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PECULIARITIES OF MICROSCOPIC AND HISTOCHEMICAL CHANGES IN THE STRUCTURE OF THE LIVER OF EXPERIMENTAL RATS UNDER THE INFLUENCE OF VIPER VENOM *VIPERA BERUS NIKOLSKII*

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Annotation. As a result of the significant distribution of poisonous animals, humanity is in constant contact with them, which often causes poisoning or fatal consequences. About 1.8-2.7 million cases of snake bites are registered every year, resulting in 81,000-138,000 deaths of the victims, and 100,000 of them are characterised by the development of irreversible physical or mental disorders. Their toxic substances show a wide range of pathological effects on most vital systems, causing damage to the lungs, heart, kidneys, and skeletal muscles. However, currently, the number of experimental works on the effect of the venom of various types of snakes and vipers on the morpho-functional changes of the liver is too limited. The study aims to study microscopic and histochemical changes in the liver of rats under the influence of viper venom *Vipera berus nikolskii*. Experimental studies were carried out on white, non-linear male rats. The animals were conditionally divided into control and experimental groups, ten individuals in each. Experimental rats were injected intraperitoneally with a semi-lethal dose (LD50) (1.576 mg·g⁻¹) of *Vipera berus nikolskii* venom in a physiological solution. Animals of the control group were injected intraperitoneally only with a physiological solution. Rats were removed from the experiment 24 hours after exposure to the poison and anesthetised by cervical dislocation. Liver samples of animals of all groups were taken for microscopic examination. Histological preparations of the liver were stained with hematoxylin and eosin. Histological preparations were examined using an SEO SCAN light microscope. Histochemical studies were carried out using the Nakhlas method to identify the key enzyme of the citric acid cycle - succinate dehydrogenase. The sections were stained with Schiff's reagent after preliminary treatment with iodic acid (PAS reaction) in Shabadash's modification to study the features of glycogen accumulation in hepatocytes. The immunohistochemical method revealed a subpopulation of CD86+ cells in the liver of experimental animals. Under the conditions of exposure to *Vipera berus nikolskii* viper venom, the animals of the research group observed the development of pronounced destructive changes in the structural elements of the liver and links of the vascular bed, which is confirmed in particular by the reliable dynamics of changes in morphometric indicators. Dilatation and filling of blood vessels, formation of blood clots, haemorrhages, and destruction of hemocapillary walls were determined. Macrophage activation was combined with leukocyte infiltration in the triad zones and locally in the periportal areas of the liver lobules. Violation of the lobular-beam structure of the organ was accompanied by hydropic dystrophy of hepatocytes, and a significant decrease in the content of succinate dehydrogenase and glycogen was also established.

Keywords: liver, vipers, hepatocytes, lymphocytes, macrophages, rats.

Introduction

As a result of the significant spread of poisonous animals, humanity is in constant contact with them, which often causes poisoning or fatal consequences [7, 13, 14]. Among the variety of poisonous animals, snakes, vipers, spiders, and scorpions are the most common. According to literary sources, about 1.8-2.7 million cases of snake bites are registered every year, resulting in 81,000 - 138,000 deaths of victims, and 100,000 of them are characterised by the development of irreversible physical or mental disorders. The most common poisonings due to snake and viper bites are in Asia, Africa, sub-Saharan Africa and Latin America [15, 18]. Such a wide distribution and a significant percentage of lethality determine the growing attention of the scientific community to this problem. In particular, WHO included poisoning due to snake bites in the list of neglected tropical diseases [22, 23, 29]. In some regions of the world, scorpionism is an urgent health problem, the number of cases of which is up to 1.2 million per year, resulting in the death of more than 3,000 victims

[1]. Several questions remain unresolved despite the achievements in studying poisonous animals' species diversity and prevalence. Yes, knowledge about the components of the poison of many of their representatives is limited. The last fact belongs to the priority since the content of animal poisons varies significantly, which is caused by geographical and ontogenetic determinants [6, 19, 35]. The wide variability of toxic components directly impacts the effectiveness of antidotes and, therefore, the frequency of development of severe complications [9, 26, 27, 30]. That is why a more in-depth study of this problem can provide valuable information to representatives of the scientific field and practising medicine, which will improve the methods of treatment and prevention of poisoning by animal bites [21, 31, 33, 34].

To date, the scientometric databases contain separate studies on the biological activity of specific venom components of snakes and vipers and the peculiarities of their tropism to particular tissues and organs [5, 8, 16, 20].

It has been established that their toxic substances exhibit a wide range of pathological effects on most vital systems, causing damage to the lungs, heart, kidneys, skeletal muscles, etc. [24, 25, 28, 32, 36]. However, the number of experimental works on the influence of the venom of various species of snakes and vipers on the morpho-functional changes of the liver is too limited.

The study *aims* to study microscopic and histochemical changes in the liver of rats under the influence of viper venom *Vipera berus nikolskii*.

Materials and methods

Experimental studies were carried out on white, non-linear male rats. For preliminary acclimatisation, the animals were kept for seven days in a particular Taras Shevchenko Kyiv National University room and later in laboratory conditions in compliance with temperature and light regimes [11]. Animals received standard food and water *ad libitum*. The National Institutes of Health Guidelines conducted all experiments for the Care and Use of Laboratory Animals and the European Council Directive of November 24, 1986, on the Care and Use of Laboratory Animals (86/609/EEC). The research was approved and confirmed by the bioethics commission of the Institute of Biology and Medicine of the Taras Shevchenko National University of Kyiv (protocol No. 2, dated August 19, 2021).

Vipera berus nikolskii viper venom was obtained from Kharkiv National University named after V. N. Karazin. The lyophilised native venom was stored at -20°C and dissolved in saline immediately before the experiment.

Animals were conditionally divided into control and experimental groups, ten individuals each. Experimental rats were injected intraperitoneally with a semi-lethal dose (LD50) (1.576 mg·g⁻¹) of *Vipera berus nikolskii* venom in a physiological solution. Animals of the control group were injected intraperitoneally with only a physiological solution. Rats were removed from the experiment 24 hours after exposure to the poison and anaesthetised by cervical dislocation.

Liver samples of animals of all groups were taken for microscopic examination. The pieces were fixed in a 10% formalin solution for one day. Next, the pieces were dehydrated in alcohols of increasing concentration and embedded in paraffin blocks. Histological preparations of the liver were stained with hematoxylin and eosin [12]. Histological preparations were studied with the help of an SEO SCAN light microscope and photo-documented with the help of a Vision CCD Camera with a system of image output from histological preparations.

To identify the key enzyme of the tricarboxylic acid cycle - succinate dehydrogenase, histochemical studies were performed according to the Nakhlas method [4]. These studies were conducted on sections made in a cryostat microtome from unfixed tissue using nitroblue tetrazole. The precipitate in the form of blue diformazan granules testified to the enzyme's presence and localisation.

Sections were stained using Schiff's reagent after preliminary treatment with iodine acid (PAS reaction) in the Shabadash modification to study the specifics of glycogen accumulation in hepatocytes.

An immunohistochemical research method was used to detect a subpopulation of CD86+ cells in the liver of experimental animals. Liver sections (thickness four μm) made from paraffin blocks using an AMR-400 rotary microtome (Amos Scientific, Australia) were deparaffinised and rehydrated. Antigen recovery was performed in the KOS histoprocessor (Milestone, Italy). In the immunohistochemical staining protocol, mouse monoclonal Anti-CD86 primary antibodies (BP2-44514-0.1 mg, Novus Biologicals, USA) and Mouse/Rabbit PolyVue™ HRP/DAB polymer detection system (Diagnostic BioSystems, USA) were used. Sections were counterstained with Mayer's hematoxylin (Biognost, Croatia).

Results. Discussion

Various damaging factors, including toxins of natural origin, lead to the development of structural and functional disorders in the organ's tissue, which are often unpredictable. To date, it has been proven that OS and the morphological and biochemical changes associated with it play a significant role in the basis of liver damage under the influence of factors of various genesis [10]. This fact is related to the extremely high sensitivity of the parenchyma cells of the organ to the action of free radicals. During their regular functional activity, organelles of the latter, such as mitochondria and peroxisomes, can produce a certain amount of ROS, which are subject to disposal by the components of the antioxidant system. However, pathological conditions are characterised by the growth and accumulation of an excessive amount of free radicals, a violation of oxidative homeostasis and the development of stress, which is a consequence not only of histological changes in the structural elements of the liver but also causes irreversible changes in the metabolism of lipids, proteins, carbohydrates, and modulates the pathways responsible for transcription, gene expression, cell apoptosis, etc. [2, 3].

Microscopic studies of the liver of rats injected with *Vipera berus nikolskii* venom revealed significant dystrophic and inflammatory changes in the organ's parenchyma. Damage to the lobular structure of the liver and disruption of the cytoarchitectonics of classic liver lobules were observed. Significant centres of chaotic arrangement of hepatocytes with loss of typical beam structure were detected. Most hepatocytes were characterised by dystrophic changes, especially in the centrilobular areas, manifested by local phenomena of hydropic dystrophy. Such cells have light, vacuolated, oxyphilic cytoplasm. The nuclei of many hepatocytes are pyknotic and hyperchromic; some have significant swelling (Fig. 1).

Most sinusoids are filled with blood, and the

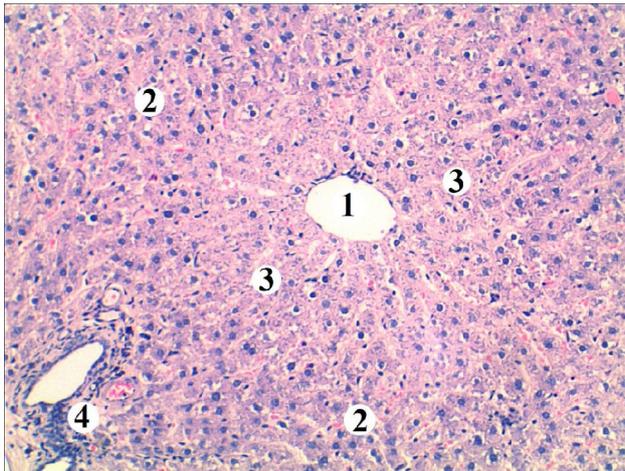


Fig. 1. Microscopic changes in the liver upon administration of *Vipera berus nikolskii* viper venom: 1 - widened lumen of the central vein, 2 - chaotic arrangement of hepatocytes, 3 - hydropic dystrophy of hepatocytes, 4 - portal tract with lymphohistiocytic infiltration. Staining with hematoxylin and eosin x 100.

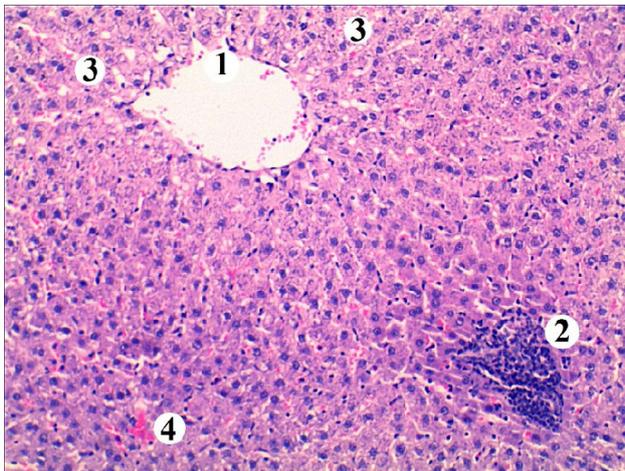


Fig. 2. Microscopic changes in the liver of animals after the introduction of *Vipera berus nikolskii* venom: 1 - damaged wall of the central vein, 2 - lymphohistiocytic infiltration in the liver lobe, 3 - vacuolated cytoplasm of hepatocytes, 4 - blood-filled sinusoidal capillaries. Staining with hematoxylin and eosin x 100.

phenomenon of coagulation of erythrocytes and the beginning of the formation of blood clots is observed. Individual hemocapillaries had significantly enlarged lumens and damaged endothelium. The integrity of the structure of the wall of the central vein was lost, and its thinning and fragmentation were noted (Fig. 1).

Significant lymphohistiocytic infiltrates were found, indicating an inflammatory process in the organ (Fig. 2). Their nodular accumulations were observed in the areas of the tracheal tracts, and lymphocytes were also observed along the liver beams in the sinusoidal capillaries.

W. Khimmaktong et al. [17] revealed the development of inflammatory processes in the liver tissue of rats that were injected intraperitoneally with the venom of *Calloselasma rhodostoma* snakes. Conglomerates of

inflammatory cells were observed around portal tracts. Blood stasis in sinusoidal capillaries, central veins, amyloidosis, diffuse necrosis of hepatocytes, and oedema were also characteristic. The number of lymphocytes and Kupffer cells increased under these conditions. With electron microscopy, swelling of the mitochondria of liver cells, destructive changes in the cytoplasm, expansion of the lumens of the sinusoids, and accumulation of cellular residue in the latter were noted. Pyknosis of hepatocyte nuclei and marked cytoplasmic eosinophilia were the defining features.

Stagnant phenomena in the vessels with the formation of blood clots were detected. The wall of the interlobular bile duct had indistinct contours. Swelling of the amorphous component of the loose connective tissue of the triads was observed (Fig. 3).

A histochemical study of succinate dehydrogenase in white rats' livers after administering *Vipera berus nikolskii*

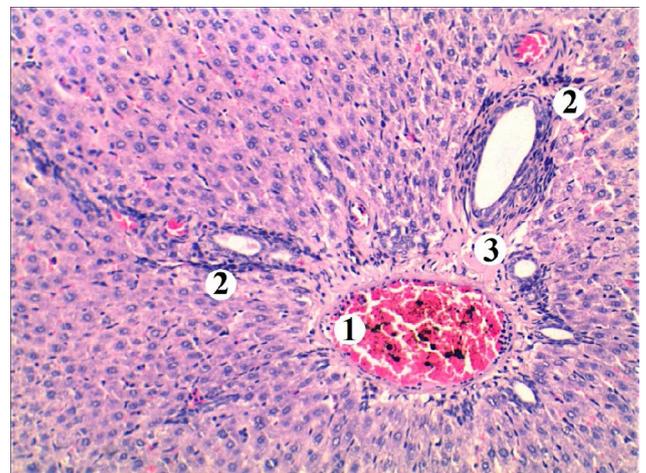


Fig. 3. Microscopic changes in the liver of animals after the introduction of *Vipera berus nikolskii* venom: 1 - venous thrombus, 2 - lymphohistiocytic infiltration, 3 - oedema of the connective tissue. Staining with hematoxylin and eosin x 100.

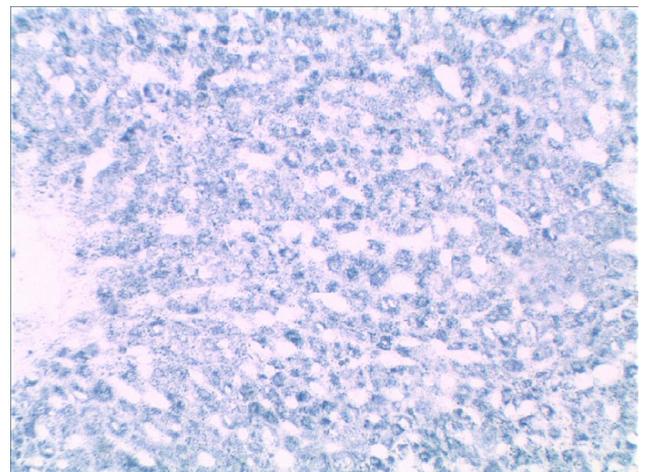


Fig. 4. Low activity of succinate dehydrogenase in hepatocytes of the liver of white laboratory rats after a Nikolsky viper bite. The Nakhlash method. Magnification: x 200.



Fig. 5. Glycogen content in white laboratory rats' liver after administering *Vipera berus nikolskii* venom. There are few lumps of trophic compounds in the cytoplasm of hepatocytes. Shabadash method. Magnification: x 400.

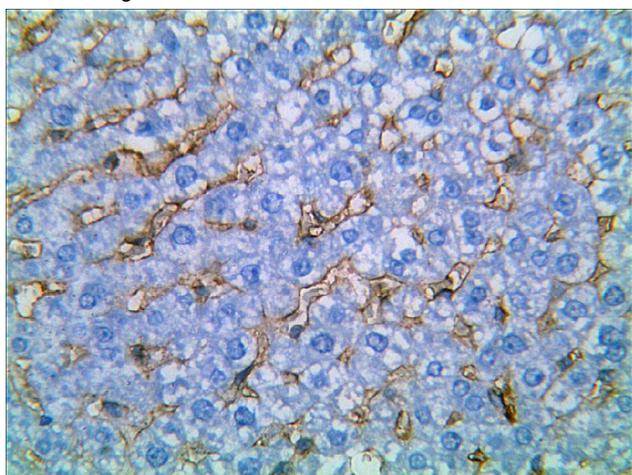


Fig. 6. High expression of CD86 in cells of the liver lobule of animals injected with *Vipera berus nikolskii* venom. Immunohistochemical reaction of CD86 antibody. Staining with Mayer's hematoxylin. Magnification: x 400.

venom showed a significant decrease in the enzyme. Few large granules were found in the cytoplasm of individual hepatocytes. In the vast majority of cells, there was a locally located powder-like amorphous precipitate of diformazan (Fig. 4). These changes indicate a violation of the process of glycolysis in cells. The average value of the cytochemical coefficient is 2.07 ± 0.09 , which is significantly ($p < 0.001$) 0.42 times lower than that of the intact group of animals.

Histochemical study of glycogen in hepatocytes of the liver of rats after administration of *Vipera berus nikolskii*

venom showed a low content of this trophic compound. In the cytoplasm of hepatocytes, local small granules are located along the edge of the plasmalemma. Single cells were characterised by the absence of glycogen (Fig. 5). The average value of the cytochemical indicator is 2.37 ± 0.11 , which is significantly ($p < 0.001$) reduced by 0.48 times compared to the similar parameter of the intact group of animals.

Immunohistochemical examination of the liver of animals injected with *Vipera berus nikolskii* venom showed that intensively stained CD86+ cells (++++) were found throughout the area of the liver lobules. Such an increase in their population in the sinusoids indicates significant inflammatory reactions caused by the influence of the poison on the organ. The cytoplasm of CD86+ cells is intensely brown, indicating secondary antibody fixation. Their appendages were numerous; they locally filled the lumen of sinusoidal hemocapillaries, and in some areas, penetration of the appendages into the lumen of Disse was noted. Also, using Mayer's hematoxylin staining, hepatocytes of the liver beams with significant destructive changes were detected, manifested by lightened vacuolated cytoplasm and pyknotisation of the nuclei. The lumens of the sinusoids were significantly dilated or collapsed. The architecture of the liver lobules was disturbed (Fig. 6)

Conclusions and prospects for further development

1. Under the conditions of exposure to *Vipera berus nikolskii* viper venom, the animals of the research group observed the development of pronounced destructive changes in the structural elements of the liver and links of the vascular bed, which is confirmed in particular by the reliable dynamics of changes in morphometric indicators.

2. Expansion and filling of blood vessels, formation of blood clots, haemorrhages, and destruction of hemocapillary walls were determined. Macrophage activation was combined with leukocyte infiltration in the triad zones and locally in the periportal areas of the liver lobules.

3. Violation of the lobular-beam structure of the organ was accompanied by hydropic dystrophy of hepatocytes, and a significant decrease in the content of succinate dehydrogenase and glycogen was also established.

A promising direction is the significant expansion and deepening of research on understanding the development of pathological processes in rats' livers under exposure to *Vipera berus viper* venom.

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ОСОБЛИВОСТІ МІКРОСКОПІЧНИХ ТА ГІСТОХІМІЧНИХ ЗМІН СТРУКТУРИ ПЕЧІНКИ ЕКСПЕРИМЕНТАЛЬНИХ ЩУРІВ ПРИ ВПЛИВІ ОТРУТИ ГАДЮК *VIPERA BERUS NIKOLSKII*

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Анотація. Внаслідок значного розповсюдження отруйних тварин людство перебуває з ними в постійному контакті, що часто стає причиною отруєнь або летальних наслідків. Щороку реєструють близько 1,8-2,7 млн випадків зміїних укусів, що призводять до 81 000 - 138 000 смертей постраждалих, а у 100 000 з них характерним є розвиток незворотних фізичних чи психічних порушень. Їх токсичні речовини проявляють широкий спектр патологічних ефектів у відношенні більшості життєво важливих систем, зумовлюючи ураження легень, серця, нирок, скелетних м'язів. Однак наразі надто лімітованою є кількість експериментальних робіт щодо впливу отрути різних видів змії і гадюк на морфо-функціональні зміни печінки. Метою дослідження є вивчення мікроскопічних і гістохімічних змін печінки щурів при впливі отрути гадюк *Vipera berus nikolskii*. Експериментальні дослідження проводили на білих нелінійних щурах-самцях. Тварин умовно розділили на дві групи - контрольну та дослідну, по 10 особин у кожній. Піддослідним щурам внутрішньоочеревинно вводили напівлетальну дозу (LD50) (1,576 мг·г-1) отрути *Vipera berus nikolskii* на фізіологічному розчині. Тваринам контрольної групи вводили внутрішньоочеревинно тільки фізіологічний розчин. Щурів виводили з експерименту через 24 години після впливу отрути, знеживлюючи шляхом цервікальної дислокації. Відбирали зразки печінки тварин усіх груп для мікроскопічного дослідження. Гістологічні препарати печінки забарвлювали гематоксиліном та еозином. Гістологічні препарати досліджували за допомогою світлового мікроскопа SEO SCAN. Для ідентифікації ключового ферменту циклу лимонної кислоти - сукцинатдегідрогенази, проводили гістохімічні дослідження за методом Нахласа. Для вивчення особливостей накопичення глікогену в гепатоцитах зрізи фарбували реактивом Шиффа після попередньої обробки йодною кислотою (PAS-реакція) у модифікації Шабадаша. Імуногістохімічним методом виявлено субпопуляцію клітин CD86+ у печінці експериментальних тварин. За умов впливу отрути гадюки *Vipera berus nikolskii* в тварин дослідної групи спостерігали розвиток виражених деструктивних змін структурних елементів печінки і ланок судинного русла, що підтверджується зокрема достовірною динамікою змін морфометричних показників. Визначались розширення і кровонаповнення судин, формування тромбів, крововиливи, деструкція стінок гемокапілярів. Активація макрофагів поєднувалась з лейкоцитарною інфільтрацією в зонах триад та локально в перипортальних ділянках печінкових часточок. Порушення часточково-балкової будови органу супроводжувалось гідропічною дистрофією гепатоцитів, а також встановлено значне зниження в них вмісту сукцинатдегідрогенази та глікогену.

Ключові слова: печінка, гадюки, гепатоцити, лімфоцити, макрофаги, щури.