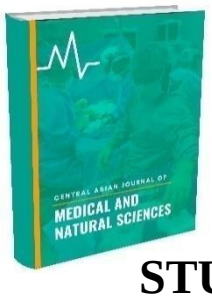
CENTRAL ASIAN JOURNAL OF MEDICAL AND NATURAL SCIENCES



Volume: 03 Issue: 04 | Jul- Aug 2022 ISSN: 2660-4159

http://cajmns.centralasianstudies.org

STUDYING THE SUB-ACUTE TOXICITY AND THE CUMULATIVE PROPERTIES OF THE DRY EXTRACT "HELMINTH-ART"

1. Yuldasheva Shahlo Khabibullaevna

2. Tukhtaev Hakim Rakhmanovich

Received 4th July 2022,
Accepted 9th July 2022,
Online 25th July 2022

[1]Basic doctoral student of the Tashkent Pharmaceutical Institute, Tashkent, Uzbekistan
[2] Doctor of Pharmacy , Professor of the Department of Inorganic, Physical and Colloidal Chemistry, Tashkent Pharmaceutical Institute, Tashkent, Uzbekistan
shaxlodr@gmail.com

Abstract: Gelmint -ART" on white rats was studied. The cumulative properties of the anthelmintic dry extract "Gelmint-ART" were studied in an experiment on mice. As a result, it was found that the anthelmintic dry extract does not have a general toxic effect at the studied doses and routes of administration. In the study of acute toxicity, it was found that the investigated anthelmintic substance belongs to the IV class of toxicity, i.e. low toxicity. It was also found that it does not have cumulative properties.

Key words: helminths, dry extract, subacute toxicity, cumulative properties, white rats, mice.

One of the important requirements for new drugs is the absence of signs of general and specific toxicity. A new drug should not have a negative effect on the body of animals and humans, not cause anomalies and not have a teratogenic effect [1]. Artemisia species are used worldwide for their antioxidant, antimicrobial, and anti-inflammatory properties. During the study, the phytochemical profile of two ethanol extracts obtained from the leaves and stems of A. absinthium L., as well as the biological potential (antioxidant activity, cytotoxic and anti-inflammatory properties). The extracts improved the anti-inflammatory effect in mice characterized by a diffuse distribution of neutrophils without exocytosis or microabscesses [2 . Preclinical toxicological research triclavascida showed what on acute toxicity acute cutaneous _ toxicity at enteral way introductions white mice and rats he applies to IV class safe substances not renders negative irritant on the intact skin, applies to group substances possessing weakly pronounced cumulative properties, not shows immunotoxic, embryotoxic and teratogenic a activity [3]. In the studies, the phytochemical profile of anthelmintic activity in vitro and in toxicity vivo of an aqueous extract of P. gonoacantha leaves [4]. In a study, an aqueous extract of the leaves of tansy vulgaris (Tanacetum vulgare L.) by determining its potential toxicity after acute and chronic administration to rodents [5]. The mass use of herbal anthelmintics eliminates environmental pollution from chemicals. Plants have a complex effect on the animal organism and are

quickly excreted, so the cumulative properties of plants are limited. [6]. Medicinal plants act in a complex way and are quickly excreted from the body [7].

The aim of the study was to study the subacute toxicity and cumulative properties of the anthelmintic dry extract "Helmint-ART".

Materials and research methods. All studies were conducted on healthy animals that were quarantined for at least 10-14 days.

The object of our study was the dry extract of Helminth-ART. " Helmint -ART" consists of a mixture of dry extracts isolated separately from anthelmintic plant materials, such as the aerial part of the wormwood (Artemisia absinthium L), pumpkin seeds (Cucurbita pepo L.), tansy flowers (Tanacetum Vulgare L.) and bulbs garlic (Allium sativum L.). This object is the substance of the Helminth-ART anthelmintic capsule.

Experiments on the study of subacute (subchronic) toxicity were carried out on 80 (40 males and 40 females) rats of the same age and weighing 160-170 g. The experiment was carried out according to the recommendations for preclinical drug trials [8,9]. The animals were kept in a vivarium with a standard diet, temperature and light conditions, with free access to water and food. All manipulations with animals were performed at the same time of day in the morning, taking into account the chronobiological dependence of most physiological processes in the body.

For the experiment, rats were divided into 4 groups of 10 males and 10 females for each study dose and control dose. The choice of doses was determined by the requirements of guidelines and literature data [9]. The studied infusion of the anthelmintic substance was administered in doses of 100 mg/kg (therapeutic), 500 mg/kg (maximum daily) and 2500 mg/kg (maximum) were administered daily intragastrically in the form of an aqueous infusion using a metal probe. The calculation of doses was carried out taking into account the content of active substances. The total observation period was 28 days. An aqueous solution of an anthelmintic substance was intragastrically injected daily for 28 days, and then the condition of the animals was observed.

The toxic effect of the preparation was judged by the general condition - survival, appearance (daily examination - the condition of the coat and skin, mucous membranes), behavior (daily), weight gain (1 time per week), food and water consumption (weekly), hematological and biochemical blood parameters, macroscopically detectable changes in internal organs and their mass coefficients.

For the analysis of hematological and biochemical parameters, biological material (blood) was collected according to standard methods [10] in the morning after a 14–15 hour fast. The study of peripheral blood parameters was carried out before the start of the experiment, on the 14th and 28th days. Registration of blood biochemical parameters was carried out using standard reagent kits on a Huma biochemical analyzer Luzer Primus 602828, Germany. The content of leukocytes, erythrocytes, platelets, hemoglobin level, hematocrit and leukocyte formula (percentage of lymphocytes, monocytes, eosinophils and granulocytes) were determined in the blood. The following indicators were determined in the blood serum : total protein, albumin, urea, creatinine, glucose, triglycerides (TG), alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (AP), (Ca 2+). After the completion of the experiment (day 28), all surviving experimental animals, including controls, were euthanized and dissected. During necroscopy , the lungs, heart, liver, kidneys, spleen, adrenal glands, and thymus were sampled. The masses of internal organs were determined on an analytical balance [9] .

An experiment to study the cumulative properties of an anthelmintic substance on 10 white mice weighing 20-22 g [9] . White mice were intragastrically injected with an aqueous solution of an anthelmintic substance. The minimum dose was 1 mg/kg . Then, every subsequent 4 days, the doses

administered were increased by 1.5 times the previous daily doses. Accounting was carried out on the basis of the general clinical and physiological state of mice [11,12] .

Results :

In the study of subacute toxicity of an aqueous solution of an anthelmintic substance after the termination of its administration, the death of rats was not observed in any of the groups.

The dynamics of changes in body weight, both males and females, was generally positive, the rate of weight gain did not differ significantly from the control group ($p>0.05$) (table 1). There were no significant changes in the amount of food and water consumed compared to control animals.

Table 1

Change in body weight of white rats after repeated administration of an aqueous solution of an anthelmintic substance, g (M ± m)

Timing research	control	aqueous solution of anthelmintic substance		
		100 mg/kg	500 mg/kg	2500 mg/kg
Before experience	164.2 ± 2.8	162.5±2.4	164.8 ± 2.6	164.5 ± 3.1
14 days	170.3±3.5	168.2 ± 2.6	170.2 ± 3.5	170.8 ± 3.2
28 days	175.8 ± 3.6	173.8±2.8	173.5 ± 3.2	174.2 ± 3.2

The results of peripheral blood studies showed that on the 14th day and on the 28th day after daily intragastric administration of an aqueous solution of an anthelmintic substance at doses of 100 mg/kg; 500 mg/kg and 2500 mg/kg, there were no significant changes in the erythrocyte and leukocyte formulas, as well as in the number of platelets, compared with the control (table 2).

table 2

Biochemical parameters of blood serum of white rats after repeated administration of an aqueous solution of an anthelmintic substance

(M ± m)

Timing research	control	aqueous solution of anthelmintic substance		
		100 mg/kg	500 mg/kg	2500 mg/kg
Leukocytes, 10^9 / l (WBC)				
14 days	7.2±0.2	7.2±0.2	7.1±0.2	7.2±0.2
28 days	7.1±0.2	7.2±0.2	7.2±0.2	7.1±0.2
Lymphocytes , %(lym)				
14 days	65.5 ± 3.4	65±2.8	62.5 ± 4.5	63.3 ± 3.4
28 days	57.6 ± 4.2	60.6 ± 2.3	60.1 ± 4.3	62.5 ± 3.5
Monocytes , % (Mon)				
14 days	2.2±0.1	2.1±0.1	2.0±0.1	2.1±0.1
28 days	2.1±0.1	2.2±0.1	2.2±0.1	2.0±0.1
Eosinophils , % (Eos)				
14 days	1.2±0.1	1.2±0.2	1.2±0.1	1.2±0.1
28 days	1.2±0.2	1.3±0.1	1.2±0.1	1.1±0.1

Basophils, % (Bas)				
14 days	0	0	0	0
28 days	0	0	0	0
Erythrocytes , 10^{-12} / l (RBC)				
14 days	7.0±0.2	7.1±0.2	7.2±0.2	7.1±0.1
28 days	7.1±0.1	7.0±0.1	7.1±0.3	7.0±0.1
Hemoglobin , g/l (HGB)				
14 days	140.5±2.5	142.5 ± 2.2	143.4 ± 3.0	142.2±2.0
28 days	142.2 ± 2.2	143.2 ± 2.3	142±2.5	142.3 ± 2.4
Platelets , 10^{9} /l (PLT)				
14 days	633±4.5	634.3 ± 3.2	630±4.5	636.6 ± 3.2
28 days	634.2±4.0	638±4.4	632.2 ± 3.2	634.2±3.5

The study of the main biochemical parameters of blood serum on the 14th day and on the 28th day after daily intragastric administration of an aqueous solution of the anthelmintic extract at doses of 100 mg/kg; 500 mg/kg and 2500 mg/kg showed no significant changes compared with the data of the control group (table 3).

Table 3

Biochemical parameters of blood serum of white rats after repeated administration of an aqueous solution of an anthelmintic substance

(M ± m)

Timing research	**control**	**Aqueous solution of anthelmintic substance**		
		100 mg/kg	**500 mg/kg**	**2500 mg/kg**
Total protein , g /l				
14 days	70.2 ± 2.4	82.5±1.5	81.5 ± 2.4	78.5 ± 3.8
28 days	72.2 ± 2.2	81.2 ± 1.3	79.5 ± 3.5	80.6 ± 3.2
Urea , mmol/l				
14 days	5.2±0.2	5.0±0.2	5.1±0.2	5.2±0.2
28 days	5.1±0.2	5.2±0.1	5.2±0.1	5.1±0.2
Creatinine , µmol /l				
14 days	2.2±0.1	2.1±0.1	2.0±0.1	2.1±0.1
28 days	2.1 ± 0.09	2.2±0.1	2.2 ± 0.07	2.0±0.1
Glucose , mmol/l				
14 days	4.2±0.1	4.2±0.1	4.0±0.2	4.1±0.1
28 days	4.2±0.2	4.1±0.2	4.1±0.2	4.0±0.1
Total cholesterol, mol/l				

14 days	2.2±0.1	2.3±0.2	2.2±0.1	2.1±0.1
28 days	2.2±0.2	2.3±0.2	2.1±0.1	2.2±0.1
Alat , U /l				
14 days	133.2 ± 3.2	133.3±2.2	134.5±2.5	134.2 ± 3.7
28 days	134.2 ± 4.2	132.5 ± 2.3	132.8±2.2	133.5 ± 3.5
Asat , U / l				
14 days	402±4.2	400.2±3.2	402.7 ± 4.2	402±4.0
28 days	398.3 ± 4.8	404.3±4.3	400.8±3.5	398.6 ± 3.8
Alkaline phosphatase , U /l				
14 days	151.6 ± 3.2	152.6 ± 4.2	153.3 ± 3.2	153.5±3.3
28 days	153.3 ± 3.6	153.5 ± 3.5	154.5 ± 2.8	154.3±3.5

When studying the effect of an aqueous solution of an anthelmintic solution at doses of 100 mg/kg; 500 mg/kg and 2500 mg/kg for diuresis and creatinine clearance after 14 and 28 days, there were no significant changes relative to the control (table 4).

Table 4

Indicators of urine analysis after repeated administration of an aqueous solution of an anthelmintic substance to white rats (M ± m)

Timing research	**control**	**Aqueous solution of anthelmintic substance**		
		100 mg/kg	**500 mg/kg**	**2500 mg/kg**
Diuresis for 18 hours , ml				
14 days	2.3±0.3	2.1±0.2	2.2±0.2	2.2±0.2
28 days	2.4±0.2	2.2±0.2	2.3±0.2	2.2±0.2
Creatinine clearance , ml/min				
14 days	0.3 ± 0.02	0.32 ± 0.01	0.31 ± 0.01	0.32 ± 0.02
28 days	0.3 ± 0.01	0.32 ± 0.02	0.3 ± 0.1	0.31 ± 0.01

The study scheme, as well as the data obtained in the study of the cumulative properties of an aqueous solution of an anthelmintic substance, are presented in Table 6.

Table 6

Cumulative properties of an aqueous solution of an anthelmintic substance

Dose of administration	**Duration of observation, days**					
	1-4	**5-8**	**9-12**	**13-16**	**17-20**	**21-24**
Daily administered dose for 4 days, ml/kg	one	1.5	2.25	3.37	5.1	7.6

Total dose, ml/kg	one	2.5	3.75	5.62	8.43	12.6
The number of dead heads from an aqueous solution of an anthelmintic substance	-	-	-	-	-	-

Discussion of the obtained results. After the end of the experiment on the study of subacute toxicity, rats of the correct physique, satisfactory nutrition, discharge from the nose and ears were not detected. The coat is shiny, foci of baldness are not determined, the teeth are preserved. Visible mucous membranes are pale in color, shiny. The genital organs of males are developed correctly, there is no deformity or swelling of the limbs.

The animals were euthanized and autopsied. There were no macroscopically distinguishable signs of pathology of internal organs. Thoracic and abdominal cavities did not contain effusion. The correct position of the internal organs of the chest and abdominal cavities. The parietal and visceral layers of the pleura and peritoneum are thin, shiny, smooth. The submandibular lymph nodes and salivary glands are oval, pale yellow or pinkish in color, with a smooth surface, a thin capsule, not soldered to each other and to the underlying tissues. When cut, the cut surface is uniform in color. The thyroid gland is reddish in color, of normal size and shape, moderately dense consistency. The thymus is triangular in shape, whitish in color, moderately dense in consistency, of normal size. The intima of the aorta is smooth, shiny, whitish in color. The aortic diameter was not changed. The leaves of the pericardium are thin, transparent, smooth. The size and shape of the heart are normal. The right and left ventricles contain a small amount of dark liquid blood. Heart valves are thin, shiny, smooth. The muscle of the heart on the cut is of a uniform cherry-brown color, moderately dense. The lumen of the trachea and large bronchi is not changed, the mucous membrane is shiny, smooth, pale in color. Light airy, without seals to the touch, pale pink in color. There are areas of fullness. The mucous membrane of the esophagus is shiny, smooth, pale in color. The stomach is of normal size and shape, filled with food contents. The mucous membranes of the stomach did not differ from the stomach lining of the control groups of animals and were folded, pink, shiny. The local irritating effect of the anthelmintic substance on the gastric mucosa was not revealed.

The mucous membrane of the small intestine is pale pink, shiny, smooth. The mucous membrane of the large intestine is grayish, shiny, smooth. The shape and size of the liver do not represent changes. The surface of the liver is smooth, of uniform dark red color, the capsule is thin, transparent. The liver tissue on the cut is plethoric, moderately dense. The pancreas is flat, pale pink, lobed, moderately dense in consistency. The spleen is of normal shape, dark cherry color, moderately dense consistency. The surface of the organ is smooth, the capsule is thin. On a section on a dark red background of the spleen, small grayish follicles are visible. The size and shape of the kidneys are not changed. The surface of the kidneys is brownish, smooth, the capsule is thin, transparent, easily removable. On the section of the organ, the cortex and medulla are clearly distinguishable. The adrenal glands are rounded, pale yellow, with a smooth surface, moderately dense. A dark-colored medulla is clearly visible on the cut. The bladder is filled with clear urine. The mucous membrane of the bladder is smooth, shiny, pale in color.

The membranes of the brain are thin, transparent. The substance of the brain is of normal density, the surface of the brain is smooth. On the frontal sections of the brain, gray and white matter are clearly distinguished. The ventricles of the brain are of normal size, there is no expansion. Ovaries (in females). Oval, dense, grape-shaped, gray-pink, compact. The testicles (males) are whitish in color and of normal size. The uterus (in females) is bifid, the surface is smooth, the color is yellowish-pink, dense. Cavity without content. The horns are thin and long.

Measurement of the mass of internal organs after the completion of the experiment showed that with

repeated intragastric administration of an aqueous solution of an anthelmintic substance at doses of 100 mg/kg; 500 mg/kg and 2500 mg/kg, the weight of the internal organs had no significant changes relative to the control. Data for determining the mass coefficients of internal organs are presented in Table 5.

Table 5

Mass coefficients of organs with repeated administration of an aqueous solution of an anthelmintic substance to white rats, mg (M ± m)

Timing research	control	aqueous solution of anthelmintic substance		
		100 mg/kg	500 mg/kg	2500 mg/kg
Heart				
28 days	3.0±0.1	3.1±0.2	3.2±0.1	3.1±0.1
thymus				
28 days	1.5±0.1	1.53±0.1	1.55±0.04	1.52±0.05
Liver				
28 days	32.2±1.5	31.1 ± 1.2	32.2 ± 1.2	31.4 ± 1.2
Spleen				
28 days	5.0±0.12	5.2±0.11	5.2±0.12	5.1±0.1
Bud				
28 days	7.3±0.1	7.1±0.2	7.2±0.2	7.1±0.1
Brain				
28 days	8.2±0.2	8.0±0.3	8.2±0.2	8.2±0.2
Testis				
28 days	7.2±0.1	7.1±0.1	7.2±0.1	7.0±0.1
Ovary				
28 days	0.45±0.01	0.47 ± 0.01	0.5±0.01	0.5±0.01
Stomach				
28 days	5.7±0.07	5.8±0.07	5.8±0.1	5.8±0.3

In the study of the study of the cumulative properties of the anthelmintic substance, in the first eight days clinically observed reduced activity, clustering of all mice for 20-30 minutes. After 45 - 60 minutes, all mice became active, freely consumed food and water.

During the entire period of administration of the preparation of an aqueous solution of an anthelmintic substance, mice did not show signs of clinical changes, such as cyanosis of the muzzle, ears, tail and limbs. Most mice had loose stools. The fur of the animals remained smooth and shiny, some animals showed lethargy and little activity, refusal of water and feed. However, after 12 hours, the animals returned to normal again. The same picture was observed on the 24th day of administration of the compared preparations.

The observed changes in the clinical manifestations and behavioral reactions of mice during the experiment were reversible, and the animals physiologically fully recovered. No death of animals was observed during the experiment.

In this regard, due to the low toxicity of the aqueous solution of the anthelmintic substance, the cumulation coefficient could not be determined.

Conclusion. Studied a subacute toxicity of an anthelmintic substance in an experiment on white rats. Thus, the obtained data on the study of subchronic toxicity showed that the studied aqueous solution of the anthelmintic substance , with repeated intragastric administration for 28 days at a therapeutic dose, at the maximum daily and maximum dose, does not cause disturbances in the functional state of the main organs and systems of the body. It does not have a locally irritating effect at the injection site (gastric mucosa). That is, an aqueous solution of the anthelmintic extract does not have a general toxic effect at the studied doses and routes of administration. The results of the study allow us to recommend the drug for clinical study. The cumulative properties of the anthelmintic substance were studied in an experiment on mice. Studies have shown that the investigated anthelmintic substance " Gelmint -ART" does not have cumulative properties. In the study of acute toxicity, it was found that the studied anthelmintic substance belongs to the IV class of toxicity, i.e. low toxicity.

References

1. Idrisov A.M., Shabalina E.V., Gainullina E.N. , Gizzatullin R.R. The results of the study of acute toxicity of the drug Dehelm - 14. Theory and practice of combating parasitic diseases . 2010. 215-217.
2. Moacă EA, Pavel IZ, Danciu C., Crăiniceanu Z., Minda D., Ardelean F., Antal DS, Ghiulai R., Cioca A., Derban M., Simu S., Chioibaş R., Szuhanek C., Dehelean CA Romanian Wormwood (Artemisia absinthium L.): Physicochemical and Nutraceutical Screening. Molecules 2019, 24 , 3087. https://doi.org/10.3390/molecules24173087
3. Musayev MB, Milenina MV, Khalikov SS, Arkhipov IA, Varlamova AI, Odoyevskaya IM, Novik TS Anthelmintic and toxicological evaluation of supramolecular complexes of triclabendazole. Meditsinskaya parazitologiya i parazitarnyye bolezni . Medical Parasitology and Parasitic Diseases. 2018; 3:15-24. (In Russ.)
4. Mariana Costa Fausto et al. Anthelmintic potential and in vivo acute toxicity study of Piptadenia gonoachanta leaf aqueous extract. Journal of Applied Pharmaceutical Science Vol. 7 (10), October, 2017. pp. 111-117. DOI: 10.7324/JAPS.2017.71016 5.
5. Lahlou S, Israili ZH, Lyoussi B. Acute and chronic toxicity of a lyophilised aqueous extract of Tanacetum vulgare leaves in rodents . Journal of Ethnopharmacology 2008, 117(2):221-7 . DOI: 10.1016/j.jep.2008.01.024
6. Magerramov, S.G. Anthelmintic actions of plants and their mixtures with a chemical preparation . News of higher educational institutions. Volga region. Natural Sciences. - 2013. - No. 2 (2). – P. 64–68.
7. Magerramov, S. G. Cumulative properties of a mixture of wormwood and harmala / S. G. Magerramov // News of natural and technical sciences. Nakhchivan branch of the National Academy of Sciences of Azerbaijan. - 2008. - No. 4. - S. 159–161.
8. Guskova T.A. Toxicology of drugs. Moscow, 2008. - S.27-30.
9. Guidelines for the study of the general toxic effect of pharmacological substances. In the guide to the experimental (preclinical) study of new pharmacological substances. Under the general editorship of Corresponding Member of the Russian Academy of Medical Sciences, Professor R. U. KHABRIEVA. Second edition, revised and enlarged. M .: - 2005. - M .: OJSC "Publishing House" Medicine ", 2005. - S. 263 - 266.
10. Nazarenko G.I. Clinical evaluation of the results of laboratory studies - M., "Medicine", 2005. - 542 p.
11. Basic methods of statistical processing of the results of ¬pharmacological experiments. /In the guide to the experimental (preclinical) study of new pharmacological substances. Under the general editorship of Corresponding Member of the Russian Academy of Medical Sciences, Professor R. U. KHABRIEVA. Second edition, revised and enlarged. M.: - 2005. - M: OJSC "Publishing House" Medicine ", 2005. - S. 763-774.

12. Stefanov A.V. Preclinical studies of drugs., Kyiv 2002. - p. 91.