

STUDY OF ACUTE TOXICITY, ALLERGIC AND IRRITANT FEATURES OF DRY EXTRACT "HELMINTH-ART"

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ABSTRACT

The acute toxicity of the anthelmintic dry extract "Helmint-ART" was studied on white male mice. As a result, it was found that the studied anthelmintic dry extract is practically non-toxic at the studied doses and routes of administration. The local irritating features of the anthelmintic dry extract "Helmint-ART" were studied in an experiment on rabbits. The allergic features of the Helmint-ART dry extract were studied on guinea pigs. Also, in the experiments on laboratory animals, it was found that it has neither local irritating nor allergic features.

KEYWORDS: *Helminths, Dry Extract, Acute Toxicity, Local Irritating Effect, Allergic Features, White Rats, Rabbits, Guinea Pigs.*

INTRODUCTION

Studying the safety of different originated medicines is an obligatory component in preclinical studies. The study of acute toxicity is one of the first stages while conducting preclinical studies, since the study of acute toxicity helps to determine the average lethal dose, as well as the obtained data on lethality, help to establish tolerated doses to conduct studies on specific activity[1]. Nowadays, the compulsory program of preclinical studies includes the assessment of allergic features of new drugs on laboratory animals [2]. Related to the current transition of national requirements for drug registration to the requirements of the Eurasian Economic Union (EAEU), the question of assessing allergic features in preclinical studies is controversial.

The study presents the results of the research of allergic features of a number of drugs with an active substance molecular weight of more than 1000 Da and less than 1000 Da in standard tests on guinea pigs, such as the reaction of systemic anaphylaxis and active cutaneous anaphylaxis [3]. The study presents the results of preclinical trials to determine the allergic features of a complex antibacterial drug intended for the treatment of mastitis in lactating cows. 4 series of experiments were carried out, including the study of active skin anaphylaxis, conjunctival test, assessment of delayed-type hypersensitivity reaction and changes in the number of eosinophils in the blood of sensitized animals [4].

The study presents the results of preclinical tests to determine the local irritating effect of the herbal remedy normotrophin by the method of conjunctival test when applied to the mucous membrane of the eyes of rabbits [5]. The massive usage of herbal anthelmintics eliminates environmental chemical pollution. Plants have a complex effect on the animal organism and are quickly excreted, therefore, the cumulative features of plants are limited [6]. Healing herbs act in a complex manner and are quickly excreted from the body [7].

The aim of the research was to study the acute toxicity, allergic and local irritant features of the anthelmintic dry extract "Helmint-ART".

Materials and research methods. All experiments were conducted on healthy animals that were quarantined for at least 10-14 days. The experiment was carried out according to the recommendations for preclinical studies of drugs [8,9].

The animals were kept in a vivarium with a standard diet, temperature and light conditions with free access to water and food. Taking into account the chronobiological dependence of most physiological processes in the body, all manipulations with animals were performed at the same time of the day in the morning. For the analysis of hematological and biochemical parameters, biological material (blood) was collected according to standard methods [10] in the morning after 14–15 hours of hunger.

The object of our study was the dry extract of "Helminth-ART". "Helminth-ART" consists of a mixture of dry extracts isolated separately from anthelmintic plant materials, such as the above the ground part of wormwood (*Artemisia absinthium* L), pumpkin seeds (*Cucurbita pepo* L.), tansy flowers (*Tanacetum Vulgare* L.) and garlic bulbs (*Allium sativum* L.). This object is the substance of the "Helmint-ART" anthelmintic capsule. The substance of the "Helmint-ART" product is a dry extract of the sum of plant collections, highly soluble in water, with a content per 0.5 g of the substance: dry extract of wormwood - 0.2 g ; dry extract of pumpkin seeds - 0.15 g; dry extract of common tansy - 0.1 g; dry garlic extract - 0.05 g. To determine "acute" toxicity, experiments were carried out on non-purebred white male mice weighing 18-20 g. The "Helmint-ART" substance was administered orally with an atraumatic probe at doses of 1000, 2000 and 3000 mg/kg as an aqueous solution. Each dose was tested on 6 mice. After a single administration of drugs, the experimental animals were observed for 14 days.

The general condition, behavior and death toll of animals in each experimental group were taken into account. Records were made on the basis of the general clinical and physiological state of mice [11,12]. The local irritating features of an aqueous solution of an anthelmintic substance were evaluated by the potential ability of the drug to irritate the mucous membrane of the eye. The experiment was carried out on rabbits of both genders, weighing 2.2-2.5 kg, which were previously under observation and had healthy eyes. 0.1 ml of a 1% aqueous solution of an anthelmintic extract was instilled into the right eye of the rabbit. The left eye served as a control, which was instilled 0.1 ml of 0.9% NaCl solution. Both eyes of the animal were examined 1, 24 and 72 hours after exposure. The state of the iris, conjunctiva was assessed by the state of the mucosa and the state of the blood-vascular graphic with the presence or absence of lacrimation.

The sensitizing properties of an aqueous solution of an anthelmintic substance were studied on 12 guinea pigs weighing 300-350 grams using the "conjunctival test" method. The conjunctival test is a very sensitive test and, in some cases, allows you to identify the reaction of animals to an allergen with weak allergization and negative skin tests. To learn the sensitizing features of

guinea pigs, they were divided into 2 groups of 6 animals each. For 20 days Guinea pigs were administrated daily with 1.0 ml of infusion intragastrically once. Testing the sensitizing features was performed on the 10th; 15th and 20th days from the beginning of the introduction of an aqueous solution of the anthelmintic substance. To set up the reaction, 2 drops of the drug was instilled with an eye dropper under the upper eyelid of experimental control guinea pigs, 1 drop of distilled water was injected into the other eye (control). After instillation, the eyelids were connected and kept in this position for 1 s. Both eyes of the animal, which was once instilled with an aqueous solution of the drug, were examined after 15 minutes (rapid reaction), after 1 hour, 24, 48 and 72 hours after exposure (delayed type hypersensitivity).

Results:

The results of the research on acute toxicity showed that with the introduction of the substance "Helminth-ART" at a dose of up to 3000 mg/kg, no special changes in the behavior of animals and their death were observed in the next 14 days (Table 1). The introduction of higher doses of the "Helminth-ART" substance turned out to be technically impossible due to the formation of a thick mass after making an aqueous solution. Due to the absence of dead mice, it was not possible to calculate the average lethal dose of the drug.

TABLE 1 THE RESULTS OF DETERMINING THE ACUTE TOXICITY OF THE DRY EXTRACT "HELMINTH-ART" IN MICE WHEN ADMINISTERED ORALLY

Dry extract "Helmint-ART"	
Doses in mg/kg	Number of mice dead / total
1000	0/6
2000	0/6
3000	0/6
LD50>3000 mg/kg	

The results of research on studying local irritant action showed that an aqueous solution of an anthelmintic substance caused a slight lacrimation on rabbits after 15 minutes, and after an hour, 24 hours; 48 hours and 72 hours signs of irritation of the mucous membrane of the eyes in the form of hyperemia, swelling, changes in the sclera and cornea were not seen. The width of the pupils also did not change, which indicates the absence of local irritating effect of the drug on mucous membrane.

When sensitization of animals was detected on the 10th, 15th and 20th days of the study, the following picture was observed: after the introduction of an aqueous solution of the anthelmintic extract into the conjunctiva, guinea pigs did not open their right eye for some time. When examining the eyes of guinea pigs after 15 minutes, an hour; 24 hours, 48 hours and 72 hours, there were no visible changes in the state of the tear duct.

Discussion of the obtained results.

It has been established that in terms of acute toxicity, the "Helmint-ART" substance, when administered orally to mice, exceeds a dose of 3000 mg/kg (Table 1), i.e. are practically non-toxic. The anthelmintic substance, when applied to the conjunctiva of the eyes of rabbits, does not have an irritating effect. Also, an aqueous solution of the anthelmintic substance, when administered daily to guinea pigs for 20 days, did not show an allergic effect.

CONCLUSION

The acute toxicity of the anthelmintic substance was studied in an experiment on white mice. Thus, the data obtained on the study of acute toxicity showed that the studied aqueous solution of the anthelmintic substance, when administered multiple times intragastrically for 14 days at a therapeutic dose, at the maximum daily dose, does not cause disturbances in the functional state of the main organs and body systems. It has been established that in terms of acute toxicity, the "Helmint-ART" substance, when administered orally to mice, exceeds a dose of 3000 mg/kg, i.e. is practically non-toxic. It does not have a local irritating effect at the injection site (gastric mucosa). The local irritating features of the anthelmintic substance were studied in an experiment on rabbits. The studies showed that the investigated anthelmintic substance "Helmint-ART" does not have irritating features. The sensitizing features of an aqueous solution of the anthelmintic substance were studied on guinea pigs. Studies have shown that the investigated anthelmintic substance "Helmint-ART" does not have allergic features.

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