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Preclinical toxicological study of the lipid concentrates of snakes of the genus Eryx.

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Abstract.
Acute and chronic toxicity of lipid concentrate of Eryx snakes was studied on sexually mature white mice and rats.
It was found that in experimental animals in the period after acute and chronic application of lipid concentrate of Eryx snakes there are no significant changes in anthropometric, biochemical, haematological and pathomorpholigical parameters, which allows to refer lipid concentrate of Eryx snakes to practically non-toxic compounds. The results of acute and chronic toxicity studies indicate the harmlessness of lipid concentrate of Eryx snakes.

Keywords: lipid concentrate of snakes of the genus Eryx, toxicity, anthropometric, haematological, biochemical and pathomorphological studies

Background. The snake has been a symbol of medicine and healing since ancient times, being associated with the Greek god of medicine Asclepius. In Greek and Roman writings, snakes were used for medicinal purposes, and the flesh of vipers was considered to have healing properties [1,2,3]. Parts and mixtures of snakes are still used in folk medicine in the Far East and other regions [4,5,6,7]. In Uzbekistan, a whole snake Eryx miliaris is used in soup to treat asthma [8]. Separately, it should be noted that aqueous solution of autolysate of E.tartaricus and E.miliaris snakes autolysate known as Eryxin (Reptilin) drug has pronounced antihyaluronidase, hepatoprotective and anti-inflammatory properties[9,10,11]. In clinical trials, this drug has shown its efficacy in the treatment of rheumatoid arthritis, brucellosis, chronic cytomegalovirus infection[12]. Lipid concentrate of snakes of the genus Eryx is used in Uzbekistan for the treatment of herpetic stomatitis [13]. Despite the popular traditional use of snakes of the genus Eryx, its preclinical toxicological study to date has not been investigated. Preclinical experimental studies are conducted under the strictest regulations to ensure that they are as safe and ethical as possible. These studies are carried out to create new means of treatment and prevention of various pathological conditions primarily to assess their safety. For these experiments, certain procedures must be followed at various levels of research, the most important of which are the evaluation of acute and chronic toxicity, pyrogenicity, cumulative, allergic and other effects at the stage of preclinical experimental studies [14,15,16,17].

The aim of the present work is to investigate the toxicity of lipid concentrate of snakes of the genus Eryx.

Methods and materials.

All experimental studies were carried out on sexually mature white male rats, white sexually mature mice and male rabbits of "Chinchilla" breed obtained from the vivarium of the Department of Sanitary and Epidemiological Surveillance of the Main Medical Department under the Administration of the President of the Republic of Uzbekistan. Before the beginning of experimental studies, laboratory animals were kept in quarantine for two weeks, and then they were carefully examined and weighed. Considering age, sex, skin condition, motor activity and body weight, groups of six animals each were created. During the whole period of preparation for the experiment, during the studies small laboratory animals were kept in vivarium in plastic cages, littered with sawdust, and rabbits in special cages at temperatures 20-24^{0}C, humidity not less than 50%, in a well-ventilated room and day/night light regime, with standard dietary regime, free access to water and feed. Feeding of animals was carried out according to age. Taking into account the chronobiological dependence of most physiological processes in the organism, all manipulations with animals were carried out at the same time of day (morning).

For toxicological studies, the lipid concentrate of snakes of the genus Eryx was obtained from SV-BIO PHARM GROUP LLC.

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Acute toxicity of lipid concentrate of Eryx snakes was studied on sexually mature white mice (weighing 18.5 - 21.5 g.), rats (weighing 155 - 175 g.) and rabbits (weighing 2.15-2.65 kg.). In rodent experiments, rodents were divided into groups of 6 individuals each by randomisation method. The absence of disease signs in laboratory animals and homogeneity of groups by sex and body weight (±20%) were considered as the criterion of acceptability of randomisation. White mice and rats were administered snake lipid concentrate intragastrically with a metal probe at doses ranging from 500 to 5000 mg/kg. To achieve high doses of the investigated lipid concentrate, it was administered to animals repeatedly at intervals of 30 minutes for 2-3 hours (up to 6 repeated administrations). Animals of control groups were administered corresponding volumes of distilled water in similar ways (up to 6 injections). Also on rabbits of Chinchilla breed with body weight of 2,1-2,5 kg the tolerability of lipid concentrate of snakes of Eryx genus was investigated. For this purpose lipid concentrate was administered intragastrically using special probes in doses of 1000, 2500 and 5000 mg/kg once. Rabbits of the control group received the corresponding volume of boiled water according to the same scheme. Observation of the general condition of experimental animals receiving different doses of lipid concentrate was carried out on the first day and the following 14 days. Clinical symptoms of intoxication and indices of general condition were recorded. Before the beginning of the experiment, as well as during the whole period of the experiment, the dynamics of body weight gain of animals, as well as their feed and water consumption, were taken into account.

Experimental studies on chronic toxicity of lipid concentrate of Eryx snakes were carried out on 40 white rats with body weight 135-150 g., which were administered intragastrically aqueous solutions of the drug daily in the morning before feeding for 60 days. The animals were divided into 4 groups of 10 animals per group: the 1st group of animals received lipid concentrate at a dose of 30 mg/kg; the 2nd group received this concentrate at a dose of 300 mg/kg; the 3rd group received a dose of 600 mg/kg; the 4th group served as a control. Before the beginning of the study and during the whole experiment, after daily intravenous administration of the studied drug, clinical signs of possible intoxication were registered: general condition of animals, every three days the change of body weight, behavioral features, motor activity, reaction to stimuli, frequency and depth of respiration, condition of hair and skin cover, mucous membranes, fecal masses, feed and water consumption were evaluated. During the entire experiment, all laboratory animals were kept under standard vivarium conditions and were fed a complete laboratory diet with free access to water. A day after the last procedure, blood was taken from the tail vein from animals of all groups and blood formula was determined on a BC-20s haematological analyser (Mindray, China). Then, under light anaesthesia, blood was collected from rats by one-stage decapitation for biochemical studies, and internal organs were fixed in 10% formalin solution for morphological studies after macroscopic examination. The activity of AsAT, AlAT, ALP and GGT; glucose, total protein, bilirubin and cholesterol, urea and creatinine (reagent kits from CYPRESS Diagnostics, Belgium) were determined in blood serum on a biochemical analyser VA-88A (Mindray, China).

Indicators of toxicity were: animal behaviour, survival rate, time of deaths, appearance of intoxication symptoms, body weight dynamics, haematological and biochemical blood parameters.

Statistical analyses of the results obtained in the experiments were carried out on the basis of standard clinical guidelines. Quantitative data are presented as arithmetic mean (M) ± standard deviation (SD) in case of normal distribution and as median (Md) and quartiles (Q) or (SD) in case of other distributions. A confidence level of $P < 0.05$ was taken as statistically significant changes. The results of the study were processed using Biostat 2009 software package.

Results and discussion.

It is known that preclinical studies of new drugs include not only chemical, physical, biological and microbiological, but also pharmacological and toxicological studies. In this case, the ultimate goal of the study is to obtain an objective assessment of the evidence of efficacy and safety of medicines.

The results of the experiments showed that in all types of laboratory animals after a single intragastric administration of lipid concentrate in doses from 500 to 5000 mg/kg for 14 days no death was found in any group of animals: rats, mice and rabbits. When observed for two weeks, the activity of experimental animals in all groups remained at a physiological level. Coordination of movements was not disturbed, skin was clean, hair was shiny, and sensitivity was present. No hair loss or other damage was found on the head, body or limbs. The condition of the hair around the natural openings was also without visible changes. Skin of scrotum

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of males without damage and swellings. Presence of skin parasites was absent and no traces of their vital activity were observed. The mucous membranes of the eyes were pink in colour, without overlaps, swellings, moist, smooth, shiny. There was no oozing from the eyes. Nasal mucosa was pink, shiny, moist without swelling. Nasal discharge was absent. Teeth were preserved in all animals, oral mucosa was pale pink in colour, no ulceration, no swelling, no haemorrhage and no other changes were observed. Feed intake was in accordance with the daily norms, accordingly, the act of defecation was regular. Faeces were sofumed, diuresis was regular. At external examination of carcasses of mice, rats and rabbits taken out of the experiment 14 days after administration of lipid concentrate, it was found that all animals were of proportional build, satisfactory fatness. From what it is possible to draw a conclusion about absence of acute toxicity of the investigated lipid concentrate of snakes of genus Eryx. Due to absence of lethal outcomes it was not possible to calculate LD50 (average lethal dose). The results obtained in the acute experiment to study the effect of lipid concentrate on three types of laboratory animals: mice rats and rabbits allowed to conclude that this lipid concentrate at enteral administration does not have a toxic effect on internal organs, which allowed to attribute the studied lipid concentrate of snakes of the genus Eryx, to relatively harmless drugs.

The results of experiments on studying chronic toxicity of lipid concentrate of snakes of genus Eryx in different doses at intragastric administration (during 60 days) showed that at external examination it was found that all rats of correct physique, satisfactory nutrition. No secretions from natural openings were found. The wool is shiny, neat appearance, foci of baldness are not determined. Teeth are preserved. Visible mucous membranes are pale in colour and shiny. Sexual organs of males are correctly developed. There is no deformation or oedema of the limbs. No skin changes, irritations, hyperaemia were observed.

During the studies in the chronic experiment, no statistically significant differences in body weight and body weight gain were found between animals receiving different doses of the investigated lipid concentrate and control animals receiving placebo (Table 1).

The results of haematological studies showed that no significant changes in blood system parameters between groups were found in rats after oral administration of the investigated lipid concentrate (Table 2).

Table 1
Body weight of animals in dynamics (M±m, n=6)

Groups of animals	Weight of animals, in grams			
	Control (placebo)	Lipid concentrate doses (mg/kg)		
		30	300	600
Initial weight of rats	133,67± 1,55	135,66 ± 1,67	136,83 ± 1,21	134,80 ± 1,57
Weight after 60 days	188,83 ± 4,73*	184,80 ± 5,51*	181,51 ± 6,11*	187,62 ± 7,43*

Note: *-statistically significant differences compared to baseline.

Table 2.
Haematological parameters of blood of rats exposed to lipid concentrate of snakes of the genus Eryx for 60 days (M±m, n=6).

Parameters	Control	30mg/kg	300mg/kg	600mg/kg
WBC(10^9/L)	14,37±1,06	14,58±1,05	14,12±1,01	14,88±1,00
Lymph# (10^9/L)	6,23±0,50	6,22±0,50	6,08±0,55	6,27±0,54
Mid# (10^9/L)	2,48±0,24	2,33±0,23	2,35±0,28	2,27±0,26
Gran# (10^9/L)	5,35±0,39	5,72±0,48	5,58±0,45	5,95±0,33
HGB(g/L)	136,7±5,41	134,33±5,10	131,50±5,35	130±5,08
RBC(10^{12}/L)	6,10±0,49	6,11±0,30	6,05±0,30	6,02±0,32

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HCT(%)	37,73±1,07	35,97±1,19	36,05±1,14	36,22±1,17
MCHC (g/L)	368±5,96	364,67±5,33	364,67±5,41	366,67±5,75
PLT(10^9/L)	608,17±52,06	603,33±40,61	595±44,84	603,50±39,42

As can be seen, there are no significant differences from the control group in animals receiving oral lipid concentrate of Eryx snakes. Haematological indices of blood of rats receiving lipid concentrate are within the acceptable generally accepted norms for this kind of animals. Also there are no deviations from the norms for these experimental animals according to the literature data [16].

As can be seen from the data of Table 3, there are no significant differences from the control group in animals receiving orally lipid concentrate of snakes of the genus Eryx.

Table 3.

Blood biochemical parameters of rats exposed to lipid concentrate of snakes of the genus Eryx for 60 days (M±m, n=6).

Parameters	Control	30mg/kg	300mg/kg	600mg/kg
ALT (U/L)	74,27±3,16	70,67±3,89	71,63±4,48	66,63±3,71
AST (U/L)	245,50±16,39	242,67±16,75	241,67±16,94	208,83±16,35
ALP (U/L)	797,17±108,98	735,98±101,10	672,88±112,35	729,02±104,68
GGT (U/L)	4,83±0,76	4,33±0,80	4,17±0,79	3,83±0,74
Glu (mmol/L)	4,69±0,42	4,77±0,41	4,65±0,38	4,76±0,41
TP (g/dL)	164,60±5,57	162,40±5,79	161,50±5,55	161,38±5,76
Alb (g/dL)	46,0±2,32	46,63±1,84	45,52±2,32	45,53±2,41
TBil (mg/dL)	6,92±1643	6,25±1,37	6,80±1,15	4,75±1,22
Chol (mmol/L)	2,87±0,51	2,93±0,60	2,76±0,79	3,31±0,55
Urea (mmol/L)	3,78±0,81	3,71±0,67	3,44±0,74	3,42±0,41

Biochemical indices of blood of rats, taking lipid concentrate, are within the acceptable generally accepted norms for this kind of animals. Also there are no deviations from the norms for these experimental animals according to the literature [16].

Conclusion.

1.Based on the results of studies lipid concentrate of Eryx snakes according to the parameters of acute toxicity during intragastric administration belongs to class 4 (low hazardous substance) [18].

2.During long-term intragastric administration of different doses of lipid concentrate of Eryx snakes no changes in haematological and biochemical parameters were revealed. The results of histomorphological studies of tissues of internal organs during long-term intragastric administration of lipid concentrate confirm the absence of toxic effects on internal organs. Further studies of teratogenicity and reproductive toxicity are recommended to obtain a complete toxicity profile of the lipid concentrate.

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