academic publishers

INTERNATIONAL JOURNAL OF ARTIFICIAL INTELLIGENCE (ISSN: 2692-5206)

Volume 04, Issue 03, 2024

Published Date: - 21-05-2024

PRECLINICAL GENERAL AND SPECIFIC TOXICOLOGY STUDY OF GLABTAN DRY EXTRACT FROM RHUS GLABRA

Karaeva N. Yu.,
Tashkent Pharmaceutical Institute, Tashkent, Republic of Uzbekistan
Rahmonova G.G.,Abdullajona N.G'.,Raimova K.V.
Institute of Biorganic Chemistry, Tashkent, Republic of Uzbekistan

Abstract
This article presents the results of the study of the general and specific toxicology of the dry extract of the drug "Glabtan" developed by the scientists of the Institute of Biorganic Chemistry named after academician A.S. Sodikov of the Academy of Sciences of the Republic of Uzbekistan.

Key words
dry extract, acute toxicity, cumulative, chronic poisoning, open field test, local irritant, conjunctival test, application.

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In order to launch the production of various drugs, they must pass a series of tests confirming the effectiveness and safety of these products. A number of these experiments are carried out in vitro and in vivo experiments. There is a huge difference between in vitro and in vivo experiments. They represent a completely different type of test. The term in vivo literally means "within the living". That is, it is research conducted on living organisms, such as insects, animals or humans. Scientific experiments on "instrument equipment" and living objects are necessary to expand the scope of knowledge about various diseases, their treatment methods, the functioning of the body and the effects of certain compounds on it. Also, experiments of this type are used to test new scientific discoveries and theoretical hypotheses in practice. [1]. Sometimes they are supplemented by other methods - for example, "ex vivo" experiments - the study of certain substances in living tissues, but outside the human or animal body. Ex vivo research means "outside the living body." In this type of experiment, living tissue is not created artificially, but is taken directly from a living organism. Then the experiment is carried out immediately in the laboratory with minimal changes in the natural conditions of the body. It can be skin cells, sebaceous glands, hair or nails, nerves, muscles or other elements.

In silico research means "done on a computer or by computer simulation." in silico was first used to describe fully computerized biological experiments. Although in silico research is a relatively new field, it has become widely used in studies that predict how drugs interact with organisms and pathogens.

In vivo refers to experiments using the whole living organism, as opposed to studying individual cells in a test tube or a dead organism. Animal studies and human clinical trials are two forms of in vivo research. In vivo testing is often used instead of in vitro testing because it is more suitable for observing the overall effect of an experiment on a living subject.

The purpose of the study: to study the general and specific toxicology of the dry extract of the drug "Glabtan" developed by the scientists of the Institute of Biorganic Chemistry named after A.S. Sodikov.

Research methods. Acute toxicity. The experiment was carried out on white laboratory mice of both sexes with a body weight of 20±2.0 g. The animals were kept in quarantine for 10-14 days before the experiment, and the injured animals were removed from the groups. The experimental animals were kept

under the same conditions with ad libitum drinking water and food on a regular diet. The study of the general effects and acute toxicity of the glabtan substance was conducted in accordance with generally accepted laboratory practices and international recommendations. [2]. In the experiments to study the acute toxicity properties of glabtan substance, the total number of mice was 50. They were divided into 5 groups of 25 male and 25 female animals of similar body weight, and each group was divided into 2 subgroups based on gender. Glabtan substance in doses of 4000, 5000 mg/kg of 16 and 20% solution was injected into the stomach of mice through a special probe in the volume of 0.5 ml to the animals of the experimental group. 12 and 16% solutions of glabtan at doses of 6000 and 8000 mg/kg were injected twice in 0.5 ml with an interval of one hour. An equal amount of distilled water was injected into the mice of the control group. Glabtan-injected animals were monitored under laboratory conditions for 14 days. During the entire experiment, the general condition and activity of the animals, changes in behavior, rate and depth of breathing, hair and skin, condition of the tail, amount and hardness of the faecal masses, urine output, changes in body weight and other indicators were compared to the indicators of the control group. The initial body weight of the animals was determined before the introduction of Glabtan substance, and then the body weight was measured every week until the end of the experiment. Animals that died during the experiment were recorded, and their mean lethal dose, LD_{50} toxicity, Hodge, Sterner and acute toxicity classification were calculated according to the modified rule of the Organization for Economic Assistance and Development. [3]. Statistical processing of the obtained results was calculated by calculating the average size (M) of the animal's body weight, the average error of the average value (m), Student's criterion (t), and the probability of error (r).

Cumulativeness. The study of the cumulative properties of glabtan substance was carried out in 10 male and female mice with a body weight of 20±2.0 g. Glabtan substance for 28 days in a strictly defined manner, the average lethal dose LD_{50}=5150 mg/kg is 0.1 of the indicator; 0.15; 0.22; 0.34; 0.50; 0.75; Each of the 1.12 shares was introduced for 4 days and continued in this way until 28 days. Its cumulative properties are determined by the methods of Lim RK and other authors, and these methods allow to evaluate not only the accumulation of the substance, but also the habituation to it.

Tests to determine the chronic toxicity of glabtan. Chronic poisoning properties of glabtan substance were studied in two types of animals - rats (determined body weight before substance introduction 160±20 g) and "Chinchilla" breed rabbits (determined body weight before substance introduction 2.5±0.5 kg). 32 rats and 16 rabbits were used for this study. Glabtan to rats 25; 50 and 100 mg/kg, 15 to rabbits; In doses of 30 and 60 mg/kg for 1 month, it was introduced into the stomach using a special probe. The animals of the control group were injected with distilled water in the same volume as the substance solution. The effect of Glabtan chronically injected into the stomach on the condition of animals according to the following indicators of peripheral (venous) blood: the amount of hemoglobin in the blood, erythrocytes, HCT - hematocrit volume fraction of erythrocytes in the blood, MSV - the average volume of erythrocytes (mkm ^{3}), MSN - the amount of hemoglobin in one erythrocyte, MSNS - the amount of hemoglobin in one erythrocyte the average concentration in erythrocytes - hemoglobin saturation level of erythrocytes in %, the number of reticulocytes, platelets, leukocytes, as well as blood glucose, total protein, ALT alanine -, AST aspartate aminotransferases and urea were evaluated. Biochemical indicators of urine were determined using a test kit from the company "CYPRESS DIAGNOSTICS" (Belgium). Renal function was assessed by 4-hour diuresis, urinary pH, urobilinogen, bilirubin, ketones, specific gravity, blood, protein, nitrites, leukocytes, and urine sediments through a 5% body weight water load.

Hematological and biochemical indicators, as well as determination of body weight, were performed every 10th and 30th days of the experiment. On the same days, the body weight of the animals was measured, and at the same time, their appearance, behavior, and the presence or absence of signs of poisoning were recorded every day. On the 30th day, some of the animals were decapitated, and their internal organs were examined with the naked eye, and it was determined whether there were any swelling or abnormal changes in color. The internal body organs of rats and rabbits that received glabtan and the control group: thymus, liver, kidneys, spleen, heart, lungs, stomach, adrenal glands were measured and compared to each other.

Open field test. To evaluate the effects of glabtan on the behavior of animals, chronic poisoning experiments were studied using the "open field" test Hall C.S. (1934). This method is very simple and convenient to evaluate the effect of glabtan on the number and quality of animal movements when

administered multiple times.
In the "open field" method, the behavior of animals was studied by introducing them to an open field that was unfamiliar to them and where they could not leave. These experiments were conducted in a 1 m^2 field facility, divided into 16 squares and containing 16 holes, enclosed by 20 cm high rat-opaque fences at the BFM Pharmacology and Screening Laboratory. Grouping of the animals was done 1 day before, and 60 min before the start of the test, they were taken to a quiet, dimly lit area. Each of the experimental animals was placed in a square in the center of the device and the latency period was recorded.
The number of squares entered by the rats - horizontal movement activity (GHF), standing freely on hind legs or standing on obstacles - vertical movement activity (VHF), the number of "holes" in the area where the animal poked its head for sniffing - learning activity (O'F), vegetative activity - usually the number of grooming movements (GHS) and the number of "feces" that may occur in stressful situations and are not for hygiene purposes. By means of these quantities, the reactions manifested in the behavior of the rats, convulsions and direction selection movements were evaluated....These values were recorded for 3 minutes after the animal was transferred to the "open field" [4]. The open field test was conducted on the 10th and 30th days of the experiment. Statistical processing of the results of all the tests used in the study of the chronic toxicity of glabtan, finding the average value of the studied parameters (M), the average error of the average value (m) using the Microsoft Excel computer program and the differences between the animal groups of the results of these parameters, Student's test (t), error probability (r) was calculated using online calculators.

Tests to evaluate the preclinical specific toxicology of glabtan

Tests to evaluate the local astringent effect of glabtan

Conjunctival test. The conjunctival swab test is very sensitive, and in some cases it detects the reaction of animals even to weak allergens and negative skin tests. Experiments 0.1 ml, 0.5 and 5.0% solutions of the studied Glabtan were instilled into the left eye of rabbits weighing 2.0-2.5 kg, and 0.1 ml of distilled water was instilled into the right eye as a control. The rapid response of animals to glabtan was evaluated after 15 minutes, and the delayed hypersensitivity after 24-48 hours on the scale points:
0 points - not affected, that is, no redness was observed.
1 point – slight redness of the tear duct.
2 points – reddening of the tear duct and sclera towards the cornea.
3 points – redness of the entire conjunctiva and sclera.
In addition, indicators such as the degree of hyperemia, swelling, and lacrimation were taken into account.
The method of application to the skin. The experiment was conducted on 10 guinea pigs with a body mass of 220±10 g, divided into 2 groups of 5 animals. Two sides of the body of guinea pigs were depilated, 0.3 ml of 0.5% solution of Glabtan and 5.0% solution in water were dripped on the left side of animals of the 1st group and 5.0% of the water for four weeks, 5 days a week for four weeks. Initially, 10 applications were made for 14 days, after no allergic reaction was observed on the skin against Glabtan, 10 applications were made for another 14 days.
The reaction to this substance on the skin was evaluated in points on the following scale:

Definition of reaction	Estemate		
	scale	Glabtan, %	
		0,5	5,0
Erythema and its spread			
Absence of erythema and spread	0		
Very faint erythema (slightly noticeable)	1		
Perceptible erythema	2		
Moderate erythema	3		
Severe erythema (bright red rash)	4		
No swelling	0		
Very weak (slightly noticeable)	1		
Perceptible swelling	2		

Moderate swelling	3		
Visible	4		
Highest scores	8		

Research results. Acute toxicity. After 3-5 minutes after glabtan was injected into the stomach of male and female mice at a dose of 4000 mg/kg, the animals showed flushing, weakness, increased breathing, narrowing of the eyes, and gathering movements. The normal state of animals in both groups was restored after 1-2 hours. The condition of hair, skin and tail, the amount and consistency of faecal masses, and the frequency of urination did not differ from control animals. Also, the condition of animals of both sexes did not differ from each other. The increase in animal mass by the end of the second week was 21.2% in male mice and 19.1% in female mice in the control group. 21.6 and 19.5% in animals receiving 4000 mg/kg glabtan, respectively. No animal deaths were observed throughout the experiment (0/5). 3-5 minutes after administration of glabtan drug at a dose of 5000 mg/kg, washing, weakness, rapid breathing, narrowing of the eyes and gathering in one place were observed in mice of both groups, and one animal died on the 1st day. The condition of the remaining animals became normal on the second day of the experiment, like the control animals. Other indicators of animals from the beginning of the experiment: hair and skin coverage, condition of the tail, amount and consistency of faecal masses, urination interval did not differ from control animals. Also, at the end of the second week, the increase in body weight was 21.0% in male mice and 19.2% in female mice compared to the control ($p>0.05$). This indicator was derived from the average body weight of 4 surviving animals. Animals receiving a dose of 6000 mg/kg of glabtan showed short-term flushing, lethargy, rapid breathing, narrowing of the eyes, and crowding, and two animals died on day 1 and one on day 2, for a total of 3 animals over two days. death observed, 3/5. The condition of the surviving mice in the groups returned to normal on the 3rd day, but the slowness of activity remained. Changes in hair and skin coverage, tail position, amount and consistency of faecal masses, urinary frequency, and live weight of these animals did not differ from control animals. It was 20.3 and 19.6% in animals receiving glabtan, respectively. When Glabtan was administered to the animals at a dose of 8000 mg/kg, after 2-3 minutes, weakness, narrowing of the eyes, acceleration of breathing, and concentration in one place were observed. Mice in the glabtan-treated male and female groups did not recover from this condition, and all animals in these groups died on day 1.

Acute toxicity studies of glabtan were conducted in male and female white laboratory mice. The results of acute toxicity and body weight dynamics are presented in Tables 1 and 2 below.

Table 1

Indications of acute toxicity after a single injection of Glabtan in laboratory mice

Sex	Groups	Doses		Death toll/ Total number of mice	LD_{50} -m+m, mg/kg
		mg/kg	ml/mouse		
Male	Glabtan	4000	0,5	0/5	5150 -700 770
		5000	0,6	1/5	
		6000	0,8	3/5	
		8000	1,0	5/5	
	Control	сув	0,5	0/5	
Female	Glabtan	4000	0,5	0/5	5150 -700 770
		5000	0,6	1/5	
		6000	0,8	3/5	
		8000	1,0	5/5	
	Control	Water	0,5	0/5	

Table 2

Changes in body weight of mice during the experiment (M±m)

Doses	Body weight, gr					Weight gain, %
	primary	7 -day		14- day		
Male / n- number of surviving animals						
Control	20,5±1,2	23,1±1,3	n=5	26,0±0,9	n=5	21,2
4000	19,6±1,3	22,8±1,2	n=5	25,0±0,8	n=5	21,6
5000	19,9±1,0	22,7±1,0	n=4	25,2±0,6	n=4	21,0
6000	20,0±1,2	23,2±1,1	n=2	25,1±0,2	n=2	20,3
8000	19,8±1,1	ўлган	n=0	ўлган	n=0	–
Female / n- number of surviving animals						
Control	20,65±1,0	23,5±1,1	n=5	25,5±0,6	n=5	19,0
4000	19,8±1,3	22,1±1,3	n=5	24,6±0,9	n=5	19,5
5000	20,1±1,1	23,0±1,0	n=4	24,9±0,7	n=4	19,2
6000	19,7±1,2	22,8±1,1	n=2	24,5±1,0	n=2	19,6
8000	20,5±1,2	died	n=0	died	n=0	–

Thus, when Glabtan was injected into the stomach of female and male mice at doses of 4000, 5000, 6000 and 8000 mg/kg, the average lethal dose was LD_{50} – 5150 (–700; +770) mg/kg, and the acute toxicity of this drug according to the classification of Hodge and Sterner It was determined that class V belongs to the class of almost non-toxic chemical compounds. The sensitivity of mice to all doses administered to determine the acute toxicity of glabtan did not depend on their gender.

Cumulativeness. To assess the subchronic toxicity of glabtan, the cumulative properties and habituation properties of this drug were studied in male mice. Based on the average lethal dose of LD_{50}=5150 mg/kg, the drug was injected into the stomach of animals for 28 days in the order and dosage sequence given in Table 3.

Table 3

Results obtained on the cumulative properties of glabtan

Dates incluted	Number of mice =10)	LD_{50}– share	LD_{50} =5150 mg/kg
1-4	0/10	0,1	515
5-8	0/10	0,15	773
9-12	0/10	0,22	1133
13-16	0/10	0,34	1751
17-20	0/10	0,50	2575
21-24	4/10	0,75	3863
25-28	6/10	1,12	5768

Conclusion. The cumulative dose administered to animals over the maximum duration of the experiment was 12.7 LD_{50}. The cumulative coefficient was calculated according to the formula K_k=.LD_{50}n/LD_{501}, where K_k is the cumulative coefficient, LD_{50} –n- is the average lethal dose when administered n times, LD_{50} 1 is the average lethal dose when administered once. The cumulative coefficient of glabtan was K= 3863/5150=0.75. Calculations according to the above formula showed that the cumulative coefficient of Glabtan was 0.75, this coefficient was K_k<1, which indicated the presence of a weak cumulative property of the drug. It also does not cause habituation to this drug in experimental animals.

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