luteal phase deficiency in women of reproductive age against the background of hypothyroidism]. Reproductive Endocrinology [Internet]. 2014 Jan 1;(19):98–101. Available from: http://reproduct-endo.com/article/view/38068/0

50. Pyrohova V, Shurliak S. Selektyvnye fytomolekuly v kombynyrovannoi terapyy dobrokachestvennykh dyshormonalnykh prolyferatyvnykh zabolevanyi zhenskoi reproduktyvnoi systemy pry dysfunktsyy shchytovydnoi zhelezy [Selective phytomolecules in the combined therapy of benign dyshormonal proliferative

diseases of the female reproductive system in thyroid dysfunction]. Zdorove zhenshchyny [Women's health]. 2014;5:12-20.

51. Potapov V, Polshchykov P. Finkova Ye. Tarhetnye mekhanyzmy antyprolyferatyvnoho effekta rastytelnoho preparata Tazalok pry leiomyome matky [Target mechanisms of antiproliferative effect of herbal drug Tazalok in uterine leiomyoma]. Zdorove zhenshchyny [Women's health]. 2013;8:38-41. Available from: http://nbuv.gov.ua/UJRN/Zdzh_2013_8_10

52. Beuers U, Trauner M, Jansen PLM, Poupon R. New paradigms in the treatment of hepatic cholestasis: From UDCA to FXR, PXR and beyond. Journal of Hepatology [Internet]. 2015 Apr 1;62(1):S25–37. Available from: https://doi.org/10.1016/j.jhep.2015.02.023

53. Uvarova KG, Saprykina MM, Ahmed MA, Oyebamire IB , Makienko NV , Maltseva MS. Polymorbidity in clinical practice. Journal of V. N. Karazin` KhNU. 2014;1141:48-51. Available from: https://cyberleninka.ru/article/n/polymorbidity-in-clinical-practice/viewer

54. Tomeno W, Kawashima K, Yoneda M, Saito S, Ogawa Y, Honda Y, et al. Non-alcoholic fatty liver disease comorbid with major depressive disorder: The pathological features and poor therapeutic efficacy. Journal of Gastroenterology and Hepatology [Internet]. 2015 Apr 27;30(6):1009–14. Available from: https://doi.org/10.1111/jgh.12897

55. Anokhina GA., Kharchenko VV, Opanasiuk ND, Lopukh IYa, Yakubovska IO. Nekotorye aspekty primeneniya preparatov ursodezoksikholevoy kisloty v sochetanii s ekstraktami rasteniy v lechenii zabolevaniy gepatobiliarnoy sistemy [Some aspects of the use of preparations of ursodeoxycholic acid, combined with plant extracts, in the treatment of the diseases of the hepatobiliary system]. Suchasna Gastroenterologiya [Modern Gastroenterology] [Internet]. 2014;1(75):49–54. Available from: http://www.vitapol.com.ua/user_files/pdfs/gastro/gas75i07.pdf

56. Zvyagintseva TD, Hrydneva SV. Mekhanyzmy Razvytyia Y Podkhody K Lechenyiu Dysfunktsyy Sfynktera Oddy Posle Kholetsystэktomyy [The Mechanisms of Development and Approaches to the Treatment of Sphincter of Oddi Dysfunction After Cholecystectomy]. Hastroenterolohiia [Gastroenterology]. 2014; 2:77–81. Available from: http://nbuv.gov.ua/UJRN/gastro_2014_2_14

57. Zviahyntseva, TD, Hamanenko YaK. Terapevtycheskye vozmozhnosty kholoplanta v lechenyy dysfunktsyy sfinktera Oddy posle kholetsystэktomyy ["Therapeutic options of holoplant in the treatment of sphincter of Oddi dysfunction after cholecystectomy"] Hastroenterolohiia [Gastroenterology]. 2014;54(4):117–21. Available from: http://nbuv.gov.ua/UJRN/gastro_2014_4_11

58. Bondarenko L, Gorchakova NA, Golembiovska O, Galkin AYu. Promising new fixed combination for the treatment of diseases of the hepatobiliar system: Substantiation of pharmacotherapeutic properties and pharmaceutical quality profile. Regulatory Mechanisms in Biosystems [Internet]. 2018 Apr 14;9(1):23–40. Available from: https://doi.org/10.15421/021804

59. Radchenko O. Ursodezoksykholevaia kyslota: dosiahnennia, perspektyvy ta problemy zastosuvannia. Ratsyonalnaia farmakoterapyia [Rational pharmacotherapy]. 2012;23(2):28–32. Available from: https://rpht.com.ua/ua/archive/2012/2%2823%29/pages-28-32/ursodezoksiholeva-kislota-dosyagnennya-perspektivi-ta-problemi-zastosuvannya

60. Derzhavnyi Formuliar Likarskykh [State Formulary of Medicinal Products] [Internet]. Available from: https://www.dec.gov.ua/materials/chinnij-vipusk-derzhavnogo-formulyara-likarskih-zasobiv/?role=ua

61. Van Der Feen C, Van Der Doef HPJ, Van Der Ent CK, Houwen RHJ. Ursodeoxycholic acid treatment is associated with improvement of liver stiffness in

cystic fibrosis patients. Journal of Cystic Fibrosis [Internet]. 2016 Nov 1;15(6):834–8. Available from: https://doi.org/10.1016/j.jcf.2016.07.009

62. Lazaridis KN, Gores GJ, Lindor KD. Ursodeoxycholic acid 'mechanisms of action and clinical use in hepatobiliary disorders.' Journal of Hepatology [Internet]. 2001 Jul 1;35(1):134–46. Available from: https://doi.org/10.1016/s0168-8278(01)00092-7

63. Jackson HK, Solaymani-Dodaran M, Card TR, Aithal GP, Logan RF, West J. Influence of ursodeoxycholic acid on the mortality and malignancy associated with primary biliary cirrhosis: A population-based cohort study. Hepatology [Internet]. 2007 Jan 1;46(4):1131–7. Available from: https://doi.org/10.1002/hep.21795

64. Lindor KD, Kowdley KV, Luketic VA, Harrison ME, McCashland TM, Befeler AS, et al. High-dose ursodeoxycholic acid for the treatment of primary sclerosing cholangitis. Hepatology [Internet]. 2009 May 20;50(3):808–14. Available from: https://doi.org/10.1002/hep.23082

65. Anokhina GA, Kharchenko VV. Vpliv preparativ zhovchnikh kislot ta lipidniy obmin u khvorikh z pislyakholetsistektomichnim sindromom [The effect of bile acid drugs and lipid metabolism in patients with postcholecystectomy syndrome]. Suchasna Gastroenterologiya [Modern Gastroenterology] [Internet]. 2016;2, 2016:29–34. Available from: http://www.irbis-nbuv.gov.ua/cgi-bin/irbis_nbuv/cgiirbis_64.exe?I21DBN=LINK&P21DBN=UJRN&Z21ID=&S21REF=10&S21CNR=20&S21STN=1&S21FMT=ASP_meta&C21COM=S&2_S21P03=FILA=&2_S21STR=SGastro_2016_2_6

66. Shcherbynyna M. Zabolevanyia pecheny s kholestatycheskym syndromom, bolezny zhelchnoho puzyria y ursodezoksykholevaia kyslota. Novые aktsenty [Liver diseases with cholestatic syndrome, gall bladder diseases and ursodeoxycholic acid. New accents] Suchasna hastroenterolohiia [Modern gastroenterology]. 2016;2:78–86.

67. Van Stijn MFM, Vermeulen M a. R, Siroen MPC, Wong LN, Van Den Tol MP, Ligthart-Melis GC, et al. Human taurine metabolism: fluxes and fractional extraction rates of the gut, liver, and kidneys. Metabolism [Internet]. 2012 Jul 1;61(7):1036–40. Available from: https://doi.org/10.1016/j.metabol.2011.12.005

68. Rezvan V, Vasilieva IS. The role of metabolic therapy in modern cardiology. Kardiologiya [Internet]. 2016 May 25; Available from: https://doi.org/10.18565/cardio.2016.5.76-80

69. Sagara M, Murakami S, Mizushima S, Liu L, Mori M, Ikeda K, et al. Taurine in 24-h urine samples is inversely related to cardiovascular risks of middle aged subjects in 50 populations of the world. In: Advances in Experimental Medicine and Biology [Internet]. 2015. p. 623–36. Available from: https://doi.org/10.1007/978-3-319-15126-7_50

70. Gebara E, Udry F, Sultan S, Toni N. Taurine increases hippocampal neurogenesis in aging mice. Stem Cell Research [Internet]. 2015 May 1;14(3):369–79. Available from: https://doi.org/10.1016/j.scr.2015.04.001

71. Toyoda A, Koike H, Nishihata K, Iio W, Goto T. Effects of chronic taurine administration on gene expression, protein translation and phosphorylation in the rat hippocampus. In: Springer eBooks [Internet]. 2015. p. 473–80. Available from: https://doi.org/10.1007/978-3-319-15126-7_37

72. Gu Y, Zhao Y, Ke Q, Sun M. Taurine attenuates hippocampal and corpus callosum damage, and enhances neurological recovery after closed head injury in rats. Neuroscience [Internet]. 2015 Apr 1;291:331–40. Available from: https://doi.org/10.1016/j.neuroscience.2014.09.073

73. Statsenko ME, Turkina SV, Shilina NN, Vinnikova AA . Endothelium protecting properties of taurin in chronic heart failure with type 2 diabetes. Cardiovascular Therapy and Prevention [Internet]. 2016 May 11; Available from: https://doi.org/10.15829/1728-8800-2016-2-38-44

74. Chien CF, Wu YT, Tsai TH. Biological analysis of herbal medicines used for the treatment of liver diseases. Biomedical Chromatography [Internet]. 2010 Dec 28;25(1–2):21–38. Available from: https://doi.org/10.1002/bmc.1568

75. Okovytii C, Sukhanov DS, Romantsov MH. Hepatotropnye sredstva: Sovremennoe sostoianye problemy. [Hepatotropic agents: Current status of the problem] Terapevtycheskyi arkhyv [Therapeutic archive]. 2012;2:62–68.

77. De Luca A, Pierno S, Camerino DC. Taurine: the appeal of a safe amino acid for skeletal muscle disorders. Journal of Translational Medicine [Internet]. 2015 Jul 25;13(1). Available from: https://doi.org/10.1186/s12967-015-0610-1

78. Tsvetkova D, Klisurov R, Pankova SA, Zlatkov BA. Investigation of some pharmacological effects of caffeine and taurine in food supplements. International Journal of Nutrition and Food Sciences [Internet]. 2015 Jan 1; Available from: https://doi.org/10.11648/j.ijnfs.s.2015040101.14

79. Salem MB, Affes H, Ksouda K, Dhouibi R, Sahnoun Z, Hammami S, et al. Pharmacological studies of artichoke leaf extract and their health benefits. Plant Foods for Human Nutrition [Internet]. 2015 Aug 27;70(4):441–53. Available from: https://doi.org/10.1007/s11130-015-0503-8.

80. Kulza M, Adamska K, Seńczuk-Przybyłowska M, Woźniak A, Wachowiak A, Miechowicz I, Horoszkiewicz M, Nowak G, Florek E. Karczoch zwyczajny--lek roślinny [Artichoke--herbal drug]. Przegl Lek. 2012;69(10):1122-6. Polish

81. Solá S, Castro RE, Kren BT, Steer CJ, Rodrigues CMP. Modulation of nuclear steroid receptors by ursodeoxycholic acid inhibits TGF-B1-Induced E2F-1/P53-Mediated apoptosis of rat hepatocytes. Biochemistry [Internet]. 2004 Jun 8;43(26):8429–38. Available from: https://doi.org/10.1021/bi049781x

82. Solá S, Amaral JD, Castro RE, Ramalho RM, Borralho PM, Kren BT, et al. Nuclear translocation of UDCA by the glucocorticoid receptor is required to reduce

TGF-β1-induced apoptosis in rat hepatocytes. Hepatology [Internet]. 2005 Jan 1;42(4):925–34. Available from: https://doi.org/10.1002/hep.20870

83. Weitzel C, Stark D, Kullmann F, Schölmerich J, Holstege A, Falk W. Ursodeoxycholic acid induced activation of the glucocorticoid receptor in primary rat hepatocytes. European Journal of Gastroenterology & Hepatology [Internet]. 2005 Feb 1;17(2):169–77. Available from: https://doi.org/10.1097/00042737-200502000-00007

84. Amaral JD, Solá S, Steer CJ, Rodrigues CMP. Role of nuclear steroid receptors in apoptosis. Current Medicinal Chemistry [Internet]. 2009 Oct 1;16(29):3886–902. Available from: https://doi.org/10.2174/092986709789178028

85. Solá S, Ma X, Castro RE, Kren BT, Steer CJ, Rodrigues CMP. Ursodeoxycholic Acid Modulates E2F-1 and p53 Expression through a Caspase-independent Mechanism in Transforming Growth Factor β1-induced Apoptosis of Rat Hepatocytes. Journal of Biological Chemistry [Internet]. 2003 Dec 1;278(49):48831–8. Available from: https://doi.org/10.1074/jbc.m300468200

86. Shi Q, Kong B, Ma K, Xiangli Z, Jiang SL. [Effects of ursodeoxycholic acid on the liver plasma membrane fluidity, hepatic glutathione concentration, hepatic estrogen receptors and progesterone receptors in pregnant rats with ethinylestradiol and progesterone induced intrahepatic cholestasis]. PubMed [Internet]. 2003 Nov 1;38(11):680–2. Available from: https://pubmed.ncbi.nlm.nih.gov/14728856

87. Sheibak, VM, Sheibak LN. Byolohycheskaia rol tauryna v orhanyzme mlekopytaiushchykh [Biological role of taurine in mammals] Медицинские новости [Medical News]. 2005;10:1-8. Available from: www.mednovosti.by/journal.aspx?article=1041.

88. Gohlke H, Schmitz B, Sommerfeld A, Reinehr R, Häussinger D. α_5 β_1 -integrins are sensors for tauroursodeoxycholic acid in hepatocytes. Hepatology [Internet]. 2012 Sep 17;57(3):1117–29. Available from: https://doi.org/10.1002/hep.25992

89. Lambert IH, Kristensen DM, Holm JB, Mortensen OH. Physiological role of taurine – from organism to organelle. Acta Physiologica [Internet]. 2014 Sep 12;213(1):191–212. Available from: https://doi.org/10.1111/apha.12365

90. Wittemer SM, Ploch M, Windeck T, Müller S, Drewelow B, Derendorf H, et al. Bioavailability and pharmacokinetics of caffeoylquinic acids and flavonoids after oral administration of Artichoke leaf extracts in humans. Phytomedicine [Internet]. 2005 Jan 1;12(1–2):28–38. Available from: https://doi.org/10.1016/j.phymed.2003.11.002

91. Mitsuyoshi H, Nakashima T, Inaba K, Ishikawa H, Nakajima Y, Sakamoto Y, et al. Ursodeoxycholic acid enhances Glucocorticoid-Induced tyrosine Aminotransferase-Gene expression in cultured RaT hepatocytes. Biochemical and Biophysical Research Communications [Internet]. 1997 Nov 1;240(3):732–6. Available from: https://doi.org/10.1006/bbrc.1997.7733

92. Úriz M, Sáez E, Prieto J, Medina JF, Bañales JM. Ursodeoxycholic Acid Is Conjugated with Taurine to Promote Secretin-Stimulated Biliary Hydrocholeresis in the Normal Rat. PLOS ONE [Internet]. 2011 Dec 14;6(12):e28717. Available from: https://doi.org/10.1371/journal.pone.0028717

93. Van Den Bossche L, Hindryckx P, Devisscher L, Devriese S, Van Welden S, Holvoet T, et al. Ursodeoxycholic acid and its taurine- or Glycine-Conjugated species reduce colitogenic dysbiosis and equally suppress experimental colitis in mice. Applied and Environmental Microbiology [Internet]. 2017 Apr 1;83(7). Available from: https://doi.org/10.1128/aem.02766-16

94. Gorchakova NA, Heimuller E, Galkin AYu. Current Safety Data of the Complex Herbal Medicine with Sedative and Cardioprotective Actions. Innovative Biosystems and Bioengineering [Internet]. 2018 Oct 2;2(3):163–74. Available from: https://doi.org/10.20535/ibb.2018.2.3.143029

95. Fillatre A, François C, Segard C, Duverlie G, Hecquet D, Pannier C, et al. Epidemiology and seasonality of acute respiratory infections in hospitalized children over four consecutive years (2012–2016). Journal of Clinical Virology [Internet]. 2018 May 1;102:27–31. Available from: https://doi.org/10.1016/j.jcv.2018.02.010

96. Tyrrell CSB, Allen JL, Carson G. Influenza and other emerging respiratory viruses. Medicine [Internet]. 2017 Dec 1;45(12):781–7. Available from: https://doi.org/10.1016/j.mpmed.2017.09.003

97. Renoux H. Methodology, importance, aims and objectives of provings carried out by homeopathic students. La Revue D'Homéopathie [Internet]. 2018 Mar 1; Available from: https://doi.org/10.1016/j.revhom.2018.01.022

98. Sarembaud A. Homeopathic therapy as everyday primary care. Observations and reflections. La Revue D'Homéopathie [Internet]. 2017 Sep 1;8(3):e43–6. Available from: https://doi.org/10.1016/j.revhom.2017.07.009

99. Bouvier NM, Palese P. The biology of influenza viruses. Vaccine [Internet]. 2008 Sep 1;26:D49–53. Available from: https://doi.org/10.1016/j.vaccine.2008.07.039

100. Matsuzaki Y, Katsushima N, Nagai Y, Miyake S, Itagaki T, Sakamoto M, et al. Clinical features of influenza C virus infection in children. The Journal of Infectious Diseases [Internet]. 2006 May 1;193(9):1229–35. Available from: https://doi.org/10.1086/502973

101. Belykh NA, Yesakova YM, Fokicheva NN, Piskunova MA, Martesheva LM, Kalashnikova ON, et al. Clinical and epidemiological characteristics of influenza in children of the Ryazan region in 2015–2016 season. Sovremennaâ Pediatriâ [Internet]. 2017 Mar 5;81(1):127–32. Available from: https://doi.org/10.15574/sp.2017.81.127

102. Brundage JF. Interactions between influenza and bacterial respiratory pathogens: implications for pandemic preparedness. Lancet Infectious Diseases [Internet]. 2006 May 1;6(5):303–12. Available from: https://doi.org/10.1016/s1473-3099(06)70466-2

103. Hageman J, Uyeki TM, Francis JS, Jernigan DB, Wheeler JG, Bridges CB, et al. Severe Community-acquired Pneumonia Due to*Staphylococcus aureus*, 2003–04 Influenza Season. Emerging Infectious Diseases [Internet]. 2006 Jun 1;12(6):894–9. Available from: https://doi.org/10.3201/eid1206.051141

104. Carrat F, Leruez-Ville M, Tonnellier M, Baudel JL, Deshayes J, Meyer P, et al. A virologic survey of patients admitted to a critical care unit for acute cardiorespiratory failure. Intensive Care Medicine [Internet]. 2005 Nov 19;32(1):156–9. Available from: https://doi.org/10.1007/s00134-005-2861-4

105. Kayne SB. Homeopathic pharmacy [Internet]. Elsevier eBooks. 2006. Available from: https://doi.org/10.1016/b978-0-443-10160-1.x5001-8

106. Michalsen A, Uehleke B, Stange R. Safety and compliance of a complex homeopathic drug (Contramutan N Saft) in the treatment of acute respiratory tract infections: A large observational (non-interventional) study in children and adults focussing on homeopathy specific adverse reactions versus adverse drug reactions. Regulatory Toxicology and Pharmacology [Internet]. 2015 Jul 1;72(2):179–84. Available from: https://doi.org/10.1016/j.yrtph.2015.04.002

107. Schmiedel V, Klein P. A complex homeopathic preparation for the symptomatic treatment of upper respiratory infections associated with the common cold: an observational study. Explore-the Journal of Science and Healing [Internet]. 2006 Mar 1;2(2):109–14. Available from: https://doi.org/10.1016/j.explore.2005.12.008

108. Klein SD, Wolf U. Comparison of homeopathic globules prepared from high and ultra-high dilutions of various starting materials by ultraviolet light spectroscopy.

Complementary Therapies in Medicine [Internet]. 2016 Feb 1;24:111–7. Available from: https://doi.org/10.1016/j.ctim.2015.12.017

109. De La Peña SS, Sothern RB, López FS, Mendoza-Lujambio I, Waizel-Bucay J, Sánchez CO, et al. Circadian aspects of hyperthermia in mice induced by Aconitum napellus. Pharmacognosy Magazine [Internet]. 2011 Jan 1;7(27):234. Available from: https://doi.org/10.4103/0973-1296.84238

110. Bellavite P, Magnani P, Marzotto M, Conforti A. Assays of homeopathic remedies in rodent behavioural and psychopathological models. Homeopathy [Internet]. 2009 Oct 1;98(4):208–27. Available from: https://doi.org/10.1016/j.homp.2009.09.005

111. Mishra N, Muraleedharan K, Paranjpe AS, Munta DK, Singh H, Nayak C. An exploratory study on scientific investigations in homeopathy using medical analyzer. Journal of Alternative and Complementary Medicine [Internet]. 2011 Aug 1;17(8):705–10. Available from: https://doi.org/10.1089/acm.2010.0334

112. Abud APR, Cesar B, Cavazzani LFM, De Oliveira CC, Gabardo J, De Freitas Buchi D. Activation of bone marrow cells treated with Canova *in vitro*. Cell Biology International [Internet]. 2006 Oct 1;30(10):808–16. Available from: https://doi.org/10.1016/j.cellbi.2006.06.011.

113. De Oliveira CC, De Oliveira SM, De Godoy LMF, Gabardo J, De Freitas Buchi D. Canova, a Brazilian medical formulation, alters oxidative metabolism of mice macrophages. Journal of Infection [Internet]. 2006 Jun 1;52(6):420–32. Available from: https://doi.org/10.1016/j.jinf.2005.08.017

114. Friese KH, Kruse S, Lüdtke R, Moêller H. The homoeopathic treatment of otitis media in children--comparisons with conventional therapy. PubMed [Internet]. 1997 Jul 1;35(7):296–301. Available from: https://pubmed.ncbi.nlm.nih.gov/9247843

115. Thurneysen A. On: Piltan D, Rist L, Simões-Wüst A, Saller R: Test of a homeopathic dilution of Aconitum napellus. A clinical, randomized, double-blind, controlled crossover study in healthy volunteers. Forsch Komplementmed 2009;16:168–173. Forschende Komplementärmedizin = [Internet]. 2009 Jan 1;16(5):349. Available from: https://doi.org/10.1159/000243809

116. Saha SK, Das S, Khuda-Bukhsh AR. Phenotypic evidence of ultra-highly diluted homeopathic remedies acting at gene expression level: a novel probe on experimental phage infectivity in bacteria. Journal of Chinese Integrative Medicine [Internet]. 2012 Apr 15;10(4):462–70. Available from: https://doi.org/10.3736/jcim20120416

117. Conforti A, Bellavite P, Bertani S, Chiarotti F, Menniti-Ippolito F, Raschetti R. Rat models of acute inflammation: a randomized controlled study on the effects of homeopathic remedies. BMC Complementary and Alternative Medicine [Internet]. 2007 Jan 17;7(1). Available from: https://doi.org/10.1186/1472-6882-7-1

118. Pedalino CMV, Perazzo FF, Carvalho JCT, Martinho KS, De Oliveira Massoco C, Bonamin LV. Effect of Atropa belladonna and Echinacea angustifolia in homeopathic dilution on experimental peritonitis. Homeopathy [Internet]. 2004 Oct 1;93(4):193–8. Available from: https://doi.org/10.1016/j.homp.2004.07.004

119. Rodionova OM, Глебов BB, Artamonova EV, Butenin MA, Anikina EV. Effect of herbal homeopathic monopreparations on the rate of tissue lymphatic drainage in healthy mice. Bulletin of Experimental Biology and Medicine [Internet]. 2016 Oct 1; Available from: https://doi.org/10.1007/s10517-016-3510-3

120. Malapane EB, Solomon EM, Pellow J. Efficacy of a homeopathic complex on acute viral tonsillitis. Journal of Alternative and Complementary Medicine [Internet]. 2014 Nov 1;20(11):868–73. Available from: https://doi.org/10.1089/acm.2014.0189

121. Danno K, Colas A, Masson JL, Bordet MF. Homeopathic treatment of migraine in Children: results of a prospective, multicenter, observational study. Journal of Alternative and Complementary Medicine [Internet]. 2013 Feb 1;19(2):119–23. Available from: https://doi.org/10.1089/acm.2011.0821

122. Berrebi A, Parant O, Ferval F, Thene M, Ayoubi JM, Connan L et al. "Traitement de la douleur de la montée laiteuse non souhaitée par homéopathie dans le post-partum immédiat" [Treatment of pain due to unwanted lactation with a homeopathic preparation given in the immediate post-partum period]. Journal de gynecologie, obstetrique et biologie de la reproduction [Journal of gynecology, obstetrics and reproductive biology] vol. 30, no. 4, 2001, pp. 353-7. Available from: http://bluegyn.com/gynorama/wp-content/uploads/2014/11/Traitement-de-la-douleur-de-la-montée-laiteuse_2001.pdf

123. Morris M, Pellow J, Solomon EM, Tsele-Tebakang T. Physiotherapy and a homeopathic complex for chronic low-back pain due to osteoarthritis: a randomized, controlled pilot study. PubMed [Internet]. 2016 Jan 17;22(1):48–56. Available from: https://pubmed.ncbi.nlm.nih.gov/26773321

124. Melchart D, Linde K, Worku F, Bauer R, Wagner H. Immunomodulation with echinacea — a systematic review of controlled clinical trials. Phytomedicine [Internet]. 1994 Dec 1;1(3):245–54. Available from: https://doi.org/10.1016/s0944-7113(11)80072-3

125. Shakeel M, Trinidade A, Jehan S, Ah-See K. The use of complementary and alternative medicine by patients attending a general otolaryngology clinic: can we afford to ignore it? American Journal of Otolaryngology [Internet]. 2010 Jul 1;31(4):252–60. Available from: https://doi.org/10.1016/j.amjoto.2009.02.016

126. Shakeel M, Little S, Bruce J, Ah-See K. Use of complementary and alternative medicine in pediatric otolaryngology patients attending a tertiary hospital in the UK.

International Journal of Pediatric Otorhinolaryngology [Internet]. 2007 Nov 1;71(11):1725–30. Available from: https://doi.org/10.1016/j.ijporl.2007.07.009

127. Shakeel M, Newton JR, Ah-See K. Complementary and alternative medicine use among patients undergoing otolaryngologic surgery. PubMed [Internet]. 2009 Jun 1;38(3):355–61. Available from: https://pubmed.ncbi.nlm.nih.gov/19476768

128. Lee KJ, Yeo MG. Homeopathic Rhus toxicodendron has dual effects on the inflammatory response in the mouse preosteoblastic cell line MC3T3-e1. Homeopathy [Internet]. 2016 Feb 1;105(1):42–7. Available from: https://doi.org/10.1016/j.homp.2015.09.004

129. Huh YH, Kim MJ, Yeo MG. Homeopathic Rhus toxicodendron treatment increased the expression of cyclooxygenase-2 in primary cultured mouse chondrocytes. Homeopathy [Internet]. 2013 Oct 1;102(4):248–53. Available from: https://doi.org/10.1016/j.homp.2013.07.001

130. Jäggi R, Würgler U, Grandjean F, Weiser M. Dual inhibition of 5-lipoxygenase/cyclooxygenase by a reconstituted homeopathic remedy; possible explanation for clinical efficacy and favourable gastrointestinal tolerability. Inflammation Research [Internet]. 2004 Mar 1;53(4):150–7. Available from: https://doi.org/10.1007/s00011-003-1236-y

131. Santos ALD, Perazzo FF, Cardoso LGV, Carvalho JCT. *In vivo* study of the anti-inflammatory effect of Rhus toxicodendron. Homeopathy [Internet]. 2007 Apr 1;96(2):95–101. Available from: https://doi.org/10.1016/j.homp.2007.03.001

132. Patel DR, Ansari I, Kachchhi YN, Patel RB, Patil K, Jadhav RB, et al. Toxicodendron pubescens retains its anti-arthritic efficacy at 1M, 10M and CM homeopathic dilutions. Homeopathy [Internet]. 2012 Jul 1;101(3):165–70. Available from: https://doi.org/10.1016/j.homp.2012.02.007

133. Patil CR, Salunkhe PS, Gaushal MH, Gadekar AR, Agrawal A, Surana SJ. Immunomodulatory activity of Toxicodendron pubescens in experimental models. Homeopathy [Internet]. 2009 Jul 1;98(3):154–9. Available from: https://doi.org/10.1016/j.homp.2009.02.011

134. Patil CR, Rambhade A, Jadhav RB, Patil K, Dubey V, Sonara BM, et al. Modulation of arthritis in rats by Toxicodendron pubescens and its homeopathic dilutions. Homeopathy [Internet]. 2011 Jul 1;100(3):131–7. Available from: https://doi.org/10.1016/j.homp.2011.01.001

135. Poitevin B. Survey of immuno-allergological ultra high dilution research. Homeopathy [Internet]. 2015 Oct 1;104(4):269–76. Available from: https://doi.org/10.1016/j.homp.2015.06.007

136. Grudianov AI, Bezrukova IV, Aleksandrovskaia IIu. [Comparative study of homeopathic remedies clinical efficacy in comprehensive treatment of inflammatory periodontal diseases in patients with burdened allergic status]. Stomatologiia (Mosk). 2006;85(2):25-8. Russian. Available from: https://pubmed.ncbi.nlm.nih.gov/16710274/

137. Gebhardt R. Antioxidative, Antiproliferative and Biochemical Effects in HepG2 Cells of a Homeopathic Remedy and its Constituent Plant Tinctures Tested Separately or in Combination. Drug Research [Internet]. 2011 Dec 25;53(12):823–30. Available from: https://doi.org/10.1055/s-0031-1299836

138. Vestweber AM, Beuth J, Ko HL, Tunggal L, Buss G, Pulverer G. *In-vitro*-Aktivität von Mercurius cyanatus-Komplex gegenüber relevanten pathogenen Bakterienisolaten [*In vitro* activity of Mercurius cyanatus complex against relevant pathogenic bacterial isolates]. Arzneimittelforschung. 1995 Sep;45(9):1018-20. Available from: https://pubmed.ncbi.nlm.nih.gov/7488303/

139. Bagheri H, Laroche M, Montastruc J. Pharmacovigilance: The new challenges. Thérapie [Internet]. 2016 Apr 1; Available from: https://doi.org/10.1016/j.therap.2016.02.019

140. Beninger P. Opportunities for collaboration at the interface of pharmacovigilance and manufacturing. Clinical Therapeutics [Internet]. 2017 Apr 1;39(4):702–12. Available from: https://doi.org/10.1016/j.clinthera.2017.03.010

141. Wise L, Parkinson J, Raine J, Breckenridge A. New approaches to drug safety: a pharmacovigilance tool kit. Nature Reviews Drug Discovery [Internet]. 2009 Sep 18;8(10):779–82. Available from: https://doi.org/10.1038/nrd3002

142. Shaw D, Ladds G, Duez P, Williamson EM, Chan K. Pharmacovigilance of herbal medicine. Journal of Ethnopharmacology [Internet]. 2012 Apr 1;140(3):513–8. Available from: https://doi.org/10.1016/j.jep.2012.01.051

143. Ocheretyanaya N. Stress y serdechno-sosudystye zabolevanyia. [Stress and heart and vascular diseases]. Medychna Hazeta "Zdorovia Ukrainy" [Medical Newspaper "Health of Ukraine"], 2015 April;8,(357):38–39. Available from: https://health-ua.com/multimedia/userfiles/files/2015/ZU_8_2015/ZU_2015_08_st38-39.pdf.

144. Shcherbatykh Yu. Psykholohyia stressa: populiarnaia entsyklopedyia [The psychology of stress: a popular encyclopaedia]. Exmo, 2008.

145. Jordan H, Stellman SD, Morabia A, Miller-Archie SA, Alper H, Laskaris Z, et al. Cardiovascular disease hospitalizations in relation to exposure to the September 11, 2001 World Trade Center disaster and posttraumatic stress disorder. Journal of the American Heart Association [Internet]. 2013 Sep 26;2(5). Available from: https://doi.org/10.1161/jaha.113.000431

146. Iso H, Date C, Yamamoto A, Toyoshima H, Tanabe N, Kikuchi S, et al. Perceived mental stress and mortality from cardiovascular disease among Japanese

men and women. Circulation [Internet]. 2002 Sep 3;106(10):1229–36. Available from: https://doi.org/10.1161/01.cir.0000028145.58654.41

147. Nabi H, Kivimäki M, Batty GD, Shipley MJ, Britton A, Brunner EJ, et al. Increased risk of coronary heart disease among individuals reporting adverse impact of stress on their health: the Whitehall II prospective cohort study. European Heart Journal [Internet]. 2013 Jun 26;34(34):2697–705. Available from: https://doi.org/10.1093/eurheartj/eht216

148. Alhurani AS, Dekker RL, Ahmad M, Miller J, Yousef K, Abdulqader B, et al. Stress, cognitive appraisal, coping, and event free survival in patients with heart failure. Heart & Lung [Internet]. 2018 May 1;47(3):205–10. Available from: https://doi.org/10.1016/j.hrtlng.2018.03.008

149. Blumenthal JA, Feger BJ, Smith PJ, Watkins LL, Jiang W, Davidson J, et al. Treatment of anxiety in patients with coronary heart disease: Rationale and design of the UNderstanding the benefits of exercise and escitalopram in anxious patients WIth coroNary heart Disease (UNWIND) randomized clinical trial. American Heart Journal [Internet]. 2016 Jun 1;176:53–62. Available from: https://doi.org/10.1016/j.ahj.2016.03.003

150. Scott SB, Thompson JP. Drugs acting on the heart: heart failure and coronary insufficiency. Anaesthesia & Intensive Care Medicine [Internet]. 2012 Aug 1;13(8):378–83. Available from: https://doi.org/10.1016/j.mpaic.2012.05.008

151. Loganovsky K. Vegetative-Vascular dystonia and osteoalgetic syndrome or chronic fatigue syndrome as a characteristic After-Effect of radioecological disaster. Journal of Chronic Fatigue Syndrome [Internet]. 2000 Jan 1;7(3):3–16. Available from: https://doi.org/10.1300/j092v07n03_02

152. Sidorenko GI. Neĭrotsirkuliatornaia distoniia [Neurocirculatory dystonia] Mezhdunarodnii medchytsyskyi zhurnal [International medical journal]. 2003; 3(10): 93-8. Available

from: http://dspace.nbuv.gov.ua/bitstream/handle/123456789/52499/04-Sidorenko.pdf?sequence=1

153. Smetanina, KI, Riazanova RM. Fitoterapevtychnyi pidkhid do likuvannia nevroziv sertsia [Phytotherapeutic approach to the treatment of heart neuroses]. Fitoterapiia. chasopys. 2004;3:7-15.

154. Frolova NL, Chutko LS, Surushkina SYu. Neurocirculatory asthenia: psychoautonomic features and treatment possibilities. Zhurnal Nevrologii i Psikhiatrii imeni S.S. Korsakova. 2012;112(12):51-54. Available from: https://www.mediasphera.ru/issues/zhurnal-nevrologii-i-psikhiatrii-im-s-s-korsakova/2012/12/031997-72982012129?lang=ru

155. Amos T, Stein DJ, Ipser J. Pharmacological interventions for preventing post-traumatic stress disorder (PTSD). The Cochrane Library [Internet]. 2014 Jul 8; Available from: https://doi.org/10.1002/14651858.cd006239.pub2

156. Hernandorena I, Duron E, Vidal J, Hanon O. Treatment options and considerations for hypertensive patients to prevent dementia. Expert Opinion on Pharmacotherapy [Internet]. 2017 May 29;18(10):989–1000. Available from: https://doi.org/10.1080/14656566.2017.1333599

157. Lackland DT, Weber MA. Global burden of cardiovascular disease and stroke: hypertension at the core. Canadian Journal of Cardiology [Internet]. 2015 May 1;31(5):569–71. Available from: https://doi.org/10.1016/j.cjca.2015.01.009

158. Parati G, Antonicelli R, Guazzarotti F, Paciaroni E, Mancia G. Cardiovascular effects of an earthquake. Hypertension [Internet]. 2001 Nov 1;38(5):1093–5. Available from: https://doi.org/10.1161/hy1101.095334

159. Tobin KJ. Stable angina pectoris: what does the current clinical evidence tell us? PubMed [Internet]. 2010 Jul 1;110(7):364–70. Available from: https://pubmed.ncbi.nlm.nih.gov/20693568

160. Hombach V, Höher M, Kochs M, Eggeling T, Schmidt A, Höpp HW, et al. Pathophysiology of unstable angina pectoris--correlations with coronary angioscopic imaging. European Heart Journal [Internet]. 1988 Dec 2;9(suppl N):40–5. Available from: https://doi.org/10.1093/eurheartj/9.suppl_n.40

161. Sedighi M, Bahmani M, Asgary S, Beyranvand F, Rafieian-Kopaei M. A review of plant-based compounds and medicinal plants effective on atherosclerosis. Journal of Research in Medical Sciences [Internet]. 2017 Jan 1;22(1):30. Available from: https://doi.org/10.4103/1735-1995.202151

162. Orekhov AN, Ivanova EA. Cellular models of atherosclerosis and their implication for testing natural substances with anti-atherosclerotic potential. Phytomedicine [Internet]. 2016 Oct 1;23(11):1190–7. Available from: https://doi.org/10.1016/j.phymed.2016.01.003

163. Dimsdale JE. Psychological stress and cardiovascular disease. Journal of the American College of Cardiology [Internet]. 2008 Apr 1;51(13):1237–46. Available from: https://doi.org/10.1016/j.jacc.2007.12.024

164. Dolzhenko MN. Try nazvanyia odnoi bolezny, yly kak lechyt vehetatyvnyi kryz? [Three names for one disease, or how to treat a vegetative crisis?] Arteryalnaia hypertenzyia [Arterial hypertension]. 2008;1:55-62.

165. Burchinsky SG. Malye neiroleptyky v terapyy psykhosomatycheskykh rasstroistv [Small neuroleptics in the pharmacotherapy of psychosomatic disorders.] Zdorovia Ukrainy [Health of Ukraine]. 2012;2(22):24-25. Available from: https://health-ua.com/article/10174-malye-nejroleptiki-v-terapii-psihosomaticheskih-rasstrojstv

166. Burchinsky SG. Vozmozhnosty kompleksnoi farmakoterapyy vehetatyvnoi dysfunktsyy y psykhoemotsyonalnoho dysbalansa [The possibilities of complex pharmacotherapy of autonomic dysfunction and psychoemotional imbalance.]

Simeina medytsyna [Family Medicine]. 2014;5(55):25-35. Available from: http://nbuv.gov.ua/UJRN/simmed_2014_5_9

167. Ghicavîi V, Podgurschi L, Pogonea I, Rakovski T. Peculiarities of using drugs in the elderly. Zenodo (CERN European Organization for Nuclear Research) [Internet]. 2017 Dec 15; Available from: https://zenodo.org/record/1106597

168. Rosić S, Kendić S, Rosic M. Phytoestrogens impact on menopausal symptomatology. Materia Socio-medica [Internet]. 2013 Jan 1;25(2):98. Available from: https://doi.org/10.5455/msm.2013.25.98-100

169. Wojtczak D, Kasznicki J, Drzewoski J. Pros and cons of polypharmacy in elderly patients with diabetes. Clinical Diabetology [Internet]. 2017 Jun 20;6(1):34–8. Available from: https://doi.org/10.5603/dk.2017.0006

170. Phipps JB, O'Kennon R, Lance RW. Hawthorns and medlars [Internet]. 2003. Available from: https://www.cabdirect.org/cabdirect/abstract/20033188981

171. Tassell MC, Kingston R, Gilroy DW, Lehane M, Furey A. Hawthorn (Crataegus spp.) in the treatment of cardiovascular disease. Pharmacognosy Reviews [Internet]. 2010 Jan 1;4(7):32. Available from: https://doi.org/10.4103/0973-7847.65324

172. Bernatonienė J, Trumbeckaitė S, Majienė D, Banienė R, Baliūtytė G, Savickas A, et al. The effect of crataegus fruit extract and some of its flavonoids on mitochondrial oxidative phosphorylation in the heart. Phytotherapy Research [Internet]. 2009 May 13;23(12):1701–7. Available from: https://doi.org/10.1002/ptr.2815

173. Fong HHS, Bauman JL. Hawthorn. Journal of Cardiovascular Nursing [Internet]. 2002 Jul 1;16(4):1–8. Available from: https://doi.org/10.1097/00005082-200207000-00002

174. Rigelsky JM, Sweet BV. Hawthorn: Pharmacology and therapeutic uses. American Journal of Health-system Pharmacy [Internet]. 2002 Mar 1;59(5):417–22. Available from: https://doi.org/10.1093/ajhp/59.5.417

175. Eggeling T, Regitz-Zagrosek V, Zimmermann A, Burkart M. Baseline severity but not gender modulates quantified Crataegus extract effects in early heart failure – A pooled analysis of clinical trials. Phytomedicine [Internet]. 2011 Nov 1;18(14):1214–9. Available from: https://doi.org/10.1016/j.phymed.2011.06.022

176. Guo RS, Pittler MH, Ernst E. Hawthorn extract for treating chronic heart failure. The Cochrane Library [Internet]. 2008 Jan 23; Available from: https://doi.org/10.1002/14651858.cd005312.pub2

177. Wiesner J. Assessment report on Crataegus spp., folium cum flore. European Medicines Agency, Oct. 2014. 159076/2014. Available from: https://www.ema.europa.eu/en/documents/herbal-report/final-assessment-report-crataegus-spp-folium-cum-flore_en.pdf

178. Rauwald H, Kuchta K, Savtschenko A, Brückner A, Rusch C, Appel K. GABAA receptor binding assays of standardized Leonurus cardiaca and L. japonicus extracts as well as their isolated constituents. Planta Medica [Internet]. 2013 Aug 21;79(13). Available from: https://doi.org/10.1055/s-0033-1352395

179. Babushkina AV. Kombynyrovannyi preparat Kratal v terapyy serdechno-sosudystykh zabolevanyi [Combination Preparation Kratal in the Treatment of Cardiovascular Diseases]. Ukrainskyi Medychnyi Chasopys [Ukrainian Medical Journal], Aug. 2010;60.

180. Zhang R, Liu Z, Yang D, Zhang XJ, Sun H, Xiao W. Phytochemistry and pharmacology of the genus Leonurus: The herb to benefit the mothers and more. Phytochemistry [Internet]. 2018 Mar 1;147:167–83. Available from: https://doi.org/10.1016/j.phytochem.2017.12.016

181. Wojtyniak K, Szymański M, Matławska I. *Leonurus cardiaca* L. (Motherwort): A Review of its Phytochemistry and Pharmacology. Phytotherapy Research [Internet]. 2012 Oct 8;27(8):1115–20. Available from: https://doi.org/10.1002/ptr.4850

182. Shang X, Hu P, Wang X, He H, Li M. Leonurus japonicus Houtt.: Ethnopharmacology, phytochemistry and pharmacology of an important traditional Chinese medicine. Journal of Ethnopharmacology [Internet]. 2014 Feb 1;152(1):14–32. Available from: https://doi.org/10.1016/j.jep.2013.12.052

183. Liu XH, Pan L, Zhu Y. Active chemical compounds of traditional Chinese medicine Herba Leonuri: implications for cardiovascular diseases. Clinical and Experimental Pharmacology and Physiology [Internet]. 2012 Feb 22;39(3):274–82. Available from: https://doi.org/10.1111/j.1440-1681.2011.05630.x

184. Committee on Herbal Medicinal Products (HMPC). "Assessment report on *Leonurus cardiaca* L., herba." London, 6 May 2010. Available from: https://www.ema.europa.eu/en/documents/herbal-report/draft-assessment-report-leonurus-cardiaca-l-herba_en.pdf

185. Korniievska VH, Sur SV, Lesyk IP Efirna Oliia Valeriany Likarskoi [Valerian Medicinal Essential Oil]. Farmatsevtychnyi Zhurnal [Pharmaceutical Journal]. 2000;3:95-97

186. Wagner H. Phytomedicine research in Germany. Environmental Health Perspectives [Internet]. 1999 Oct 1;107(10):779–81. Available from: https://doi.org/10.1289/ehp.99107779

187. Leach M, Page A. Herbal medicine for insomnia: A systematic review and meta-analysis. Sleep Medicine Reviews [Internet]. 2015 Dec 1;24:1–12. Available from: https://doi.org/10.1016/j.smrv.2014.12.003

188. Bega D, Malkani RG. Alternative treatment of restless legs syndrome: an overview of the evidence for mind–body interventions, lifestyle interventions, and neutraceuticals. Sleep Medicine [Internet]. 2016 Jan 1;17:99–105. Available from: https://doi.org/10.1016/j.sleep.2015.09.009

189. Waldschütz R, Klein P. The Homeopathic Preparation Neurexan® vs. Valerian for the Treatment of Insomnia: An Observational Study. The Scientific World Journal [Internet]. 2008 Jan 1;8:411–20. Available from: https://doi.org/10.1100/tsw.2008.61

190. Hubner R, Van Haselen R, Klein P. Effectiveness of the Homeopathic Preparation Neurexan®Compared with that of Commonly used Valerian-Based Preparations for the Treatment of Nervousness/Restlessness – an Observational Study. The Scientific World Journal [Internet]. 2009 Jan 1;9:733–45. Available from: https://doi.org/10.1100/tsw.2009.95

191. Taavoni S, Ekbatani NN, Haghani H. Valerian/lemon balm use for sleep disorders during menopause. Complementary Therapies in Clinical Practice [Internet]. 2013 Nov 1;19(4):193–6. Available from: https://doi.org/10.1016/j.ctcp.2013.07.002

192. Committee on Herbal Medicinal Products (HMPC). "Assessment report on *Valeriana officinalis* L., *radix* and *Valeriana officinalis* L., *aetheroleum*" London, 7 July 2015. Available from: https://www.ema.europa.eu/en/documents/herbal-report/final-assessment-report-valeriana-officinalis-l-radix-valeriana-officinalis-l-aetheroleum_en.pdf

193. Kennedy DO, Scholey A, Tildesley NTJ, Perry EK, Wesnes K. Modulation of mood and cognitive performance following acute administration of Melissa officinalis (lemon balm). Pharmacology, Biochemistry and Behavior [Internet]. 2002 Jul 1;72(4):953–64. Available from: https://doi.org/10.1016/s0091-3057(02)00777-3

194. Shakeri A, Sahebkar A, Javadi B. *Melissa officinalis* L. – A review of its traditional uses, phytochemistry and pharmacology. Journal of Ethnopharmacology [Internet]. 2016 Jul 1;188:204–28. Available from: https://doi.org/10.1016/j.jep.2016.05.010.

195. Committee on Herbal Medicinal Products (HMPC). "Community Herbal Monograph on *Melissa officinalis* L., *Folium*." London, 8 May 2007. Available from: http://www.teinture-mère.fr/files/boutique/produits/doc/186603-3-melissa-officinalis.pdf

196. Prihodko VYu, Voloschiuk TR, Prihodko VM Klynycheskyi Opyt Prymenenyia Preparata Karvelys U Patsyentov Razlychnykh Vozrastnykh Hrupp S Vehetatyvnoi Dysfunktsyei [Clinical Experience of Using the Drug Karvelis in Patients of Different Age Groups With Autonomic Dysfunction]. Zdorovia Ukrainy [Health of Ukraine]. 2014;19:38-39. Available from: https://health-ua.com/pics/pdf/ZU_2014_19/38-39.pdf

197. Ross SM. Psychophytomedicine. Holistic Nursing Practice [Internet]. 2014 Jul 1;28(4):275–80. Available from: https://doi.org/10.1097/hnp.0000000000000040

198. Luo S. Separation and determination of alkaloids of Leonurus sibiricus. Bulletin of Chinese Materia Medica [Internet]. 1985 Jan 1;10(1):32–5. Available from: https://pubmed.ncbi.nlm.nih.gov/3157476/

199. Kosyakova LYe. Rastenyia-tselytely: spravochnyk po narodnoi y praktycheskoi fytoterapyy [Healing Plants: A Handbook of Folk and Practical Phytotherapy]. Russia, Verkhne-Volzhskoye Publishing House, 1993.

200. Wang C, Dong H, Tan L, Sun XX, Wu MC. Surgicopathological classification of hepatic space-occupying lesions: A single-center experience with literature review. World Journal of Gastroenterology [Internet]. 2011 Jan 1;17(19):2372. Available from: https://doi.org/10.3748/wjg.v17.i19.2372

201. Özkardeş AB, Tokaç M, Dumlu EG, Bozkurt B, Çiftçi AB, Yetışır F, et al. Early versus Delayed laparoscopic cholecystectomy for acute cholecystitis: A Prospective, randomized study. International Surgery [Internet]. 2014 Jan 1;99(1):56–61. Available from: https://doi.org/10.9738/intsurg-d-13-00068.1

202. Coats M, Shimi SM. Cholecystectomy and the risk of alimentary tract cancers: A systematic review. World Journal of Gastroenterology [Internet]. 2015 Jan 1;21(12):3679. Available from: https://doi.org/10.3748/wjg.v21.i12.3679

203. Tsai MC, Chen CH, Lee HC, Lin HC, Lee CZ. Increased Risk of Depressive Disorder following Cholecystectomy for Gallstones. PLOS ONE [Internet]. 2015 Jun 8;10(6):e0129962. Available from: https://doi.org/10.1371/journal.pone.0129962

204. Chhabra S, Ahmed Z, Massey AC, Agarwal S, Vij V, Agarwal B, et al. Laparoscopic cholecystectomy in a 2 year old male child with choleilithiasis and recurrent right hypochondrial pain: Case report and review of literature. International Journal of Surgery Case Reports [Internet]. 2016 Jan 1; Available from: https://doi.org/10.1016/j.ijscr.2016.07.038

205. Pallayová M, Taheri S. Non-alcoholic fatty liver disease in obese adults: clinical aspects and current management strategies. Clinical Obesity [Internet]. 2014 Jul 28;4(5):243–53. Available from: https://doi.org/10.1111/cob.12068

206. Kim HY, Lee K, Lee KW, Yi NJ, Lee HW, Hong G, et al. Histologically proven non-alcoholic fatty liver disease and clinically related factors in recipients after liver transplantation. Clinical Transplantation [Internet]. 2014 Apr 16;28(5):521–9. Available from: https://doi.org/10.1111/ctr.12343

207. Chan W, Ida NH, Cheah P, Goh K. Progression of liver disease in non-alcoholic fatty liver disease: A prospective clinicopathological follow-up study. Journal of Digestive Diseases [Internet]. 2014 Oct 1;15(10):545–52. Available from: https://doi.org/10.1111/1751-2980.12175

209. Borum MML, Fromm H. Expression of HLA molecules on hepatocytes: Does ursodeoxycholic acid prevent immunologic injurY? Gastroenterology [Internet]. 1990 Aug 1;99(2):561–2. Available from: https://doi.org/10.1016/0016-5085(90)91045-8

210. Cheng K, Ashby D, Smyth RL. Ursodeoxycholic acid for cystic fibrosis-related liver disease. The Cochrane Library [Internet]. 2017 Sep 11;2021(4). Available from: https://doi.org/10.1002/14651858.cd000222.pub4

211. Buko V, Lukivskaya O, Naruta E, Belonovskaya E, Tauschel HD. Protective effects of norursodeoxycholic acid versus ursodeoxycholic acid on thioacetamide-induced rat liver fibrosis. Journal of Clinical and Experimental Hepatology [Internet]. 2014 Dec 1;4(4):293–301. Available from: https://doi.org/10.1016/j.jceh.2014.02.001

212. Tiedemann F, Gmelin L. Einige neue Bestandtheile der Galle des Ochsen. Annalen Der Physik [Internet]. 1827 Jan 1;85(2):326–37. Available from: https://doi.org/10.1002/andp.18270850214

213. Sumizu K. Oxidation of hypotaurine in rat liver. Biochimica Et Biophysica Acta [Internet]. 1962 Sep 1;63(1):210–2. Available from: https://doi.org/10.1016/0006-3002(62)90357-8

214. Loseva NV, Moiseyenko YeYe. Opyt prymenenyia preparata Dybykor v kompleksnoi terapyy nealkoholnoi zhyrovoi bolezny pecheny [Grounds of Ursodeoxycholic Acid, Taurine and Extractum Folii Artishoke Combine Use Safety in the Hepatobiliar System Diseases]. Pharmateka [Pharmateca]. 2010;15:63–67. Available from: https://pharmateca.ru/ru/archive/article/7946

215. Kraft KA. Artichoke leaf extract — Recent findings reflecting effects on lipid metabolism, liver and gastrointestinal tracts. Phytomedicine [Internet]. 1997 Dec 1;4(4):369–78. Available from: https://doi.org/10.1016/s0944-7113(97)80049-9

216. Guarrera PM, Salerno G, Caneva G. Folk phytotherapeutical plants from Maratea area (Basilicata, Italy). Journal of Ethnopharmacology [Internet]. 2005 Jul 1;99(3):367–78. Available from: https://doi.org/10.1016/j.jep.2005.01.039

217. Mootoosamy A, Mahomoodally MF. Ethnomedicinal application of native remedies used against diabetes and related complications in Mauritius. Journal of Ethnopharmacology [Internet]. 2014 Jan 1;151(1):413–44. Available from: https://doi.org/10.1016/j.jep.2013.10.069

218. Giacosa A, Guido D, Grassi M, Riva A, Morazzoni P, Bombardelli E, et al. The Effect of Ginger (*Zingiber officinalis*) and Artichoke (*Cynara cardunculus*) Extract Supplementation on Functional Dyspepsia: A Randomised, Double-Blind, and Placebo-Controlled Clinical Trial. Evidence-based Complementary and Alternative Medicine [Internet]. 2015 Jan 1;2015:1–9. Available from: https://doi.org/10.1155/2015/915087

219. Wei W, Zeng R, Gu CM, Qu Y, Huang L. Angelica sinensis in China-A review of botanical profile, ethnopharmacology, phytochemistry and chemical analysis. Journal of Ethnopharmacology [Internet]. 2016 Aug 1;190:116–41. Available from: https://doi.org/10.1016/j.jep.2016.05.023

220. Simoni P, Cerrè C, Cipolla A, Polimeni C, Pistillo A, Ceschel GC, et al. Bioavailability study of a new, sinking, enteric-coated ursodeoxycholic acid formulation. Pharmacological Research [Internet]. 1995 Jan 1;31(2):115–9. Available from: https://doi.org/10.1016/1043-6618(95)80056-5

221. Podda M, Ghezzi C, Battezzati PM, Bertolini E, Crosignani A, Petroni ML, et al. Effect of different doses of ursodeoxycholic acid in chronic liver disease. Digestive Diseases and Sciences [Internet]. 1989 Dec 1;34(S12):S59–65. Available from: https://doi.org/10.1007/bf01536665

222. Hwang S, Chan C, Lee SD, Wu J, Tsay S, Lo K. Ursodeoxycholic acid in the treatment of primary biliary cirrhosis: A short-term, randomized, double-blind

controlled, cross-over study with long-term follow up. Journal of Gastroenterology and Hepatology [Internet]. 1993 Jun 1;8(3):217–23. Available from: https://doi.org/10.1111/j.1440-1746.1993.tb01189.x

223. Battezzati PM, Podda M, Bianchi FB, Naccarato R, Orlandi F, Surrenti C, et al. Ursodeoxycholic acid for symptomatic primary biliary cirrhosis. Journal of Hepatology [Internet]. 1993 Jan 1;17(3):332–8. Available from: https://doi.org/10.1016/s0168-8278(05)80214-4

224. Angulo P, Dickson ER, Therneau TM, Jorgensen RA, Smith CI, DeSotel CK, et al. Comparison of three doses of ursodeoxycholic acid in the treatment of primary biliary cirrhosis: a randomized trial. Journal of Hepatology [Internet]. 1999 May 1;30(5):830–5. Available from: https://doi.org/10.1016/s0168-8278(99)80136-6

225. Harnois DM, Angulo P, Jorgensen RA, LaRusso NF, Lindor KD. High-Dose ursodeoxycholic acid as a therapy for patients with primary sclerosing cholangitis. The American Journal of Gastroenterology [Internet]. 2001 May 1;96(5):1558–62. Available from: https://doi.org/10.1111/j.1572-0241.2001.03777.x

226. Hess LM, Krutzsch M, Guillen J, Chow HS, Einspahr JG, Batta AK, et al. Results of a Phase I Multiple-Dose Clinical Study of ursodeoxycholic acid. Cancer Epidemiology, Biomarkers & Prevention [Internet]. 2004 May 1;13(5):861–7. Available from: https://doi.org/10.1158/1055-9965.861.13.5

227. Keiding S, Höckerstedt K, Bjøro K, Bondesen S, Hjortrup A, Isoniemi H, et al. The nordic multicenter double-blind randomized controlled trial of prophylactic ursodeoxycholic acid in liver transplant patients1. Transplantation [Internet]. 1997 Jun 1;63(11):1591–4. Available from: https://doi.org/10.1097/00007890-199706150-00009

228. Colombo C, Battezzati PM, Podda M, Bettinardi N, Giunta A. Ursodeoxycholic acid for liver disease associated with cystic fibrosis: A double-blind multicenter trial.

Hepatology [Internet]. 1996 Jun 1;23(6):1484–90. Available from: https://doi.org/10.1002/hep.510230627

229. Heubi JE, Wiechmann DA, Creutzinger V, Setchell KDR, Squires RH, Couser RJ, et al. Tauroursodeoxycholic acid (TUDCA) in the prevention of total parenteral nutrition-associated liver disease. The Journal of Pediatrics [Internet]. 2002 Aug 1;141(2):237–2. Available from: https://doi.org/10.1067/mpd.2002.125802

230. Vettorazzi JF, Kurauti MA, Soares GM, Borck PC, Ferreira SM, Branco RCS, et al. Bile acid TUDCA improves insulin clearance by increasing the expression of insulin-degrading enzyme in the liver of obese mice. Scientific Reports [Internet]. 2017 Nov 1;7(1). Available from: https://doi.org/10.1038/s41598-017-13974-0

231. Lupattelli G, Marchesi S, Lombardini R, Roscini AR, Trinca F, Gemelli F, et al. Artichoke juice improves endothelial function in hyperlipemia. Life Sciences [Internet]. 2004 Dec 1;76(7):775–82. Available from: https://doi.org/10.1016/j.lfs.2004.07.018

232. Ferracane R, Pellegrini N, Visconti A, Graziani G, Chiavaro E, Miglio C, et al. Effects of different cooking methods on antioxidant profile, antioxidant capacity, and physical characteristics of artichoke. Journal of Agricultural and Food Chemistry [Internet]. 2008 Aug 30;56(18):8601–8. Available from: https://doi.org/10.1021/jf800408w

233. Betancor-Fernández AJ, Pérez-Gálvez A, Sies H, Stahl W. Screening pharmaceutical preparations containing extracts of turmeric rhizome, artichoke leaf, devil's claw root and garlic or salmon oil for antioxidant capacity. Journal of Pharmacy and Pharmacology [Internet]. 2003 Jul 1;55(7):981–6. Available from: https://doi.org/10.1211/0022357021468

234. Sannia A. Un'esperienza di fitoterapia con una associazione di fitocomplessi ad attività epatoprotettrice contenente un estratto di foglie di carciofo per il controllo dei sintomi della dispepsia funzionale [Phytotherapy with a mixture of dry extracts

with hepato-protective effects containing artichoke leaves in the management of functional dyspepsia symptoms]. Minerva Gastroenterologica E Dietologica [Internet]. 2010 Jun 1;56(2):93–9. Available from: http://europepmc.org/abstract/MED/20485248

235. Barrat E, Zaïr Y, Sirvent P, Chauveau P, Maudet C, Housez B, et al. Effect on LDL-cholesterol of a large dose of a dietary supplement with plant extracts in subjects with untreated moderate hypercholesterolaemia: a randomised, double-blind, placebo-controlled study. European Journal of Nutrition [Internet]. 2012 Dec 25;52(8):1843–52. Available from: https://doi.org/10.1007/s00394-012-0486-2

236. Hirata J, Swiersz LM, Zell B, Small R, Ettinger B. Does dong quai have estrogenic effects in postmenopausal women? A double-blind, placebo-controlled trial. Fertility and Sterility [Internet]. 1997 Dec 1;68(6):981–6. Available from: https://doi.org/10.1016/s0015-0282(97)00397-x

237. Wu YC, Hsieh CL. Pharmacological effects of Radix Angelica Sinensis (Danggui) on cerebral infarction. Chinese Medicine [Internet]. 2011 Jan 1;6(1):32. Available from: https://doi.org/10.1186/1749-8546-6-32

238. Ko WC. A newly isolated antispasmodic—butylidenephthalide. Japanese Journal of Pharmacology [Internet]. 1980 Jan 1;30(1):85–91. Available from: https://doi.org/10.1254/jjp.30.85

239. Ma J, Guo ZB, Jin L, Li Y. Phytochemical progress made in investigations of Angelica sinensis (Oliv.) Diels. Chinese Journal of Natural Medicines [Internet]. 2015 Apr 1;13(4):241–9. Available from: https://doi.org/10.1016/s1875-5364(15)30010-8

240. Dilger K, Hohenester S, Winkler–Budenhofer U, Bastiaansen BA, Schaap FG, Rust C, et al. Effect of ursodeoxycholic acid on bile acid profiles and intestinal detoxification machinery in primary biliary cirrhosis and health. Journal of

Hepatology [Internet]. 2012 Jul 1;57(1):133–40. Available from: https://doi.org/10.1016/j.jhep.2012.02.014

241. Wang DQ -h., Cohen DE, Carey MC. Biliary lipids and cholesterol gallstone disease. Journal of Lipid Research [Internet]. 2009 Apr 1;50:S406–11. Available from: https://doi.org/10.1194/jlr.r800075-jlr200

242. Miyazaki T, Matsuzaki Y. Taurine and liver diseases: a focus on the heterogeneous protective properties of taurine. Amino Acids [Internet]. 2012 Aug 24;46(1):101–10. Available from: https://doi.org/10.1007/s00726-012-1381-0

243. Ghandforoush-Sattari M, Mashayekhi S, Krishna C, Thompson JP, Routledge PA. Pharmacokinetics of oral taurine in healthy volunteers. Journal of Amino Acids [Internet]. 2010 Jun 29;2010:1–5. Available from: https://doi.org/10.4061/2010/346237

244. Li W, Guo J, Tang Y, Wang H, Huang M, Qian D, et al. Pharmacokinetic Comparison of Ferulic Acid in Normal and Blood Deficiency Rats after Oral Administration of Angelica sinensis, Ligusticum chuanxiong and Their Combination. International Journal of Molecular Sciences [Internet]. 2012 Mar 16;13(3):3583–97. Available from: https://doi.org/10.3390/ijms13033583

208. Wisher D. Martindale: The Complete Drug Reference. 37th ed. Journal of the Medical Library Association [Internet]. 2012 Jan 1;100(1):75–6. Available from: https://doi.org/10.3163/1536-5050.100.1.018

245. Jin Y, Tang Y, Zhu Z, Shang E, Pang H, Shi XQ, et al. Pharmacokinetic Comparison of Seven Major Bio-Active Components in Normal and Blood Stasis Rats after Oral Administration of Herb Pair Danggui-Honghua by UPLC-TQ/MS. Molecules [Internet]. 2017 Oct 17;22(10):1746. Available from: https://doi.org/10.3390/molecules22101746

246. Lo A, Chan K, Yeung JHK, Woo KS. Danggui (Angelica sinensis) affects the pharmacodynamics but not the pharmacokinetics of warfarin in rabbits. European Journal of Drug Metabolism and Pharmacokinetics [Internet]. 1995 Mar 1;20(1):55–60. Available from: https://doi.org/10.1007/bf03192289

247. Abebe W. Herbal medication: potential for adverse interactions with analgesic drugs. Journal of Clinical Pharmacy and Therapeutics [Internet]. 2002 Dec 1;27(6):391–401. Available from: https://doi.org/10.1046/j.1365-2710.2002.00444.x.

248. Tian Y, Yang Z, Li Y, Qiao Y, Yang J, Jia Y, et al. Pharmacokinetic comparisons of hydroxysafflower yellow A in normal and blood stasis syndrome rats. Journal of Ethnopharmacology [Internet]. 2010 May 1;129(1):1–4. Available from: https://doi.org/10.1016/j.jep.2010.02.023

249. Jin Y, Wu L, Tang Y, Cao YJ, Li S, Shen J, et al. UFLC-Q-TOF/MS based screening and identification of the metabolites in plasma, bile, urine and feces of normal and blood stasis rats after oral administration of hydroxysafflor yellow A. Journal of Chromatography B [Internet]. 2016 Feb 1;1012–1013:124–9. Available from: https://doi.org/10.1016/j.jchromb.2016.01.023

250. Grohmann M. The activity of the neuronal and extraneuronal catecholamine-metabolizing enzymes of the perfused rat heart. Naunyn-Schmiedeberg's Archives of Pharmacology [Internet]. 1987 Aug 1;336(2):139–47. Available from: https://doi.org/10.1007/bf00165797

251. Azuma K, Ippoushi K, Nakayama M, Ito H, Higashio H, Terao J. Absorption of Chlorogenic Acid and Caffeic Acid in Rats after Oral Administration. Journal of Agricultural and Food Chemistry [Internet]. 2000 Oct 7;48(11):5496–500. Available from: https://doi.org/10.1021/jf000483q

252. Lafay S, Morand C, Manach C, Besson C, Scalbert A. Absorption and metabolism of caffeic acid and chlorogenic acid in the small intestine of rats. British

Journal of Nutrition [Internet]. 2006 Jul 1;96(1):39–46. Available from: https://doi.org/10.1079/bjn20061714

253. Zeng H, Xue P, Su S, Huang X, Shang E, Guo J, et al. Comparative Pharmacokinetics of three major bioactive components in rats after oral administration of Typhae Pollen-Trogopterus Feces drug pair before and after compatibility. DARU Journal of Pharmaceutical Sciences [Internet]. 2016 Jan 20;24(1). Available from: https://doi.org/10.1186/s40199-016-0140-2

254. Dymowski W. Committee on Herbal Medicinal Products (HMPC). Assessment report on Angelica sinensis (Oliv.) Diels, radix. London, 2013. Available from: https://www.ema.europa.eu/en/documents/herbal-report/final-assessment-report-angelica-sinensis-oliv-diels-radix-first-version_en.pdf

255. Luo H, Li X, Xiao F, Gu F. [Study on pharmacokinetics of ferulic acid and paeoniflorin in Angelic-Paeonia root powder]. Zhong Yao Cai. 2003 Mar;26(3):189-92. Chinese. Available from: https://pubmed.ncbi.nlm.nih.gov/12856471/

256. Yan R, Lin G, Ko NL, Tam YK. Low Oral Bioavailability and Pharmacokinetics of Senkyunolide A, a Major Bioactive Component in Rhizoma Chuanxiong, in the Rat. Therapeutic Drug Monitoring [Internet]. 2007 Feb 1;29(1):49–56. Available from: https://doi.org/10.1097/ftd.0b013e31802c5862

257. Rost D, Rudolph G, Kloeters-Plachky P, Stiehl A. Effect of high-dose ursodeoxycholic acid on its biliary enrichment in primary sclerosing cholangitis. Hepatology [Internet]. 2004 Jan 1;40(3):693–8. Available from: https://doi.org/10.1002/hep.20370

258. Fang C, Filipp FV, Smith JW. Unusual binding of ursodeoxycholic acid to ileal bile acid binding protein: role in activation of FXRα. Journal of Lipid Research [Internet]. 2012 Apr 1;53(4):664–73. Available from: https://doi.org/10.1194/jlr.m021733

259. Sturman JA, Hepner GW, Hofmann AF, Thomas PJ. Metabolism of [35S]Taurine in man. Journal of Nutrition [Internet]. 1975 Sep 1;105(9):1206–14. Available from: https://doi.org/10.1093/jn/105.9.1206

260. Yan R, Ko NL, Li SL, Tam YK, Lin G. Pharmacokinetics and metabolism of ligustilide, a major bioactive component in rhizoma chuanxiong, in the rat. Drug Metabolism and Disposition [Internet]. 2007 Nov 26;36(2):400–8. Available from: https://doi.org/10.1124/dmd.107.017707

261. Moridani MY, Scobie H, Jamshidzadeh A, Salehi P, O'Brien PJ. Caffeic acid, chlorogenic acid, and dihydrocaffeic acid metabolism: glutathione conjugate formation. PubMed [Internet]. 2001 Nov 1;29(11):1432–9. Available from: https://pubmed.ncbi.nlm.nih.gov/11602518

262. Zhao Z, Moghadasian MH. Bioavailability of hydroxycinnamates: a brief review of *in vivo* and *in vitro* studies. Phytochemistry Reviews [Internet]. 2009 Sep 1;9(1):133–45. Available from: https://doi.org/10.1007/s11101-009-9145-5

263. Pei K, Ou J, Huang J, Ou S. *p*-Coumaric acid and its conjugates: dietary sources, pharmacokinetic properties and biological activities. Journal of the Science of Food and Agriculture [Internet]. 2016 Jan 25;96(9):2952–62. Available from: https://doi.org/10.1002/jsfa.7578

264. Jacobsen J, Smith LH. Biochemistry and physiology of taurine and taurine derivatives. Physiological Reviews [Internet]. 1968 Apr 1;48(2):424–511. Available from: https://doi.org/10.1152/physrev.1968.48.2.424

265. Chesney RW. Taurine: its biological role and clinical implications. PubMed [Internet]. 1985 Jan 1;32:1–42. Available from: https://pubmed.ncbi.nlm.nih.gov/3909770

266. Naismith DJ, Rana SK, Emery PW. Metabolism of taurine during reproduction in women. PubMed [Internet]. 1987 Jan 1;41(1):37–45. Available from: https://pubmed.ncbi.nlm.nih.gov/3570861

267. Santoveña A, Sánchez-Negrín E, Charola L, Llabrés M, Fariña JB. Study of quality and stability of ursodeoxycholic acid formulations for oral pediatric administration. International Journal of Pharmaceutics [Internet]. 2014 Dec 1;477(1–2):32–8. Available from: https://doi.org/10.1016/j.ijpharm.2014.10.011

268. Aerts L, Van Assche FA. Taurine and taurine-deficiency in the perinatal period. Journal of Perinatal Medicine [Internet]. 2002 Jan 26;30(4). Available from: https://doi.org/10.1515/jpm.2002.040.

269. Raphael BP, Duggan C. Prevention and treatment of Intestinal Failure-Associated liver Disease in children. Seminars in Liver Disease [Internet]. 2013 Feb 8;32(04):341–7. Available from: https://doi.org/10.1055/s-0032-1329903

270. Chernobrovy VM. Biliarnyi refliuks-hastryt ta refliuks-ezofahit: aktualni pytannia zastosuvannia preparatu «Ukrliv» — ursodezoksykholevoi kysloty (perspektyva dobovoho ezofaho-impedans-pH-monitorynhu). [Bile reflux gastritis and esophagitis: the urgent questions of treatment with Ukrliv – ursodeoxycholic acid (future trends in diagnosis using 24-hour multichannel intraluminal impedance-pH-monitoring).] Suchasna hastroenterolohiia [Modern Gastroenterology]. 2013;2:47-53.

271. Pelletier G, Roulot D, Davion T, Masliah C, Causse X, Oberti F, et al. A randomized controlled trial of ursodeoxycholic acid in patients with alcohol-induced cirrhosis and jaundice. Hepatology [Internet]. 2003 Apr 1;37(4):887–92. Available from: https://doi.org/10.1053/jhep.2003.50118

272. Venneman NG, Besselink MG, Keulemans YCA, vanBerge-Henegouwen GP, Boermeester MA, Broeders I a. MJ, et al. Ursodeoxycholic acid exerts no beneficial effect in patients with symptomatic gallstones awaiting cholecystectomy.

Hepatology [Internet]. 2006 Jan 1;43(6):1276–83. Available from: https://doi.org/10.1002/hep.21182

273. Guarino MPL, Cong P, Cicala M, Alloni R, Carotti S, Behar J. Ursodeoxycholic acid improves muscle contractility and inflammation in symptomatic gallbladders with cholesterol gallstones. Gut [Internet]. 2007 Jun 1;56(6):815–20. Available from: https://doi.org/10.1136/gut.2006.109934

274. Roma MG, Toledo FD, Boaglio AC, Basiglio CL, Crocenzi FA, Pozzi EJS. Ursodeoxycholic acid in cholestasis: linking action mechanisms to therapeutic applications. Clinical Science [Internet]. 2011 Aug 22;121(12):523–44. Available from: https://doi.org/10.1042/cs20110184

275. Portincasa P, Di Ciaula A, Bonfrate L, Wang DQ -h. Therapy of gallstone disease: What it was, what it is, what it will be. World Journal of Gastrointestinal Pharmacology and Therapeutics [Internet]. 2012 Jan 1;3(2):7. Available from: https://doi.org/10.4292/wjgpt.v3.i2.7

276. Guarino MPL, Cocca S, Altomare A, Emerenziani S, Cicala M. Ursodeoxycholic acid therapy in gallbladder disease, a story not yet completed. World Journal of Gastroenterology [Internet]. 2013 Jan 1;19(31):5029. Available from: https://doi.org/10.3748/wjg.v19.i31.5029

277. Amaral JD, Viana RJS, Ramalho RM, Steer CJ, Rodrigues CMP. Bile acids: regulation of apoptosis by ursodeoxycholic acid. Journal of Lipid Research [Internet]. 2009 Sep 1;50(9):1721–34. Available from: https://doi.org/10.1194/jlr.r900011-jlr200

278. Tomida S, Abei M, Yamaguchi T, Matsuzaki Y, Shoda J, Tanaka N, et al. Long-term ursodeoxycholic acid therapy is associated with reduced risk of biliary pain and acute cholecystitis in patients with gallbladder stones: A cohort analysis. Hepatology [Internet]. 1999 Jul 1;30(1):6–13. Available from: https://doi.org/10.1002/hep.510300108

279. Maev YV, Kucheriavyi YuA, Morozov SV, Stukova NIu. Vlyianye preparatov ursodezoksykholevoi kyslotы na byokhymycheskye pokazately krovy y rezultaty elastohrafyy pecheny u patsyentov s alkoholnym tsyrrozom pecheny [Effect of ursodeoxycholic acid drugs on blood biochemical parameters and results of liver elastography in patients with alcohol-induced liver cirrhosis.] Klynycheskye perspektyvy hastroenterolohyy y hepatolohyy [Clinical perspectives in gastroenterology and hepatology]. 2010;4:43-48.

280. Sital RR, Kusters JG, De Rooij FWM, Kuipers EJ, Siersema PD. Bile acids and Barrett's oesophagus: A sine qua non or coincidence? Scandinavian Journal of Gastroenterology [Internet]. 2006 Jan 1;41(sup243):11–7. Available from: https://doi.org/10.1080/00365520600664219

281. Huo X, Juergens S, Zhang X, Rezaei D, Yu C, Strauch ED, et al. Deoxycholic acid causes DNA damage while inducing apoptotic resistance through NF-κB activation in benign Barrett's epithelial cells. American Journal of Physiology-gastrointestinal and Liver Physiology [Internet]. 2011 Aug 1;301(2):G278–86. Available from: https://doi.org/10.1152/ajpgi.00092.2011

282. Gérard P. Metabolism of cholesterol and bile acids by the gut microbiota. Pathogens [Internet]. 2013 Dec 30;3(1):14–24. Available from: https://doi.org/10.3390/pathogens3010014

283. Marcinkiewicz J, Kontny E. Taurine and inflammatory diseases. Amino Acids [Internet]. 2012 Jul 19;46(1):7–20. Available from: https://doi.org/10.1007/s00726-012-1361-4

284. Salem MB, Kolsi RBA, Dhouibi R, Ksouda K, Charfi S, Yaich M, et al. Protective effects of Cynara scolymus leaves extract on metabolic disorders and oxidative stress in alloxan-diabetic rats. BMC Complementary and Alternative Medicine [Internet]. 2017 Jun 19;17(1). Available from: https://doi.org/10.1186/s12906-017-1835-8

285. Babak OYa, Frolov VM, Fadeyenko GD. Vplyv kombinatsii artikholu ta vitaminu E na stan perekysnoho okyslennia lipidiv u khvorykh na nealkoholnyi steatohepatyt, poiednanyi z khronichnym nekalkuloznym kholetsystytom, na tli vtorynnykh imunodefitsytnykh staniv [Influence of the combination of articholes and vitamin E on the state of lipid peroxidation in patients with non-alcoholic steatohepatitis, combined with chronic noncalculous cholecystitis, against secondary immunodeficiency states.] Problemy ekoloho-medychnoi henetyky ta klinichnoi imunolohii [Problems of ecological and medical genetics and clinical immunology]. 2006;6(75):111-118.

286. Frolov VM, Peresadin MO, Khomutianska NI, Sydorova VS, Bykadorov VI. Vplyv hlutarhinu v kombinatsii z artyshokom ekstraktom Zdorovia ta enterosorbtsiieiu na pokaznyky mikrohemotsyrkuliatsii ta lipoperoksydatsii u pidlitkiv, khvorykh na khronichnyi nekalkuloznyi kholetsystyt. [Effect of glutargin in combination with artichoke extract Health and enterosorption on indicators of microhemocirculation and lipoperoxidation in adolescents with chronic noncalculous cholecystitis] Ukrainskyi morfolohichnyi almanakh [Ukrainian morphological almanac], 2009;4:185-191. Available from: http://nbuv.gov.ua/UJRN/Umora_2009_7_4_35

287. Fang L, Xiao X, Liu C, He X. Recent Advance in Studies on Angelica sinensis. Chinese Herbal Medicines [Internet]. 2012 Feb 1;4(1):12–25. Available from: https://doi.org/10.3969/j.issn.1674-6384.2012.01.004.

288. Wang J, Du JR, Wang Y, Kuang X, Wang C. Z-ligustilide attenuates lipopolysaccharide-induced proinflammatory response via inhibiting NF-κB pathway in primary rat microglia. Acta Pharmacologica Sinica [Internet]. 2010 Jun 28;31(7):791–7. Available from: https://doi.org/10.1038/aps.2010.71

289. Su YW, Chiou WF, Chao SH, Lee MH, Chen CC, Tsai YC. Ligustilide prevents LPS-induced iNOS expression in RAW 264.7 macrophages by preventing ROS production and down-regulating the MAPK, NF-κB and AP-1 signaling

pathways. International Immunopharmacology [Internet]. 2011 Sep 1;11(9):1166–72. Available from: https://doi.org/10.1016/j.intimp.2011.03.014

290. Wu X, Qian ZM, Zhu L, Du F, Yung W, Gong Q, et al. Neuroprotective effect of ligustilide against ischaemia-reperfusion injury via up-regulation of erythropoietin and down-regulation of RTP801. British Journal of Pharmacology [Internet]. 2011 Aug 22;164(2):332–43. Available from: https://doi.org/10.1111/j.1476-5381.2011.01337.x

291. Chen XD, Zhao SM, Hua XYu, Yu H. Angelica effects on proliferation and differentiation of neural stem cells from neonatal rats with intrauterin hypoxia. Journal of Clinical Rehabilitative Tissue Engineering Research, 2010, (27): 5050 - 5053 . DOI: 10.3969/j.issn.1673-8225.2010.27.025

292. Hua Y, Xue W, Zhang M, Wei Y, Ji P. Metabonomics study on the hepatoprotective effect of polysaccharides from different preparations of Angelica sinensis. Journal of Ethnopharmacology [Internet]. 2014 Feb 1;151(3):1090–9. Available from: https://doi.org/10.1016/j.jep.2013.12.011

293. Zaretskyi MM, Chernikova NM, Lobachevskaya TV. Vozmozhnosty prymenenyia ursodezoksykholevoi kysloty v lechenyy zhelchnokamennoi bolezny [Possibilities of using ursodeoxycholic acid in the treatment of cholelithiasis]. Sovremennaia gastroenterolohyia [Modern Gastroenterology], 2011;2:135-140. Available
from: http://www.vitapol.com.ua/user_files/pdfs/gastro/965243157577232_08052011144629.pdf

294. May GR, Sutherland LR, Shaffer EA. Efficacy of bile acid therapy for gallstone dissolution: a meta-analysis of randomized trials. Alimentary Pharmacology & Therapeutics [Internet]. 2007 Mar 31;7(2):139–48. Available from: https://doi.org/10.1111/j.1365-2036.1993.tb00082.x

295. Wu S, Li L, Wang J. Ursodeoxycholic acid for nonalcoholic steatohepatitis. European Journal of Gastroenterology & Hepatology [Internet]. 2012 Aug 1;1. Available from: https://doi.org/10.1097/meg.0b013e3283572ec0

296. Rudić JS, Poropat G, Krstić M, Bjelaković G, Gluud C. Ursodeoxycholic acid for primary biliary cirrhosis. The Cochrane Library [Internet]. 2012 Dec 12; Available from: https://doi.org/10.1002/14651858.cd000551.pub3

297. Triantos C, Koukias N, Nikolopoulou V, Burroughs AK. Meta-analysis: ursodeoxycholic acid for primary sclerosing cholangitis. Alimentary Pharmacology & Therapeutics [Internet]. 2011 Aug 22;34(8):901–10. Available from: https://doi.org/10.1111/j.1365-2036.2011.04822.x

298. Chen WD, Gluud C. Bile acids for primary sclerosing cholangitis [A cochrane systematic review]. Journal of Hepatology [Internet]. 2003 Apr 1;38:205. Available from: https://doi.org/10.1016/s0168-8278(03)80086-7

299. Hopf C, Grieshaber R, Hartmann H, Hinrichsen H, Eisold M, Cordes HJ, et al. Therapeutic equivalence of ursodeoxycholic acid tablets and ursodeoxycholic acid capsules for the treatment of primary biliary cirrhosis. Clinical Pharmacology in Drug Development [Internet]. 2013 Apr 15;2(3):231–6. Available from: https://doi.org/10.1002/cpdd.24

300. Gubska O.Yu. Rol y mesto preparatov ursodezoksykholevoi kysloty v lechenyy khronycheskoi patolohyy pecheny y hepatobylyarnoi systemy [The Role and Place of Ursodeoxycholic Acid Drugs in Treatment of Chronic Liver Disease and Pathology of Hepatobiliary System]. Ukrainskyi medychnyi chasopys [Ukrainian Medical Journal]. 2013;2:10-13.

301. Khokhlacheva NA, Sergeeva NN, Vakhrushev YaM . Age and gender peculiarities of development of gallstone disease. Arhiv″ Vnutrennej Mediciny [*Archive of internal medicine*] [Internet]. 2016 Mar 4;6(1):34–9. Available from: https://doi.org/10.20514/2226-6704-2016-6-1-34-39

302. Lammert F, Neubrand M, Bittner R, Feußner H, Greiner L, Hagenmüller F, et al. S3-Leitlinie der Deutschen Gesellschaft für Verdauungs- und Stoffwechselkrankheiten und der Deutschen Gesellschaft für Viszeralchirurgie zur Diagnostik und Behandlung von Gallensteinen. Zeitschrift Fur Gastroenterologie [Internet]. 2007 Sep 1;45(9):971–1001. Available from: https://doi.org/10.1055/s-2007-963437

303. Tran TDH, Ryu HC, Yoo SH, Rhee D. Antibacterial and anti-atrophic effects of a highly soluble, acid stable UDCA formula in Helicobacter pylori-induced gastritis. Biochemical Pharmacology [Internet]. 2008 Jun 1;75(11):2135–46. Available from: https://doi.org/10.1016/j.bcp.2008.03.008

304. Alberts DS, MartíNez ME, Hess LM, Einspahr JG, Green SB, Bhattacharyya AK, et al. Phase III Trial of Ursodeoxycholic Acid to Prevent Colorectal Adenoma Recurrence. Journal of the National Cancer Institute [Internet]. 2005 Jun 1;97(11):846–53. Available from: https://doi.org/10.1093/jnci/dji144

305. Larghi A, Crosignani A, Battezzati PM, Valle G, Allocca M, Invernizzi P, et al. Ursodeoxycholic and tauro-ursodeoxycholic acids for the treatment of primary biliary cirrhosis: a pilot crossover study. Alimentary Pharmacology & Therapeutics [Internet]. 1997 Apr 1;11(2):409–14. Available from: https://doi.org/10.1046/j.1365-2036.1997.124295000.x

306. Crosignani A, Battezzati PM, Setchell KDR, Invernizzi P, Covini G, Zuin M, et al. Tauroursodeoxycholic acid for treatment of primary biliary cirrhosis. Digestive Diseases and Sciences [Internet]. 1996 Apr 1;41(4):809–15. Available from: https://doi.org/10.1007/bf02213140

307. Mizushima S, Nara Y, Sawamura M, Yamori Y. Effects of oral taurine supplementation on lipids and sympathetic nerve tone. In: Advances in Experimental Medicine and Biology [Internet]. 1996. p. 615–22. Available from: https://doi.org/10.1007/978-1-4899-0182-8_68

308. Zhang M, Bi L, Fang J, Su X, Da G, Kuwamori T, et al. Beneficial effects of taurine on serum lipids in overweight or obese non-diabetic subjects. Amino Acids [Internet]. 2003 Dec 15;26(3). Available from: https://doi.org/10.1007/s00726-003-0059-z

309. De La Puerta C, Arrieta F, Balsa JA, Botella-Carretero JI, Zamarrón I, Vázquez C. Taurine and glucose metabolism: a review. PubMed [Internet]. 2011 Apr 27;25(6):910–9. Available from: https://pubmed.ncbi.nlm.nih.gov/21519760

310. Kirchhoff R, Beckers C, Kirchhoff GM, Trinczek-Gärtner H, Petrowicz O, Reimann HJ. Increase in choleresis by means of artichoke extract. Phytomedicine [Internet]. 1994 Sep 1;1(2):107–15. Available from: https://doi.org/10.1016/s0944-7113(11)80027-9

311. Bundy R, Walker AF, Middleton RW, Marakis G, Booth J. Artichoke Leaf Extract Reduces Symptoms of Irritable Bowel Syndrome and Improves Quality of Life in Otherwise Healthy Volunteers Suffering from Concomitant Dyspepsia: A Subset Analysis. Journal of Alternative and Complementary Medicine [Internet]. 2004 Aug 1;10(4):667–9. Available from: https://doi.org/10.1089/acm.2004.10.667

312. Englisch W, Beckers C, Unkauf M, Ruepp M, Zinserling V. Efficacy of Artichoke Dry Extract in Patients with Hyperlipoproteinemia. Drug Research [Internet]. 2011 Dec 27;50(03):260–5. Available from: https://doi.org/10.1055/s-0031-1300196

313. Sahebkar A, Pirro M, Banach M, Mikhailidis DP, Atkin SL, Cicero AFG. Lipid-lowering activity of artichoke extracts: A systematic review and meta-analysis. Critical Reviews in Food Science and Nutrition [Internet]. 2017 Aug 24;58(15):2549–56. Available from: https://doi.org/10.1080/10408398.2017.1332572

314. Wider B, Pittler MH, Thompson-Coon J, Ernst E. Artichoke leaf extract for treating hypercholesterolaemia. The Cochrane Library [Internet]. 2016 May 19; Available from: https://doi.org/10.1002/14651858.cd003335.pub4

315. Dong W, Liu S, Zhu H, Luo H, Yu JP. Abnormal function of platelets and role of angelica sinensis in patients with ulcerative colitis. World Journal of Gastroenterology [Internet]. 2004 Jan 1;10(4):606. Available from: https://doi.org/10.3748/wjg.v10.i4.606

316. Gan T, Liu Y, Wang Y, Yang J. Traditional Chinese Medicine herbs for stopping bleeding from haemorrhoids. The Cochrane Library [Internet]. 2010 Oct 6; Available from: https://doi.org/10.1002/14651858.cd006791.pub2

317. Dhami N, Mishra AD. Phytochemical variation: How to resolve the quality controversies of herbal medicinal products? Journal of Herbal Medicine [Internet]. 2015 Jun 1;5(2):118–27. Available from: https://doi.org/10.1016/j.hermed.2015.04.002

318. Galkin, AYu, Kotov, AG. Rozrobka metodiv kontroliu yakosti ta doslidzhennia halenovoho preparatu dlia likuvannia ta profilaktyky alopetsii. [Development of quality control methods and analysis of herbal preparation for treatment and prevention of alopecia]. Ukrainskyi zhurnal klinichnoi ta laboratornoi medytsyny [Ukrainian Journal of Clinical and Laboratory Medicine]. 2011;6(2):115-119.

319. Galkin AYu, Solovjova VF, Dugan A. Anti-inflammatory and immunomodulating properties of the herbal preparation indicated for prevention and treatment of alopecia. Botanics : Targets and Therapy [Internet]. 2013 Oct 1;49. Available from: https://doi.org/10.2147/btat.s45420

320. Gontova TM, Ilyinska N, Golembiovska O, Mashtaler VV . A study of the component composition of phenolic compounds obtained from Dahlia varieties Ken’s Flame herb. Der Pharma Chemica [Internet]. 2016 Jan 1;8(18):455–9. Available from: https://www.derpharmachemica.com/abstract/a-study-of-the-

component-composition-of-phenolic-compounds-obtained-from-dahlia-varieties-kens-flame-herb-6352.html

321. Lutsenko T, Kovalenko M, Galkin AYu. Validation of biological activity testing procedure of recombinant human interleukin-7. Ukrainian Biochemical Journal [Internet]. 2017 Feb 21;89(1):82–9. Available from: https://doi.org/10.15407/ubj89.01.082

322. Ramaekers JG. Drugs and driving research in medicinal drug development. Trends in Pharmacological Sciences [Internet]. 2017 Apr 1;38(4):319–21. Available from: https://doi.org/10.1016/j.tips.2017.01.006

323. European Pharmacopoeia 2020: European Pharmacopoeia. 9th edition. Strasbourg (FR): Directorate for the Quality of Medicines and HealthCare of the Council of Europe (EDQM); 2019.

324. Ben-Shabat S, Yarmolinsky L, Porat D, Dahan A. Antiviral effect of phytochemicals from medicinal plants: Applications and drug delivery strategies. Drug Delivery and Translational Research [Internet]. 2019 Dec 1;10(2):354–67. Available from: https://doi.org/10.1007/s13346-019-00691-6

325. Jassim S, Naji MA. Novel antiviral agents: a medicinal plant perspective. Journal of Applied Microbiology [Internet]. 2003 Sep 1;95(3):412–27. Available from: https://doi.org/10.1046/j.1365-2672.2003.02026.x

326. Ojo OO, Oluyege JO, Famurewa O. Antiviral properties of two Nigerian plants. African Journal of Plant Science [Internet]. 2009 Jul 31;3(7):157–9. Available from: https://academicjournals.org/journal/AJPS/article-abstract/5DA05F310994

327. Liu A, Du G. Antiviral properties of phytochemicals. In: Springer eBooks [Internet]. 2012. p. 93–126. Available from: https://doi.org/10.1007/978-94-007-3926-0_3

328. Sun Z, Zhao T, Lü N, Yang Y, Zhu H. Research Progress of glycyrrhizic acid on antiviral activity. Mini-reviews in Medicinal Chemistry [Internet]. 2019 May 29;19(10):826–32. Available from: https://doi.org/10.2174/1389557519666190119111125

329. Michelini F, Alché LE, Bueno CA. Virucidal, antiviral and immunomodulatory activities of β-escin and *Aesculus hippocastanum* extract. Journal of Pharmacy and Pharmacology [Internet]. 2018 Aug 30;70(11):1561–71. Available from: https://doi.org/10.1111/jphp.13002

330. Ghoke SS, Sood R, Kumar N, Pateriya AK, Bhatia S, Mishra A, et al. Evaluation of antiviral activity of Ocimum sanctum and Acacia arabica leaves extracts against H9N2 virus using embryonated chicken egg model. BMC Complementary and Alternative Medicine [Internet]. 2018 Jun 5;18(1). Available from: https://doi.org/10.1186/s12906-018-2238-1

331. Khwaza V, Oyedeji OO, Aderibigbe BA. Antiviral activities of oleanolic acid and its analogues. Molecules [Internet]. 2018 Sep 9;23(9):2300. Available from: https://doi.org/10.3390/molecules23092300

332. Akram M, Tahir IM, Shah SMA, Mahmood Z, Altaf A, Ahmad K, et al. Antiviral potential of medicinal plants against HIV, HSV, influenza, hepatitis, and coxsackievirus: A systematic review. Phytotherapy Research [Internet]. 2018 Jan 22;32(5):811–22. Available from: https://doi.org/10.1002/ptr.6024

333. De Oliveira JR, Camargo SEA, De Oliveira LD. Rosmarinus officinalis L. (rosemary) as therapeutic and prophylactic agent. Journal of Biomedical Science [Internet]. 2019 Jan 9;26(1). Available from: https://doi.org/10.1186/s12929-019-0499-8

334. Choi HJ. Chemical Constituents of Essential Oils Possessing Anti-Influenza A/WS/33 Virus Activity. Osong Public Health and Research Perspectives [Internet].

2018 Dec 31;9(6):348–53. Available from: https://doi.org/10.24171/j.phrp.2018.9.6.09

335. Tohmé MJ, Giménez MC, Peralta A, Colombo M, Delgui LR. Ursolic acid: A novel antiviral compound inhibiting rotavirus infection *in vitro*. International Journal of Antimicrobial Agents [Internet]. 2019 Nov 1;54(5):601–9. Available from: https://doi.org/10.1016/j.ijantimicag.2019.07.015

336. Li BY, Hu Y, Li J, Shi K, Shen Y, Zhu B, et al. Ursolic acid from Prunella vulgaris L. efficiently inhibits IHNV infection *in vitro* and *in vivo*. Virus Research [Internet]. 2019 Nov 1;273:197741. Available from: https://doi.org/10.1016/j.virusres.2019.197741

337. Guo SS, Bao L, Li C, Sun J, Zhao R, Cui X. Antiviral activity of iridoid glycosides extracted from Fructus Gardeniae against influenza A virus by PACT-dependent suppression of viral RNA replication. Scientific Reports [Internet]. 2020 Feb 5;10(1). Available from: https://doi.org/10.1038/s41598-020-58443-3

338. Qian W, Zhang R, Wang Q, Yan XJ, Yu QW, Yan FX, et al. Iridoid, phenylethanoid and flavonoid glycosides from *Forsythia suspensa*. Natural Product Research [Internet]. 2019 Jan 24;34(9):1320–5. Available from: https://doi.org/10.1080/14786419.2018.1560288

339. Wang Z, Xia Q, Liu X, Liu W, Huang W, Xue M, et al. Phytochemistry, pharmacology, quality control and future research of Forsythia suspensa (Thunb.) Vahl: A review. Journal of Ethnopharmacology [Internet]. 2018 Jan 1;210:318–39. Available from: https://doi.org/10.1016/j.jep.2017.08.040

340. Huh J, Song J, Kim SR, Cho HM, Ko H, Yang H, et al. Lignan Dimers from *Forsythia viridissima* Roots and Their Antiviral Effects. Journal of Natural Products [Internet]. 2019 Jan 24;82(2):232–8. Available from: https://doi.org/10.1021/acs.jnatprod.8b00590

341. Dias MM, Zuza O, Riani LR, De Faria Pinto P, Pintó P, Silva MP, et al. *In vitro* schistosomicidal and antiviral activities of Arctium lappa L. (Asteraceae) against Schistosoma mansoni and Herpes simplex virus-1. Biomedicine & Pharmacotherapy [Internet]. 2017 Oct 1;94:489–98. Available from: https://doi.org/10.1016/j.biopha.2017.07.116

342. Hu Y, Chen W, Shen Y, Zhu B, Wang G. Synthesis and antiviral activity of a new arctigenin derivative against IHNV *in vitro* and *in vivo*. Fish & Shellfish Immunology [Internet]. 2019 Sep 1;92:736–45. Available from: https://doi.org/10.1016/j.fsi.2019.07.006

343. Zhong Y, Jin C, Wang X, Li X, Han J, Wei X, et al. Protective effects of apigenin against 3-MCPD-induced renal injury in rat. Chemico-Biological Interactions [Internet]. 2018 Dec 1;296:9–17. Available from: https://doi.org/10.1016/j.cbi.2018.08.005

344. Khandelwal N, Chander Y, Kumar R, Riyesh T, Dedar RK, Kumar M, et al. Antiviral activity of Apigenin against buffalopox: Novel mechanistic insights and drug-resistance considerations. Antiviral Research [Internet]. 2020 Sep 1;181:104870. Available from: https://doi.org/10.1016/j.antiviral.2020.104870

345. Yan H, Ma L, Wang H, Wu S, Hua H, Gu Z, et al. Luteolin decreases the yield of influenza A virus *in vitro* by interfering with the coat protein I complex expression. Journal of Natural Medicines [Internet]. 2019 Feb 13;73(3):487–96. Available from: https://doi.org/10.1007/s11418-019-01287-7

346. Yu R, Liang C, Liu R, Shen R, Li P. Computational screening of antagonists against the SARS-CoV-2 (COVID-19) coronavirus by molecular docking. International Journal of Antimicrobial Agents [Internet]. 2020 Aug 1;56(2):106012. Available from: https://doi.org/10.1016/j.ijantimicag.2020.106012

347. Ferracane R, Graziani G, Gallo M, Fogliano V, Ritieni A. Metabolic profile of the bioactive compounds of burdock (Arctium lappa) seeds, roots and leaves. Journal

of Pharmaceutical and Biomedical Analysis [Internet]. 2010 Jan 1;51(2):399–404. Available from: https://doi.org/10.1016/j.jpba.2009.03.018

348. Yao C, Xi C, Hu K, Gao W, Cai X, Qin J, et al. Inhibition of enterovirus 71 replication and viral 3C protease by quercetin. Virology Journal [Internet]. 2018 Jul 31;15(1). Available from: https://doi.org/10.1186/s12985-018-1023-6

349. Biancatelli RMLC, Berrill M, Catravas JD, Marik PE. Quercetin and vitamin C: An experimental, synergistic therapy for the prevention and treatment of SARS-COV-2 related disease (COVID-19). Frontiers in Immunology [Internet]. 2020 Jun 19;11. Available from: https://doi.org/10.3389/fimmu.2020.01451

350. Kim CH, Kim JE, Song YB. Antiviral activities of quercetin and isoquercitrin against human herpesviruses. Molecules [Internet]. 2020 May 20;25(10):2379. Available from: https://doi.org/10.3390/molecules25102379

351. Ninfali P, Antonelli A, Magnani M, Scarpa ES. Antiviral properties of flavonoids and delivery strategies. Nutrients [Internet]. 2020 Aug 21;12(9):2534. Available from: https://doi.org/10.3390/nu12092534

352. Di Sotto A, Checconi P, Celestino I, Locatelli M, Carissimi S, De Angelis M, et al. Antiviral and Antioxidant Activity of a Hydroalcoholic Extract from *Humulus lupulus* L. Oxidative Medicine and Cellular Longevity [Internet]. 2018 Jul 24;2018:1–14. Available from: https://doi.org/10.1155/2018/5919237

353. Das P, Majumder R, Mandal M, Basak P. In-Silico approach for identification of effective and stable inhibitors for COVID-19 main protease (M^{pro}) from flavonoid based phytochemical constituents of *Calendula officinalis*. Journal of Biomolecular Structure & Dynamics [Internet]. 2020 Jul 24;39(16):6265–80. Available from: https://doi.org/10.1080/07391102.2020.1796799

354. Wang L, Song J, Liu A, Xiao B, Li S, Zhang W, et al. Research progress of the antiviral bioactivities of natural flavonoids. Natural Products and Bioprospecting

[Internet]. 2020 Sep 18;10(5):271–83. Available from: https://doi.org/10.1007/s13659-020-00257-x

355. Katanić J, Pferschy-Wenzig E, Mihailović V, Boroja T, Pan SP, Nikles S, et al. Phytochemical analysis and anti-inflammatory effects of Filipendula vulgaris Moench extracts. Food and Chemical Toxicology [Internet]. 2018 Dec 1;122:151–62. Available from: https://doi.org/10.1016/j.fct.2018.10.001

356. Chen H, Ishfaq M, Zhang Y, Ren Y, Zhang R, Huang X, et al. Antiviral Activity Against Infectious Bronchitis Virus and Bioactive Components of Hypericum perforatum L. Frontiers in Pharmacology [Internet]. 2019 Oct 29;10. Available from: https://doi.org/10.3389/fphar.2019.01272

357. Barboza RS, Valente LMM, Wolff T, Assunção-Miranda I, Neris RLS, Guimarães-Andrade IP, et al. Antiviral Activity of Faramea hyacinthina and Faramea truncata Leaves on Dengue Virus Type-2 and Their Major Compounds. Chemistry & Biodiversity [Internet]. 2018 Feb 1;15(2):e1700393. Available from: https://doi.org/10.1002/cbdv.201700393

358. Yonekawa M, Shimizu M, Kaneko A, Matsumura J, Takahashi H. Suppression of R5-type of HIV-1 in CD4+ NKT cells by Vδ1+ T cells activated by flavonoid glycosides, hesperidin and linarin. Scientific Reports [Internet]. 2019 May 17;9(1). Available from: https://doi.org/10.1038/s41598-019-40587-6

359. Luo E, Zhang D, Luo H, Liu B, Keming Z, Zhao Y, et al. Treatment efficacy analysis of traditional Chinese medicine for novel coronavirus pneumonia (COVID-19): an empirical study from Wuhan, Hubei Province, China. Chinese Medicine [Internet]. 2020 Apr 15;15(1). Available from: https://doi.org/10.1186/s13020-020-00317-x

360. Xu L, Jiang W, Jia H, Zheng L, Xing J, Liu A, et al. Discovery of Multitarget-Directed Ligands Against Influenza A Virus From Compound Yizhihao Through a Predictive System for Compound-Protein Interactions. Frontiers in Cellular and

Infection Microbiology [Internet]. 2020 Feb 11;10. Available from: https://doi.org/10.3389/fcimb.2020.00016

361. Aly SH, Elissawy AM, Eldahshan OA, El-Shanawany MA, Efferth T, Singab ANB. The pharmacology of the genus Sophora (Fabaceae): An updated review. Phytomedicine [Internet]. 2019 Nov 1;64:153070. Available from: https://doi.org/10.1016/j.phymed.2019.153070

362. Ren G, Ding G, Zhang H, Wang H, Jin Z, Yang G, et al. Antiviral activity of sophoridine against enterovirus 71 *in vitro*. Journal of Ethnopharmacology [Internet]. 2019 May 1;236:124–8. Available from: https://doi.org/10.1016/j.jep.2019.02.045

363. Stierle AA, Stierle DB, Decato DA, Priestley ND, Alverson JB, Hoody J, et al. The Berkeleylactones, Antibiotic Macrolides from Fungal Coculture. Journal of Natural Products [Internet]. 2017 Mar 22;80(4):1150–60. Available from: https://doi.org/10.1021/acs.jnatprod.7b00133

364. Deyrup M, Sidwell RW, Little RF, Druzgala P, Bodor N, Brewster ME. Improved Delivery through Biological Membranes. Synthesis and Antiviral Activity of a Series of Ribavirin Chemical Delivery Systems: 2′ and 3′ Derivatives. Antiviral Chemistry & Chemotherapy [Internet]. 1991 Dec 1;2(6):337–55. Available from: https://doi.org/10.1177/095632029100200603

365. Ryu YB, Long MJC, Kim JH, Jeong SH, Yang M, Lee KW, et al. Pterocarpans and flavanones from Sophora flavescens displaying potent neuraminidase inhibition. Bioorganic & Medicinal Chemistry Letters [Internet]. 2008 Dec 1;18(23):6046–9. Available from: https://doi.org/10.1016/j.bmcl.2008.10.033

366. Dimeglio C, Mansuy J, Charpentier S, Claudet I, Izopet J. Children are protected against SARS-CoV-2 infection. Journal of Clinical Virology [Internet]. 2020 Jul 1;128:104451. Available from: https://doi.org/10.1016/j.jcv.2020.104451

367. Naithani R, Huma LC, Holland LE, Shukla D, McCormick DL, Mehta RG, et al. Antiviral activity of Phytochemicals: A Comprehensive review. Mini-reviews in Medicinal Chemistry [Internet]. 2008 Oct 1;8(11):1106–33. Available from: https://doi.org/10.2174/138955708785909943

368. Russo M, Moccia S, Spagnuolo C, Tedesco I, Russo GL. Roles of flavonoids against coronavirus infection. Chemico-Biological Interactions [Internet]. 2020 Sep 1;328:109211. Available from: https://doi.org/10.1016/j.cbi.2020.109211

369. Fujimoto K, Nema D, Ninomiya M, Koketsu M, Sadanari H, Takemoto M, et al. An in silico-designed flavone derivative, 6-fluoro-4′-hydroxy-3′,5′-dimetoxyflavone, has a greater anti-human cytomegalovirus effect than ganciclovir in infected cells. Antiviral Research [Internet]. 2018 Jun 1;154:10–6. Available from: https://doi.org/10.1016/j.antiviral.2018.03.006

370. Dmytrenko O, Arkhypova M, Starosyla D, Рыбалко СЛ, Gevorkyan M, Galkin AYu. Biological evaluation of medical devices in the form of suppositories for rectal and vaginal use. Innovative Biosystems and Bioengineering [Internet]. 2021 Dec 26;5(4):228–37. Available from: https://doi.org/10.20535/ibb.2021.5.4.249082

371. Peter L, Hájek L, Marešová P, Augustýnek M, Penhaker M. Medical Devices: Regulation, risk classification, and open innovation. Journal of Open Innovation: Technology, Market, and Complexity [Internet]. 2020 Jun 1;6(2):42. Available from: https://doi.org/10.3390/joitmc6020042

372. Green J. Medical device legislation for custom-made devices after the UK has left the EU: answers to ten important questions. British Dental Journal [Internet]. 2021 Oct 22;231(8):513–21. Available from: https://doi.org/10.1038/s41415-021-3530-x

373. Bedi O, Krishan P, Singh G. Regulatory Requirements for Medical Devices: An insight. Applied Clinical Research, Clinical Trials and Regulatory Affairs

[Internet]. 2017 Feb 27;4(1):16–25. Available from: https://doi.org/10.2174/2213476x03666160804153513

374. Parvizi N, Woods KL. Regulation of medicines and medical devices: contrasts and similarities. Clinical Medicine [Internet]. 2014 Feb 1;14(1):6–12. Available from: https://doi.org/10.7861/clinmedicine.14-1-6

375. Godlee F. Why aren't medical devices regulated like drugs? BMJ [Internet]. 2018 Nov 29;k5032. Available from: https://doi.org/10.1136/bmj.k5032

376. Kramer DB, Tan YT, Sato C, Kesselheim AS. Ensuring medical device effectiveness and safety: a cross--national comparison of approaches to regulation. PubMed [Internet]. 2014 Jan 1; Available from: https://pubmed.ncbi.nlm.nih.gov/24772683

377. Miclăuş T, Valla V, Koukoura A, Nielsen AA, Dahlerup B, Tsianos GI, et al. Impact of design on medical device safety. Therapeutic Innovation & Regulatory Science [Internet]. 2019 Dec 9;54(4):839–49. Available from: https://doi.org/10.1007/s43441-019-00022-4

378. Palojoki S, Saranto K, Lehtonen L. Reporting medical device safety incidents to regulatory authorities: An analysis and classification of technology-induced errors. Health Informatics Journal [Internet]. 2017 Jul 27;25(3):731–40. Available from: https://doi.org/10.1177/1460458217720400

379. Geavlete P, Multescu R, Geavlete B. *Serenoa repens* extract in the treatment of benign prostatic hyperplasia. Therapeutic Advances in Urology [Internet]. 2011 Aug 1;3(4):193–8. Available from: https://doi.org/10.1177/1756287211418725

380. Spréa RM, Fernandes Â, Finimundy TC, Pereira C, Alves MJ, Calhelha RC, et al. Lovage (Levisticum officinale W.D.J. Koch) Roots: A Source of Bioactive Compounds towards a Circular Economy. Resources [Internet]. 2020 Jun 30;9(7):81. Available from: https://doi.org/10.3390/resources9070081

381. Ashwlayan VD, Kumar A, Verma M, Garg V, Gupta S. Therapeutic Potential of Calendula officinalis. Pharmacy & Pharmacology International Journal [Internet]. 2018 Apr 20;6(2). Available from: https://doi.org/10.15406/ppij.2018.06.00171

382. Stern AD. Innovation Under Regulatory Uncertainty: Evidence from Medical Technology. Social Science Research Network [Internet]. 2016 Jan 1; Available from: https://doi.org/10.2139/ssrn.2853173

383. Kramer DB, Xu S, Kesselheim AS. How does medical device regulation perform in the United States and the European Union? A systematic review. PLOS Medicine [Internet]. 2012 Jul 31;9(7):e1001276. Available from: https://doi.org/10.1371/journal.pmed.1001276

384. Van Norman GA. Drugs and devices. JACC: Basic to Translational Science [Internet]. 2016 Aug 1;1(5):399–412. Available from: https://doi.org/10.1016/j.jacbts.2016.06.003

385. Van Norman GA. Drugs, Devices, and the FDA: Part 1. JACC: Basic to Translational Science [Internet]. 2016 Apr 1;1(3):170–9. Available from: https://doi.org/10.1016/j.jacbts.2016.03.002

387. Tian J, Xu S, Wang Y, Cheng M, Lu S, Xu W, et al. Regulatory perspectives of combination products. Bioactive Materials [Internet]. 2022 Apr 1;10:492–503. Available from: https://doi.org/10.1016/j.bioactmat.2021.09.002

386. Van Norman GA. Drugs, Devices, and the FDA: Part 2. JACC: Basic to Translational Science [Internet]. 2016 Jun 1;1(4):277–87. Available from: https://doi.org/10.1016/j.jacbts.2016.03.009

388. Contardi M. Changes in the medical device's regulatory framework and its impact on the medical device's industry: from the medical device directives to the medical device regulations. Erasmus Law Review [Internet]. 2019 Dec 1;12(2):166–77. Available from: https://doi.org/10.5553/elr.000139

389. Di Pierro F, Di Paola G, Risso P. Role of a medical device for intra-vaginal use in improving the quality of the colposcopic examination and the anatomical/pathological reading of the cytological test and biopsy. PubMed [Internet]. 2014 Aug 20;85(2):121–6. Available from: https://pubmed.ncbi.nlm.nih.gov/25245647

390. Plummer EL, Bradshaw CS, Doyle M, Fairley CK, Murray GL, Bateson D, et al. Lactic acid-containing products for bacterial vaginosis and their impact on the vaginal microbiota: A systematic review. PLOS ONE [Internet]. 2021 Feb 11;16(2):e0246953. Available from: https://doi.org/10.1371/journal.pone.0246953

391. Papa R, Troncone MG, Altruda F, Rullo V, Saponati G. Clinical Evaluation of the Efficacy and Safety of a Medical Vaginal Device Containing Rigenase® for the Treatment of Vaginosis: A Randomized Study. Journal of Clinical Gynecology and Obstetrics [Internet]. 2017 Jan 1;6(1):6–11. Available from: https://doi.org/10.14740/jcgo424w

392. Oliva MM, Poverini R, Lisi R, Carra MC, Lisi F. Treating Woman with Myo-Inositol Vaginal Suppositories Improves Partner's Sperm Motility and Fertility. International Journal of Endocrinology [Internet]. 2016 Jan 1;2016:1–5. Available from: https://doi.org/10.1155/2016/7621942

393. Ham AS, Buckheit RW. Designing and developing suppository formulations for anti-HIV drug delivery. Therapeutic Delivery [Internet]. 2017 Aug 1;8(9):805–17. Available from: https://doi.org/10.4155/tde-2017-0056

394. Sibona M, Destefanis P, Agnello M, Lillaz B, Giuliano M, Cai T, et al. The association of Boswellia resin extract and propolis derived polyphenols can improve quality of life in patients affected by prostatitis-like symptoms. Archivio Italiano Di Urologia, Andrologia [Internet]. 2020 Jan 14;91(4):251–5. Available from: https://doi.org/10.4081/aiua.2019.4.251

395. Montrone S, Gonnelli A, Cantarella M, Sainato A. Use of Proktis-M suppositories in patients undergoing neoadjuvant radiochemotherapy for adenocarcinoma of the rectum. PubMed [Internet]. 2015 Dec 1;61(4):293–7. Available from: https://pubmed.ncbi.nlm.nih.gov/26657928

396. Marešová P, Režný L, Peter L, Hájek L, Lefley F. Do regulatory changes seriously affect the medical devices industry? Evidence from the Czech Republic. Frontiers in Public Health [Internet]. 2021 Apr 28;9. Available from: https://doi.org/10.3389/fpubh.2021.666453

397. Iguchi K, Okumura N, Usui S, Sajiki H, Hirota K, Hirano K. Myristoleic acid, a cytotoxic component in the extract fromSerenoa repens, induces apoptosis and necrosis in human prostatic LNCaP cells. The Prostate [Internet]. 2001 Jan 1;47(1):59–65. Available from: https://doi.org/10.1002/pros.1047

398. Shimada H, Tyler VE, McLaughlin JL. Biologically Active Acylglycerides from the Berries of Saw-Palmetto (*Serenoa repens*). Journal of Natural Products [Internet]. 1997 Apr 1;60(4):417–8. Available from: https://doi.org/10.1021/np960552o

399. Li YL, Yeung C, Chiu LCM, Cen YZ, Ooi VEC. Chemical composition and antiproliferative activity of essential oil from the leaves of a medicinal herb,*Schefflera heptaphylla*. Phytotherapy Research [Internet]. 2009 Jan 1;23(1):140–2. Available from: https://doi.org/10.1002/ptr.2567

400. Sargazi S, Saravani R, Galavi H, Mollashahee-Kohkan F. Effect of Levisticum officinale Hydroalcoholic Extract on DU-145 and PC-3 Prostate Cancer Cell Lines. Gene, Cell and Tissue [Internet]. 2017 Oct 31;4(4). Available from: https://doi.org/10.5812/gct.66094

401. Cruceriu D, Diaconeasa Z, Socaci S, Socaciu C, Rákosy-Tican E, Bălăcescu O. Biochemical profile, selective cytotoxicity and molecular effects of Calendula officinalis extracts on breast cancer cell lines. Notulae Botanicae Horti Agrobotanici

Cluj-napoca [Internet]. 2020 Mar 31;48(1):24–39. Available from: https://doi.org/10.15835/nbha48111778

402. Jiménez-Medina E, García-Lora A, Paco L, Algarra I, Collado A, Garrido F. A new extract of the plant calendula officinalis produces a dual *in vitro* effect: cytotoxic anti-tumor activity and lymphocyte activation. BMC Cancer [Internet]. 2006 May 5;6(1). Available from: https://doi.org/10.1186/1471-2407-6-119

403. Zaichenko GV, Horchakova NO, Stryha OA, Ruban OI. Farmakolohichne obhruntuvannia rozrobky novykh likarskykh preparativ na osnovi resveratrolu [Pharmacological rationale for the development of new drugs based on resveratrol]. Visnyk problem biolohii i medytsyny [Herald of problems of biology and medicine]. 2017; 4(1):21-30. Available from: http://repository.pdmu.edu.ua/handle/123456789/13647

404. Xue Y, Di J, Luo Y, Cheng K, Xing W, Shi Z. Resveratrol oligomers for the prevention and treatment of cancers. Oxidative Medicine and Cellular Longevity [Internet]. 2014 Jan 1;2014:1–9. Available from: https://doi.org/10.1155/2014/765832

405. Shifren JL, Crandall CJ, Manson JE. Menopausal hormone therapy. JAMA [Internet]. 2019 Jun 25;321(24):2458. Available from: https://doi.org/10.1001/jama.2019.5346

406. Sarmento ACA, Costa APF, Lírio J, Eleutério J, Vieira-Baptista P, Gonçalves AK. Efficacy of hormonal and nonhormonal approaches to vaginal atrophy and sexual dysfunctions in postmenopausal Women: a Systematic review. Revista Brasileira De Ginecologia E Obstetrícia [Internet]. 2022 Oct 1;44(10):986–94. Available from: https://doi.org/10.1055/s-0042-1756148

407. Gentry-Maharaj A, Karpinskyj C, Glazer CH, Burnell M, Ryan A, Fraser L, et al. Use and perceived efficacy of complementary and alternative medicines after

discontinuation of hormone therapy. Menopause [Internet]. 2015 Apr 1;22(4):384–90. Available from: https://doi.org/10.1097/gme.0000000000000330

408. Liang R, Knight K, Easley DC, Palcsey S, Abramowitch SD, Moalli P. Towards rebuilding vaginal support utilizing an extracellular matrix bioscaffold. Acta Biomaterialia [Internet]. 2017 Jul 1;57:324–33. Available from: https://doi.org/10.1016/j.actbio.2017.05.015

409. Jeong CH, Kim DH, Yune JH, Kwon HC, Shin DM, Sohn H, et al. *In vitro* toxicity assessment of crosslinking agents used in hyaluronic acid dermal filler. Toxicology in Vitro [Internet]. 2021 Feb 1;70:105034. Available from: https://doi.org/10.1016/j.tiv.2020.105034

410. Liu H, Lightfoot R, Stevens J. Activation of heat shock factor by alkylating agents is triggered by glutathione depletion and oxidation of protein thiols. Journal of Biological Chemistry [Internet]. 1996 Mar 1;271(9):4805–12. Available from: https://doi.org/10.1074/jbc.271.9.4805

411. Asadi S, Moradi M, Khyripour N, Goodarzi MT, Mahmoodi M. Resveratrol attenuates copper and zinc homeostasis and ameliorates oxidative stress in type 2 diabetic rats. Biological Trace Element Research [Internet]. 2016 Oct 15;177(1):132–8. Available from: https://doi.org/10.1007/s12011-016-0861-6

412. Lu X, Ma L, Ruan L, Kong Y, Mou H, Zhang Z, et al. Resveratrol differentially modulates inflammatory responses of microglia and astrocytes. Journal of Neuroinflammation [Internet]. 2010 Aug 17;7(1). Available from: https://doi.org/10.1186/1742-2094-7-46

413. Zaychenko GV, Stryga O, Sinitsyna OO, Doroshenkoo AN, Sulaieva O, Falalyeyeva T, et al. Resveratrol effects on the reproductive system in ovariectomized rats: Deciphering possible mechanisms. Molecules [Internet]. 2022 Aug 2;27(15):4916. Available from: https://doi.org/10.3390/molecules27154916

414. Naumova I, Castelo-Branco C. Current treatment options for postmenopausal vaginal atrophy. International Journal of Women's Health [Internet]. 2018 Jul 1;Volume 10:387–95. Available from: https://doi.org/10.2147/ijwh.s158913

415. Roby KF. Vaginitis. In: Elsevier eBooks [Internet]. 2019. Available from: https://doi.org/10.1016/b978-0-12-801238-3.11379-0

416. Tsoumani M, Nikolaou P, Argyropoulou A, Tseti I, Mitakou S, Andreadou I. Novel Evidence-Based Combination of Plant Extracts with Multitarget Mechanisms of Action for the Elimination of Hot Flashes during Menopause. Molecules [Internet]. 2022 Feb 11;27(4):1221. Available from: https://doi.org/10.3390/molecules27041221

417. Shirasuna K, Iwata H. Effect of aging on the female reproductive function. Contraception and Reproductive Medicine [Internet]. 2017 Oct 3;2(1). Available from: https://doi.org/10.1186/s40834-017-0050-9

418. Kenda M, Glavač NK, Nagy M, Dolenc MS, Oemonom OBO. Herbal products used in menopause and for gynecological disorders. Molecules [Internet]. 2021 Dec 8;26(24):7421. Available from: https://doi.org/10.3390/molecules26247421

419. Baber R, Panay N, Fenton A. 2016 IMS Recommendations on women's midlife health and menopause hormone therapy. Climacteric [Internet]. 2016 Feb 12;19(2):109–50. Available from: https://doi.org/10.3109/13697137.2015.1129166

420. Rossouw JE, Anderson GL, Prentice RL, LaCroix AZ, Kooperberg C, Stefanick ML, et al. Risks and benefits of estrogen plus progestin in healthy postmenopausal women: principal results from the Women's Health Initiative randomized controlled trial. JAMA [Internet]. 2002 Jul 17;288(3):321–33. Available from: https://doi.org/10.1001/jama.288.3.321

421. Ameye L, Antoine C, Paesmans M, De Azambuja E, Rozenberg S. Menopausal hormone therapy use in 17 European countries during the last decade. Maturitas

[Internet]. 2014 Nov 1;79(3):287–91. Available from: https://doi.org/10.1016/j.maturitas.2014.07.002

422. Ruan X, Mueck AO. Hormonal treatment of vulvar vaginal atrophy (VVA): Are there options to reduce or avoid systemic adverse effects and risks? Clinical Research and Trials [Internet]. 2018 Jan 1;4(6). Available from: https://doi.org/10.15761/crt.1000239

423. Santen RJ. Vaginal administration of estradiol: effects of dose, preparation and timing on plasma estradiol levels. Climacteric [Internet]. 2014 Oct 18;18(2):121–34. Available from: https://doi.org/10.3109/13697137.2014.947254

424. Gustavino C, Sala P, Cusini N, Gravina B, Ronzini C, Marcolin D, et al. Efficacy and safety of prolonged-release hyaluronic acid derivative vaginal application in the postpartum period: a prospective randomised clinical trial. Annals of Medicine [Internet]. 2021 Jan 1;53(1):1589–97. Available from: https://doi.org/10.1080/07853890.2021.1974083

425. Carter J, Goldfarb S, Baser RE, Goldfrank DJ, Seidel B, Milli L, et al. A single-arm clinical trial investigating the effectiveness of a non-hormonal, hyaluronic acid-based vaginal moisturizer in endometrial cancer survivors. Gynecologic Oncology [Internet]. 2020 Aug 1;158(2):366–74. Available from: https://doi.org/10.1016/j.ygyno.2020.05.025

426. Sinha AK, Ewies A. Non-hormonal topical treatment of vulvovaginal atrophy: an up-to-date overview. Climacteric [Internet]. 2013 Jan 8;16(3):305–12. Available from: https://doi.org/10.3109/13697137.2012.756466

427. De Seta F, Caruso S, Di Lorenzo G, Romano F, Mirandola M, Nappi RE. Efficacy and safety of a new vaginal gel for the treatment of symptoms associated with vulvovaginal atrophy in postmenopausal women: A double-blind randomized placebo-controlled study. Maturitas [Internet]. 2021 May 1;147:34–40. Available from: https://doi.org/10.1016/j.maturitas.2021.03.002

428. Novaković R, Rajković J, Gostimirović M, Gojković-Bukarica L, Radunović N. Resveratrol and reproductive health. Life [Internet]. 2022 Feb 16;12(2):294. Available from: https://doi.org/10.3390/life12020294

429. Kisková T, Kubatka P, Büsselberg D, Kassayová M. The Plant-Derived compound Resveratrol in brain Cancer: a review. Biomolecules [Internet]. 2020 Jan 19;10(1):161. Available from: https://doi.org/10.3390/biom10010161

430. Galiniak S, Aebisher D, Bartusik-Aebisher D. Health benefits of resveratrol administration. Acta Biochimica Polonica [Internet]. 2019 Feb 28; Available from: https://doi.org/10.18388/abp.2018_2749

431. Gašparová I, Kubatka P, Opatřilová R, Čaprnda M, Filipová S, Rodrigo L, et al. Perspectives and challenges of antioxidant therapy for atrial fibrillation. Naunyn-Schmiedeberg's Archives of Pharmacology [Internet]. 2016 Nov 29;390(1):1–14. Available from: https://doi.org/10.1007/s00210-016-1320-9

432. Model JFA, Lima MV, Ohlweiler R, Vogt ÉL, Rocha DS, De Souza SK, et al. Liraglutide improves lipid and carbohydrate metabolism of ovariectomized rats. Molecular and Cellular Endocrinology [Internet]. 2021 Mar 1;524:111158. Available from: https://doi.org/10.1016/j.mce.2021.111158

433. Ko SH, Kim H. Menopause-Associated Lipid Metabolic Disorders and Foods Beneficial for postmenopausal women. Nutrients [Internet]. 2020 Jan 13;12(1):202. Available from: https://doi.org/10.3390/nu12010202

434. Monteleone P, Mascagni G, Giannini A, Genazzani AR. Symptoms of menopause — global prevalence, physiology and implications. Nature Reviews Endocrinology [Internet]. 2018 Feb 2;14(4):199–215. Available from: https://doi.org/10.1038/nrendo.2017.180

435. Minkin MJ. Menopause. Obstetrics and Gynecology Clinics of North America [Internet]. 2019 Sep 1;46(3):501–14. Available from: https://doi.org/10.1016/j.ogc.2019.04.008

436. Garg A, Robinson L. Surgical menopause: A toolkit for healthcare professionals. British Menopause Society Journal [Internet]. 2021 Nov 11;27(4):222–5. Available from: https://doi.org/10.1177/20533691211038455

437. İlhan G, Aslan M, Cevrioğlu AS, Yıldırım M, Erkorkmaz Ü. Clinical efficacy of hormonal and nonhormonal agents in the treatment of vulvovaginal atrophy. Journal of Menopausal Medicine [Internet]. 2021 Jan 1;27(1):15. Available from: https://doi.org/10.6118/jmm.20026

438. Pasquariello R, Verdile N, Brevini T a. L, Gandolfi F, Boiti C, Zerani M, et al. The role of resveratrol in mammalian reproduction. Molecules [Internet]. 2020 Oct 5;25(19):4554. Available from: https://doi.org/10.3390/molecules25194554

439. Fabrício V, Oishi J, Biffe BG, Ruffoni LDG, Da Silva KA, Nonaka KO, et al. Resveratrol treatment normalizes the endothelial function and blood pressure in ovariectomized rats. Arquivos Brasileiros De Cardiologia [Internet]. 2017 Jan 1; Available from: https://doi.org/10.5935/abc.20170012

440. Zaw JJT, Howe P, Wong RHX. Long-term resveratrol supplementation improves pain perception, menopausal symptoms, and overall well-being in postmenopausal women: findings from a 24-month randomized, controlled, crossover trial. Menopause [Internet]. 2020 Aug 31;28(1):40–9. Available from: https://doi.org/10.1097/gme.0000000000001643

441. Klinge CM, Blankenship KA, Risinger KE, Bhatnagar S, Noisin EL, Sumanasekera W, et al. Resveratrol and Estradiol Rapidly Activate MAPK Signaling through Estrogen Receptors α and β in Endothelial Cells. Journal of Biological Chemistry [Internet]. 2005 Mar 1;280(9):7460–8. Available from: https://doi.org/10.1074/jbc.m411565200

442. Meng T, Xiao D, Muhammed A, Deng J, Chen L, He J. Anti-Inflammatory action and mechanisms of resveratrol. Molecules [Internet]. 2021 Jan 5;26(1):229. Available from: https://doi.org/10.3390/molecules26010229

443. Zhou DD, Luo M, Huang S, Saimaiti A, Shang A, Gan R, et al. Effects and Mechanisms of Resveratrol on aging and Age-Related Diseases. Oxidative Medicine and Cellular Longevity [Internet]. 2021 Jul 11;2021:1–15. Available from: https://doi.org/10.1155/2021/9932218

444. Belemets N, Kobyliak N, Virchenko O, Falalyeyeva T, Tsyryuk O, Bodnar P, et al. Effects of polyphenol compounds melanin on NAFLD/NASH prevention. Biomedicine & Pharmacotherapy [Internet]. 2017 Apr 1;88:267–76. Available from: https://doi.org/10.1016/j.biopha.2017.01.028

445. Mongioì LM, La Vignera S, Cannarella R, Cimino L, Compagnone M, Condorelli RA, et al. The role of resveratrol administration in human obesity. International Journal of Molecular Sciences [Internet]. 2021 Apr 22;22(9):4362. Available from: https://doi.org/10.3390/ijms22094362

446. Yakovlev P, Гарманчук ЛВ, Falalyeyeva T, Берегова ТВ, Ostapchenko L, Nehelia A. Cytostatic effect of polyphenol compound melanin in combination with Cisplatin on bladder cancer cell line T24/83. European Urology Supplements [Internet]. 2018 Oct 1;17(10):e2553–4. Available from: https://doi.org/10.1016/s1569-9056(18)33031-8

447. Li H, Horke S, Förstermann U. Vascular oxidative stress, nitric oxide and atherosclerosis. Atherosclerosis [Internet]. 2014 Nov 1;237(1):208–19. Available from: https://doi.org/10.1016/j.atherosclerosis.2014.09.001

448. Xia N, Daiber A, Förstermann U, Li H. Antioxidant effects of resveratrol in the cardiovascular system. British Journal of Pharmacology [Internet]. 2016 May 6;174(12):1633–46. Available from: https://doi.org/10.1111/bph.13492

449. O'Hanlon DE, Come RA, Moench TR. Vaginal pH measured *in vivo*: lactobacilli determine pH and lactic acid concentration. BMC Microbiology [Internet]. 2019 Jan 14;19(1). Available from: https://doi.org/10.1186/s12866-019-1388-8

450. Santos CCMD, Uggioni MLR, Colonetti T, Colonetti L, Grande AJ, Da Rosa MI. Hyaluronic acid in Postmenopause Vaginal Atrophy: A Systematic Review. The Journal of Sexual Medicine [Internet]. 2021 Jan 1;18(1):156–66. Available from: https://doi.org/10.1016/j.jsxm.2020.10.016

451. Liu T, Li N, Yan Y, Liu Y, Xiong K, Liu Y, et al. Recent advances in the anti-aging effects of phytoestrogens on collagen, water content, and oxidative stress. Phytotherapy Research [Internet]. 2019 Nov 20;34(3):435–47. Available from: https://doi.org/10.1002/ptr.6538

452. Levenson AS, Gehm BD, Pearce ST, Horiguchi J, Simons L, Ward JC, et al. Resveratrol acts as an estrogen receptor (ER) agonist in breast cancer cells stably transfected with ER ? International Journal of Cancer [Internet]. 2003 Feb 19;104(5):587–96. Available from: https://doi.org/10.1002/ijc.10992

453. Polito F, Marini H, Bitto A, Irrera N, Vaccaro M, Adamo EB, et al. Genistein aglycone, a soy-derived isoflavone, improves skin changes induced by ovariectomy in rats. British Journal of Pharmacology [Internet]. 2012 Jan 25;165(4):994–1005. Available from: https://doi.org/10.1111/j.1476-5381.2011.01619.x

454. Khayum MA, De Vries EFJ, Glaudemans AWJM, Dierckx R, Doorduin J. *In Vivo* Imaging of Brain Estrogen Receptors in Rats: A 16α-^{18}F-Fluoro-17β-Estradiol PET Study. The Journal of Nuclear Medicine [Internet]. 2014 Jan 30;55(3):481–7. Available from: https://doi.org/10.2967/jnumed.113.128751.

455. Zaychenko G, Stryga O, Myronenko O, Ivaniuk O. Doslidzhennya farmakologichnikh vlastivostey vaginal'nogo gelyu z resveratrolom ta gialuronovoyu kislotoyu v umovakh yeksperimental'nogo gipoestrogenovogo stanu

u shchuriv .[Pharmacological substantiation for choice the optimal composition of a new vaginal gel with resveratrol and gialuronic acid], Pharmacol. Drug Toxicol, 2020; 14:397-412. Available from: https://doi.org/10.33250/14.06.397.

456. Iwasa T, Matsuzaki T, Kinouchi R, Gereltsetseg G, Murakami M, Munkhzaya M, et al. Changes in central and peripheral inflammatory responses to lipopolysaccharide in ovariectomized female rats. Cytokine [Internet]. 2014 Jan 1;65(1):65–73. Available from: https://doi.org/10.1016/j.cyto.2013.10.004

457. Harada T, Kaponis A, Iwabe T, Taniguchi F, Makrydimas G, Sofikitis N, et al. Apoptosis in human endometrium and endometriosis. Human Reproduction Update [Internet]. 2004 Jan 1;10(1):29–38. Available from: https://doi.org/10.1093/humupd/dmh007

458. Fan D, Liu C, Guo Z, Huang K, Peng M, Li N, et al. Resveratrol promotes angiogenesis in a FoxO1-Dependent manner in hind limb ischemia in mice. Molecules [Internet]. 2021 Dec 12;26(24):7528. Available from: https://doi.org/10.3390/molecules26247528

459. Breuss JM, Atanasov AG, Uhrín P. Resveratrol and its effects on the vascular system. International Journal of Molecular Sciences [Internet]. 2019 Mar 27;20(7):1523. Available from: https://doi.org/10.3390/ijms20071523

460. Ilovayskaya I. Menopausal hormone treatments and risk of cardiovascular diseases: modern view. Ginekologiya [*Gynecology]* [Internet]. 2018 Aug 15;20(4):40–3. Available from: https://doi.org/10.26442/2079-5696_2018.4.40-43

461. Shestakova IG, Bettikher OA, Aleev IA. Nadezhde na oazys – byt! Urohenytalnaia atrofyia kak sledstvye defytsyta estrohenov: yzlechyma y predotvratyma [Hope for an oasis to be! Urogenital atrophy as a consequence of estrogen deficiency: curable and preventable.] StatusPraesens. Гинекология [StatusPraesens. Gynecology]. 2015;5(28):52-59.

462. Plaksina ND, Simonovskaya KhYu. Vozmozhnosty nehormonalnoi korrektsyy vazomotornykh paroksyzmov v postmenopauze [Possibilities of non-hormonal correction of vasomotor paroxysms in postmenopause]. StatusPraesens.Hynekolohyia [StatusPraesens. Gynecology]. 2014;2(19):60-65.

463. Radzinskiy VE, Khamoshina MB. Nereshennyie problemy v sovremennoi hynekolohyy:Quo vadis? [Unsolved Problems in Modern Gynecology:Quo vadis?]. Доктор Ру [Doktor Ru], 2016;7(124):4-9. Available from: https://journaldoctor.ru/catalog/ginekologiya/quo-vadis/

464. Smetnik VP. Starenye reproduktyvnoi systemы zhenshchyn: klynyko-hormonalnoe obosnovanye, stadyy, termynolohyia [Female Reproductive Aging: Describing Terminology and Identifying Different Stages Based on Clinical and Hormonal Characteristics] Zhenskoe zdorove y reproduktsyia [Women's health and reproduction]. 2014;12(100): 13-16. Available from: https://journalgynecology.ru/wp-content/uploads/2019/02/Starenie-reproduktivnoj-sistemy-zhenshhiny.pdf

465. Anishchenko A. Фитоэстрогены как альтернатива заместительной гормональной терапии при гипоэстрогенемии. [Phytoestrogens as an alternative to hormone replacement therapy for hypoestrogenemia] [doctoral dissertation]. Научно-исследовательский институт фармакологии имени Е.Д. Гольдберга [E.D. Goldberg Research Institute of Pharmacology], 2014.

466. Rietjens IMCM, Louisse J, Beekmann K. The potential health effects of dietary phytoestrogens. British Journal of Pharmacology [Internet]. 2016 Oct 20;174(11):1263–80. Available from: https://doi.org/10.1111/bph.13622

467. Zaychenko G, Stryga O, Belenichev IF, Gorchakova N, Kuchkovskyi O. Effect of resveratrol on no/sh-mechanisms of neuron damage in experimental VCD-hypoestrogenemia. Fìtoterapìâ [Internet]. 2022 Jan 1;(2):13–20. Available from: https://doi.org/10.33617/2522-9680-2022-2-13

468. Glišić M, Kastrati N, González-Jaramillo V, Bramer WM, Ahmadizar F, Chowdhury R, et al. Associations between Phytoestrogens, Glucose Homeostasis, and Risk of Diabetes in Women: A Systematic Review and Meta-Analysis. Advances in Nutrition [Internet]. 2018 Nov 1;9(6):726–40. Available from: https://doi.org/10.1093/advances/nmy048

469. Belenichev IF, Aliyeva OG, Kamyshny OM, Bukhtiyarova NV, Ryzhenko VP, Gorchakova NO. Pharmacological Modulation of Endogenous Neuroprotection after Experimental Prenatal Hypoxia. Neurochemical Journal [Internet]. 2022 Mar 1;16(1):68–75. Available from: https://doi.org/10.1134/s1819712422010044

470. Belenichev IF, Odnokoz OV, Pavlov SV, Belenicheva OI, Polyakova EN. The neuroprotective activity of tamoxifen and tibolone during glutathione depletion *in vitro*. Neurochemical Journal [Internet]. 2012 Jul 1;6(3):202–12. Available from: https://doi.org/10.1134/s181971241203004x

471. Belenychev IF, Chernyi VI, Nahornaia OA, Pavlov SV, Chernyi TV, Horchakova NA et al. Neiroprotektsyia y neiroplastychnost [Neuroprotection and neuroplasticity]. Kyiv, Polihrafplius. [Poligrafplus], 2014. Available from: https://cp-medical.com/downloads/monograph3.pdf

472. Chekman IS, Belenichev IF, Nahorna OO, Gorchakova NA, Lukianchuk BD, Bukhtiyarova NV et al. Doklinichne vyvchennia spetsyfichnoi aktyvnosti potentsiinykh likarskykh zasobiv pervynnoi ta vtorynnoi neiroprotektsii : metodychni rekomendatsii [Preclinical study of the specific activity of potential drugs for primary and secondary neuroprotection: methodological recommendations], Ukraine, Kyiv, 2016, 93 p.

473. Pavlov SV, Belenichev IF. Molecular and biochemical aspects of the neuroprotective effect of the selective estrogen receptor modulator tamoxifen in a model of acute cerebral ischemia. Neurochemical Journal [Internet]. 2014 Jan 1;8(1):28–32. Available from: https://doi.org/10.1134/s1819712413040077

474. Dinger J, Möhner S, Heinemann K. Cardiovascular risks associated with the use of drospirenone-containing combined oral contraceptives. Contraception [Internet]. 2016 May 1;93(5):378–85. Available from: https://doi.org/10.1016/j.contraception.2016.01.012

475. Roach REJ, Helmerhorst FM, Lijfering WM, Stijnen T, Algra A, Dekkers OM. Combined oral contraceptives: the risk of myocardial infarction and ischemic stroke. The Cochrane Library [Internet]. 2015 Aug 27;2018(3). Available from: https://doi.org/10.1002/14651858.cd011054.pub2

476. Abolaji AO, Omozokpia MU, Oluwamuyide OJ, Akintola TE, Farombi EO. Rescue role of hesperidin in 4-vinylcyclohexene diepoxide-induced toxicity in the brain, ovary and uterus of wistar rats. Journal of Basic and Clinical Physiology and Pharmacology [Internet]. 2020 Mar 1;31(2). Available from: https://doi.org/10.1515/jbcpp-2018-0115.

Наукове видання

Галкін Олександр
Горчакова Надія
Зайченко Ганна
Голембіовська Олена
Бондаренко Лариса
Дмитренко Олександра
Архипова Марина

Питання ефективності та безпеки сучасних багатокомпонентних фітопрепаратів

Монографія

(Англійською мовою)

У монографії узагальнено дослідження щодо безпеки та ефективності багатокомпонентних рослинних лікарських засобів у лікуванні гінекологічних, гепатобіліарних та серцево-судинних захворювань, а також рослинних противірусних засобів тощо. Охоплено доказову фітотерапію, фармакотерапію, профілі безпеки рослинних комбінацій та розробку лікарських засобів на їх основі.

Ця монографія є важливим ресурсом для дослідників і медичних працівників, які цікавляться перевагами рослинних лікарських засобів.

В авторській редакції

Надруковано з оригінал-макета замовника

Національний технічний університет України
«Київський політехнічний інститут імені Ігоря Сікорського»
Свідоцтво про державну реєстрацію: серія ДК № 5354 від 25.05.2017 р.
просп. Берестейський, 37,
м. Київ, 03056

Підп. до друку 19.02.2024. Формат 60×84 1/16. Папір офс. Гарнітура Times.
Спосіб друку – електрографічний. Ум. друк. арк. 15,4. Обл.-вид. арк. 13,37.
Поз. 24-3-1-003. Зам. 24-010. Наклад 50 пр.

Видавництво «Політехніка» КПІ ім. Ігоря Сікорського
вул. Політехнічна, 14, корп. 15
03056, м. Київ
тел. (044) 204-81-78