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EVALUATION OF ACUTE TOXICITY OF CITRULLUS COLOCYNTHIS (L.) SHRAD. FRUITS DRY EXTRACT

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Abstract. *Citrullus Colocynthis (L.) Shrad. is a perennial plant that belongs to Cucurbitaceae family widespread in African regions and Mediterranean countries. It is mostly used for treatment of diabetes mellitus, inflammation, joint pain, fever, bacterial and fungal infections. Citrullus colocynthis (L.) Shrad. fruits dry extract was obtained for the first time in Bogomolets National Medical University and standardized with the content of ellagic acid. However further investigation of its pharmacological properties and application in medicine is hardly possible without evaluation of toxicological parameters.*
Acute toxicity of Citrullus colocynthis (L.) Shrad. fruits dry extract was studied on outbred white mice and rats of both sexes for dose range of 1000-6310 mg/kg body weight orally. Plant extract was administered in the form of aqueous solution at a single dose of 1000,0 mg/kg, 1260,0 mg/kg, 1580,0 mg/kg, 2000,0 mg/kg, 2500,0 mg/kg, 3160,0 mg/kg, 3980,0 mg/kg, 5000,0 mg/kg, 6310,0 mg/kg to each group (I,II,III,IV,V,VI,VII,VIII,IX) respectively. The main parameters such as general behavior, body and organs weight, macroscopic analysis of organs, and animals mortality were monitored.
Administration of plant extract caused mortality in mice and rats at doses 2000,0 mg/kg and above. According to obtained results LD_{50} was determined at doses 2240 mg/kg and 2180 mg/kg for mice and rats respectively and the substance was put into class IV (low toxic substances) by K. Sidorov classification. While conducting the study of acute toxicity negative changes in animals' behavior and breathing difficulty were registered. In addition to this, minor hemorrhagia in intestinal mucous membrane and diarrhea were observed.
Conclusions. Having analyzed the data it is possible to draw the conclusions that an extract belongs to class IV (low toxic substances) according to standard classification; it doesn't demonstrate neither gender nor species specificity; the most probable toxic effects with overdose of an extract are watery waste and petechial hemorrhage in intestine
Keywords*: Citrullus colocynthis (L.) Shrad, dry extract, acute toxicity, LD_{50}, mortality, hemorrhagia, diarrhea.*

Introduction. Citrullus colocynthis (L.) Shrad. is well known as a plant with wide range of biologically active substances which determine its certain pharmacological activities and medical value. Thus C. Colocynthis has attracted the attention of many scientists who study its biological properties, mechanisms of action, effective doses and, of course, safety. Study of toxicity is the first link in further pharmacological research [1-4].

Citrullus colocynthis (L.) Shrad. fruits dry extract was obtained at pharmacognosy and botany department of Bogomolets National Medical University for the first time. It means that for investigation of its pharmacological action study of acute toxicity is needed.

Purpose of the study: to investigate the acute toxicity of Citrullus colocynthis (L.) Shrad. fruits dry extract.

Material and methods.

Plant material

Dry fruits of Citrullus colocynthis (L.) Shrad. were imported from Cairo (Egypt). Plant samples were ground to pieces of 0,5 inch that is an optimal size for extraction for most plant raw material [5, 6].

Preparation of plant extract

Powdered sample was loaded into Soxhlet apparatus and extracted by chloroform [7]. Obtained extaction cake was dried and continued to be extracted with purified water (in the ratio of 1:10) for 30 minutes in a water bath [6]. Filtered off water extract was evaporated and dried to a residual moisture content of 5%. Dry fruits extract was powdered and then divided into certain doses for use in acute toxicity test after dilution each of doses in water for injection.

Experimental animals

Acute toxicity of C.Colocynthis fruits dry extract was tested on two species of rodents of both sexes such as outbred adult white mice weighing 19-2 g and rats weighing 150-180 g. Laboratory animals were raised and kept under vivarium standard conditions of the State Institution «Institute of Pharmacology and Toxicology of NAMS of Ukraine» having free access to food and wate.

Acute toxicity test

Acute toxicity of the extract was studied by its single oral administration as this administration route is going to be used in further studies. Each dose of test substance was calculated taking into consideration the animal body weight of and prepared separately for each rodent. According to the method of V. Prozorovsky, mice and rats were administered a number of doses of extract from 1000.0 mg/kg to 6310.0 mg/kg body weight and from 1000.0 mg/kg to 3980.0 mg/kg body weight respectively. The control group of mice and rats received only a particular ammount of water for injection.

Observations of clinical signs of toxic effect and animals (mice and rats) mortality were performed on the first day after the administration of the plant extract for the first hour continuously, and then after two, three, four, five hours respectively. For the next fortnight each animal was examined twice a day and animals (mice and rats) mortality and macroscopic studies of their internal organs, absolute and relative weight of rats' organs, rats body weight were studied as well.

Macroscopic analysis.

All survived animals (mice and rats) were euthanized under mild ether anesthesia in 14 days from the beginning of the experiment and they had to starve for three hours before it. Before animals necropsy they were subjected to a full external examination. Macroscopic examination included a detailed study of the internal organs as well as the stomach where the substance was injected. If animals died during the experimental study macroscopic examination was carried out as soon as the fact of death was registered or in the morning of the following day if the animal died at night.

Body weight

The individual body weight of each rat was recorded before the extract administration and on the third and fourteenth days after its administration. Changes in body weight were calculated in comparison with basic data of weight. In order to balance the number of rats in the control groups according to their number in the experimental groups the animals of the control groups were euthanized.

Weight of organs

The study was conducted on rats so in order to study absolute and relative weight the following organs were weighed: liver, kidneys, spleen, lungs, heart. The paired organs were weighed together. The relative weight of organs was calculated in *g* per *100 g* of body weight.

Statistical analysis

A comparative analysis of the data was carried out using Student's t-criterion. The processing of the obtained data was performed using the statistical program package of «Statistica for Windows, Release 6.0.».

Research results. The details of acute toxicity study on outbred adult white mice after single orally administration of plant extract at doses of 2500.0 mg/kg, 3160.0 mg/kg, 3980.0 mg/kg, 5000.0 mg/kg and 6310.0 mg/kg are presented in Table 1.

Table 1. Survival rate of white mice after single oral administration of C. Colocynthis extract at the range of doses (n=4)

Group	I	II	III	IV	V	VI	VII	VIII	IX
Dose of extract (mg/kg)	1000,0	1260,0	1580,0	2000,0	2500,0	3160,0	3980,0	5000,0	6310,0
Effect* (female)	0/2	0/2	0/2	0/2	2/2	2/2	2/2	2/2	2/2
Effect* (male)	0/2	0/2	0/2	0/2	2/2	2/2	2/2	2/2	2/2

* – numerator includes number of dead animals, denominator includes number of animals in group with a certain dose, n – number of animals in group

Animals treated with C. Colocynthis extract at all doses that exceeded 2500.0 mg/kg died within 3 h 15 min - 3 h 30 min after substance administration. Administration of plant extract at doses lower than 2000.0 mg/kg didn't cause death during the whole observation period (14 days after a single administration of the plant extract) (Table 1).

Immediately after the administration of C. Colocynthis extract (2-5 min) breathing difficulty, irregular surface breath, negative chronotropism, huddling of animals in the cage were recorded. In some animals that were overdosed it was possible to observe flat body posture, stretching of hind limbs, slow move in the cage. Animals that were treated with the extract at doses of 2000.0 mg/kg and above had tremor of the anterior and posterior extremities within 3 h after the administration of the plant extract. All animals suffered from watery waste on the first day of examination. In the surviving animals diarrhea disappeared after 1-3 days.

External examination and also autopsy and macroscopic examination of mice of both sexes, which died within 1-2 days after a single oral administration of plant extract at the range of doses, revealed no pathological changes of the internal organs except the small and large intestine, where petechial hemorrhages were observed.

According to the data obtained the LD_{50} for white mice was determined at a dose of 2240 (1900-2500) mg/kg. By K. Sidorov classification C. Colocynthis extract belongs to class IV (low toxic substances).

After treatment with C. colocynthis dry extract at the dose range 1000.0-1260.0 mg/kg no mortality in rats was reported. The death of one animal was reported at the dose of 1580.0 mg/kg. At the same time, animals of both sexes receiving a plant extract at a dose of 2000.0 mg/kg did not die. Administration of extract at dose of 2500.0 mg/kg and above resulted in rats mortality within 3 h-3 h 30 min as well as within 1-2 days after tratment (Table 2).

Table 2. Survival rate of white rats after single oral administration of C. Colocynthis extract at the range of doses (n=4)

Group	I	II	III	IV	V	VI	VII
Dose of extract (mg/kg)	1000,0	1260,0	1580,0	2000,0	2500,0	3160,0	3980,0
Effect* (female)	0/2	0/2	1/2	0/2	1/2	2/2	2/2
Effect* (male)	0/2	0/2	1/2	0/2	1/2	2/2	2/2

* – numerator includes number of dead animals, denominator includes number of animals in group with a certain dose, n – number of animals in group

The toxicity of the plant extract was manifested as breathing difficulty, irregular surface breath negative chronotropism, ataxia, hyperreflexia, huddling and trembling. These clinical signs developed within 15-30 min after administration of extract. In 1 h when a single dose was given tremor of front and hind limbs, stretching of the hind limbs (flat body posture) were observed, frequency of breathing decreased, deep breaths changed to frequent surface irregular inspiration-expiration. The visible mucous membranes had a cyanosed tint. In 3 h the watery waste was recorded.

Clinical signs of intoxication among survived rats disappeared within 3-6 h, diarrhea remained for 1-3 days. Thus, there were no abnormalities in external features and toxic manifestations of plant extract within 1-3 days after its administration, as well as throughout the observation period (14 days) in white rats that remained alive. Animals moved actively, consumed food. There were no behavioral changes, changes in motor responses, state of reflexes, reactions to external stimuli. All animals had smooth fur and clean skin.

According to the obtained data average lethal dose of C. Colocynthis fruits dry extract while being orally administered to rats was 2180 mg/kg, which allowed to place the plant extract to class IV (low toxic substances).

Single oral administration of C. colocynthis dry extract at the dose range 1000.0-3980.0 mg/kg did not significantly affect the body weight dynamics of the surviving rats compared to controled ones (Table 3). Experimental and control animals gained weight in accordance to the physiological norm, at the same time, it could be observed that the animals of the experimental group gained weight a little

more slowly within 3 days after administration of the plant extract, which is apparently explained by the symptoms of diarrhea during the first 3 days.

Table 3. Dynamics of body weight of white rats after single oral administration of C. Colocynthis extract (M ± m)

Group	Body weight (M±m, g) on the relevant day of observation		
	Basic data	3 days	14 days
Experimental	165,4± 2,31	167,2±3,45	207,2±2,46*
Control	162,6±1,08	173,3±2,60*	213,1±1,13*

* – P<0,05 statistically significant in relation to basic data in group

The study of the absolute and relative organs weight in dead rats and euthanized rats after 1-2 days of observation, and the absolute weight of organs of rats in the experimental and control groups after 14 days of observation was conducted.

The absolute weight of internal organs in the dead rats of the experimental group did not have significant difference in relation to the corresponding indicator in rats of the control group, which were euthanized in the planned terms of observation for comparison with the indicators in rats of the experimental group (Table 4).

Table 4. Absolute weight of internal organs of rats in experimental group and rats in control group euthanized after 1-2 days of observation (M±m, n=6)

	Weight of organ (g)			
Organ	Experimental (female)	Control (female)	Experimental (male)	Control (male)
Heart	0,89± 0,01	0,90± 0,01	0,87± 0,01	0,90± 0,01
Lungs	1,31±0,02	1,29±0,2	1,20±0,2	1,24±0,1
Liver	10,54± 1,30	10,34±1,1	10,13± 1,2	9,86±0,8
Spleen	1,27±0,02	1,19± 0,03	1,18±0,03	1,19± 0,3
Kidneys	2,29 ±0,07	2,16± 0,1	2,14 ±0,08	2,15± 0,09

n – number of animals in group

The relative weight of the internal organs of dead rats (after 1-2 days) in the experimental group did not differ significantly from the relative weight of the internal organs of rats in the control group (Table 5).

Table 5. Relative weight of internal organs of rats in experimental group and rats in control group euthanized after 1-2 days of observation (M±m)

	Weight of organ (g/100 g body weight)		
Organ	Experimental (female+male) (n=12)	Control (female) (n=6)	Control (male) (n=6)
Heart	0,40±0,01	0,41±0,01	0,41± 0,01
Lungs	0,68± 0,03	0,67±0,03	0,64±0,02
Liver	5,50±0,15	6,00±0,2	5,10± 0,24
Spleen	0,67±0,01	0,62±0,01	0,62±0,02
Kidneys	1,20±0,03	1,10±0,01	1,10±0,04

n – number of animals in group

Table 6. Absolute weight of internal organs of rats in experimental group and rats in control group euthanized after 14 days of observation (M±m, n=16)

	Weight of organ (g)	
Organ	Experimental (female+male)	Control (female+male)
Heart	0,95± 0,10	0,90± 0,01
Lungs	1,54±0,2	1,29±0,4
Liver	11,0± 0,34	10,34±0,17
Spleen	1,26±0,6	1,19± 0,3
Kidneys	2,2±0,09	2,1± 0,1

n – number of animals in group

In acute toxicity study of C. Colocynthis dry fruit extract absence of gender and species specificity of the plant extract was noticed. The toxic effect of an extract was apparent in animals (mice and rats)

behavioral changes that were more explicit within 2-5 min after administration of substance. Also having injected the substance breathing difficulty was observed. Tremor of extremities was noted within 3 h after administration of the plant extract in mice and rats treated with C. colocynthis extract at doses of 2000.0 mg/kg and above. All animals had watery waste in the first day of examination that disappeared within 1-3 days of observation. Macroscopic examination of the internal organs of rodents demonstrated no alternative changes except of petechial hemorrhages in the mucous membrane of the colon and small intestine. Indicators of body weight, as well as the absolute and relative weight of organs of the experimental animals did not differ from the similar indicators of animals of the control group.

It should be noted that the signs of poisoning that we observed in the study of acute toxicity are closely correlated with the data of other authors [10-12].

The main signs of toxicity resulting from taking a single therapeutic dose of C. colocynthis were diarrhea, rough hair, palpitations, shortness of breath, soft stool, changes in the behavior of animals like huddling. The toxic effect of C. colocynthis was also manifested by a decrease in appetite and body weight, rectorrhagia, colitis and tremor, general depression, activation, convulsions, mortality of animals [10, 13-16]. Javadzadeh H. R. and co-authors found that the plant causes the following side effects: toxic acute colitis, infertility and hepatotoxicity in rats. These disorders are exacerbated with increasing dose of C.colocynthis [17]. It is believed that the main mechanism of damaging effect on the intestine is the membranolytic action of C. colocynthis saponins, which interact with cholesterol and phospholipids of the cell membrane, disrupt the structure of the plasmolemma cause degeneration of microvilli and endothelial layer of blood capillary result in hemorrhagia. Experimental trials have shown that these biologically active substances provide the enterohepatorenal effects of C. colocynthis extracts [18]. The presence of cucurbitacins in the C. colocynthis raw material causes the development of diarrhea as a result of impaired transport of water and nutrients in the intestine [19, 20].

Conclusions. Having analyzed the data it is possible to draw the conclusions that an extract belongs to class IV (low toxic substances) according to standard classification; it doesn't demonstrate neither gender nor species specificity; the most probable toxic effects with overdose of an extract are watery waste and petechial hemorrhage in intestine

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