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Study on the Specific Toxicology of Dorusim

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Abstract Experimental studies have shown a distinct diuretic activity of the phytocompositiondorusim which activity was equal to Ecustim. It has been established that Dorusim in terms of its pharmacological activity is not inferior to the phytomedicine canephron in experimental aseptic cystitis. Dorusim does not have a local irritant, skin-resorptive effect or cumulative properties. The authors believe that the phytocomposition Dorusim can be recommended as a pathogenetic medicine for the treatment of acute cystitis.

Keywords Phytocomposition, Diuretic, Skin-resorptive, Local irritant effect, Cumulative properties

1. Introduction

One of the important problems of modern pharmacology is the development of effective medicines for the treatment of the most common human diseases [1,2,3]. A large arsenal of medicines has been developed over the last two centuries has significantly changed the outcome of many pathologies, which has led to an increase in life expectancy, working capacity and well-being of the population [4,5]. It is with regret that we have to state that, along with the beneficial effect of medicines has been developed and introduced into practical medicine, especially synthetic ones, the number of various side effects from their use has clearly increased. Therefore, it will have to stop further pharmacotherapy. According to many researchers, a way out of this state can be achieved by developing new effective, low-toxic and affordable medicines. This problem is largely solved by the development of medicines from plant raw materials, which in their properties are close to the animal and human body [6]. With high biological activity, they rarely cause the development of side effects. We have developed a phyto-composition from plant extracts: Herbaalhagi, Folium Uvaeursi, Fructus Rosae, Glycyrrhiza glabra and Flores chamomillae, which have distinct anti-inflammatory activity [7,8]. However, preclinical studies of the specific toxicity of Dorusim have not been fully studied, which determined the need for this work.

The purpose of this work was preclinical study of the specific toxicity of Dorusim.

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2. Material and Methods

2.1. Experiments

All experimental studies were carried out on adult white male rats, herd bred, weighing 180-205 g. Before the start of experimental studies, laboratory animals were quarantined for two weeks, after which they were carefully examined and weighed. We took into account animals' age, gender, skin condition, motor activity and body weight. Animals divided by six individuals in each group. Laboratory animals were kept in a vivarium in plastic cages, bedding made of sawdust at a temperature of 20-24℃, humidity of at least 50%, in a well-ventilated room and day/night light mode, with a standard diet, free access to water and food during the entire period of preparation for the experiment and the research,. Animals were fed according to age. All manipulations with animals were carried out at the same time of day (in the morning) taking into account the chronobiological dependence of most physiological processes in the body. The diuretic activity of the prepared phytocompositions was studied according to the approved methodological recommendations [9,10]. 1 hour before urine collection, healthy rats of the experimental groups were administered the phytocomposition Dorusim at doses of 50, 100 and 200 mg/kg, Ecustim at a dose of 150 mg/kg, and canephron at a dose of 100 mg/kg. Then all animals received a water load at the rate of 3 ml per 100 g of body weight, placed in individual metabolic cages, and the amount of excreted urine was collected during 3, 6 and 24 hours of observation.

In another series of experiments, we studied the effect of Dorusim on diuresis in animals with aseptic cystitis. A model of acute aseptic cystitis in rats was developed by double intraperitoneal administration of cyclophosphamide at a dose of 80 mg/kg every other day [11]. A day later, the animals

were divided into three groups. Animals of the first group were administered Dorusim at a dose of 50 mg/kg, the second group - Canephron - 100 mg/kg, and the third group - an equivolume saline solution. Animals were then placed in individual metabolic cages to collect urine the total volume of excreted urine and the content of epithelium, erythrocytes, leukocytes and protein were determined in a three-hour urine sample.

The local irritant effect of Dorusim was assessed using a conjunctival test. The experiments were carried out on 9 rabbits weighing 2.0-2.5 kg, in which 0.1 ml of 0.5; 2.5 and 5.0% solutions of Dorusim was instilled into the right eye, and 0.1 ml of distilled water was instilled into the left eye (control). The reaction was taken into account after 15 minutes (rapid reaction) and after 24-48 hours (delayed hypersensitivity) and was assessed on the following scale (in points):

- slight redness of the tear duct;
- redness of the tear duct and sclera towards the cornea;
- redness of the entire conjunctiva and sclera.

In addition, the degree of hyperemia, swelling, and lacrimation were taken into account.

The local irritant effect of various concentrations of Dorusim was also studied in another series of experiments on 18 rats, weighing 150 - 165 g. The animals hair cover was clipped on both sides of the spinal column (4 fields) size 2x2 cm. On the right side of rats on 2 clipped areas of their backs, 0.5 ml of Dorusim solution was applied in concentrations (0.5, 2.5 and 5.0%) for 10 days. To the control clipped areas (2 left fields) the distilled water was applied in the same volume. Observation was carried out hourly for 6 hours on the first day and for the next 13 days (14 days in total).

The skin-resorptive effect of the medicine Dorusim in various concentrations was studied on 18 white outbred male rats weighing 150-170 g, which were fixed in special fixing apparatus and 2/3 length of the their tails were immersed in a test tube with 0.5; 2.5 and 5.0% Dorusim solution. The test tubes were placed in a water bath with a temperature of 28-30℃. The exposure time was 4 hours. Then, after exposure, the rats' tails were pulled out and washed with warm water and soap.

The cumulative properties of the medicine studied in repeated administration, which contributes to the accumulation of toxic substances and it is determined on the basis of a quantitative indicator - the cumulation coefficient [12].

To solve this problem, the cumulative properties of Dorusim was studied under conditions of subchronic repeated enteral administration of medicine to experimental animals according to Lim. The experiments were carried out on adult rats with an initial body weight of 150-165 g. Aqueous solutions of Dorusim were administered intragastrically with a special metal canula daily 1 hour before feeding. Control animals were administered distilled water in an equivalent volume at the same time. The experiment was carried out for 28 days; Throughout the entire experiment, the animals were monitored, their condition and degree of activity were taken into account. We also assessed the general condition (excitement, depression), the nature and degree of activity and coordination of movements, the reaction of animals to painful stimuli, the presence of tremors, convulsions, paresis, paralysis, discharge from the eye, nose, urinary tract, change in skin color, change in weight body, appetite.

All experiments were carried out in compliance with the requirements of the European Convention for the Protection of Vertebrate Animals Used for Experimental or Other Scientific Purposes (Strasbourg 1986).

2.2. Statistical Analysis

The data obtained were processed by the method of variation statistics using the paired Student's test and one-way analysis of variance using the standard software package BIOSTAT 2009 with an assessment of the significance of indicators (Mean±Std error). Differences in the compared groups were considered significant at a significance level of 95% $p < 0.05$.

3. Results and Discussion

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

One of the common diseases of the urinary system is acute cystitis [13,14]. Despite important advances in the field of pharmacotherapy for this pathology, its effectiveness is not satisfactory, which has a negative impact on the ability to work and quality of life of patients. Considering the insufficient arsenal of medicines used in the treatment of cystitis and the lack of domestic medicines, it is currently actual to develop medicines for the treatment of inflammatory diseases of the urinary tract. Biologically active compounds contained in medicinal plants: Herbaalhagi, Folium Uvaeursi, Fructus Rosae, Glycyrrhiza glabra and Flores chamomillae, conventionally called “Dorusim”, have an antioxidant and membrane stabilizing effect [15,16,17,18], which allows us to assume the presence of anti-inflammatory effects in this phytocomplex. The expected effect was compared with the well-known herbal medicines – Ecustim and canephron [19,20].

The results of conducted experimental studies in this regard have showed that Dorusim in healthy rats increased diuresis in the first three hours after water load by 37.7% at a dose of 50 mg/kg, by 46.5% at a dose of 100 mg/kg and 41.4% at a dose of 200 mg/kg (Table 1). In this observation period, Ekustim caused an increase in diuresis by only 9.2%, and canephron did not have a significant effect. The volume of urine excreted during the first six hours under the influence of Dorusimincreased by 21.1; 25.7 and 22.2%, respectively, from doses of 50, 100 and 200 mg/kg. Ekustim in this period, that is, six hours after the water load, increased

diuresis compared to intact animals by 37.3%, and canephron did not lead to statistically significant changes, although there was an increase in diuresis by 6.9%. It should be noted that by the end of the first day of observation, diuresis increased under the influence of dorusim by 10.0-12.0%, and under the influence of Ekustim increased by 35.6%, while canephron did not have a noticeable effect on the volume of excreted daily urine. From the data in Table 1, it is clear that in terms of its effectiveness, Dorusim significantly increased diuresis especially at an average dose and it surpassed Ekustim in its activity. However, diuretic activity of Ekustim did not appear in the first hours of observation, but it appeared six hours after the water load and remained at this level until the end of the experiment. The results of this series of experiments suggest that Canephron does not have diuretic activity.

Table 1. A comparative study of the effect of different doses of Dorusim, Ekustim and Canephron on the urinary excretion of rats (M±m, n=6)

Groups	Dose, mg/kg	Hours of examination (volume of excreted urine in ml.)		
		3 hours	6 hours	24 hours
Intact	-	4,32 ± 0,28 2,21 ± 0,14	5,63 ± 0,44 2,88 ± 0,22	8,22 ± 0,36 4,21 ± 0,17
Dorusim	50	5,95 ± 0,31* 3,06 ± 0,17*	6,76 ± 0,38 3,46 ± 0,20	9,15 ± 0,34 4,68 ± 0,19
Dorusim	100	6,33 ± 0,25* 3,34 ± 0,16*	7,08 ± 0,33* 3,74 ± 0,20*	9,23 ± 0,22* 4,88± 0,13*
Dorusim	200	6,11 ± 0,38* 3,22 ± 0,21*	6,88 ± 0,30 3,64 ± 0,18	9,05 ± 0,45 4,79 ± 0,28
Ekustim	150	4,72 ± 0,16 2,58 ± 0,11	7,73 ± 0,22* 4,22 ± 0,11*	11,15± 0,22* 6,08 ± 0,15*
Canephron	100	4,20 ± 0,10 2,25 ± 0,07	6,02 ± 0,11 3,23 ± 0,09	8,53 ± 0,30 4,59 ± 0,22

*Note: in the numerator is the volume of absolutely excreted urine in milliliters, and in the denominator is the volume of excreted urine in terms of 100 grams of animal body weight, * - statistical differences in relation to intact animals.*

Thus, the studied new phytocomposition, Dorusim, clearly stimulates diuresis in healthy rats and its activity is superior to Ekustim.

The results of the next series of experiments conducted on animals with acute aseptic cystitis confirmed the validity of the conclusions made in the first series of experiments. Thus, in acute aseptic cystitis, there was an almost threefold decrease in the volume of excreted urine in the first 3 hours of observation. At the same time, the content of epithelium in the urine increased by 298.2%, amount of erythrocytes increased by more than 10 times, and leukocytes increased by more than 20 times, with a high protein content (increase by more than 16.6 times).

Consequently, the obtained material clearly proves the development of acute aseptic cystitis in rats under influence of cyclophosphamide.

In contrast, 5-day experimental therapy with Canephron had a clear pharmacotherapeutic effect, which was manifested in an increase in the volume of excreted urine by 140%, in which the content of epithelium, erythrocytes, leukocytes and protein was low compared to untreated groups of animals by 43.0; 48.0; 42.0 and 58.0% respectively. We found an almost the same effect in animals treated with a new phytocomposition – Dorusim. Thus, the volume of excreted urine compared to untreated animals increased by 137.4%, the content of epithelium, erythrocytes and leukocytes in the urine decreased by 46.3%, 39.2 and 34.8%, respectively. On this background, the level of proteinuria decreased by 54.1%. It can be seen that the effectiveness of new phytocomposition is not inferior to Canephron and it can be recommended as a pathogenetic medicine in the treatment of acute cystitis, which requires special pharmacological and biochemical studies in order to introduce it into nephrological practice.

Subsequently, in order to study specific toxicity, we have conducted separate series of experiments. Thus, a conjunctival test of the studying medicines carrying out on laboratory animals is a very sensitive test and, in some cases, even allows to detect the reaction of animals to an allergen with weak allergization and negative skin tests [21].

The results of observations have showed that preparations containing various concentrations of Dorusim did not cause even slight redness after 15 minutes, nor after 24 and 48 hours. Based on the results of experimental studies, we can conclude that the studied extract was isolated from medicinal plants: Herbaalhagi, Folium Uvaeursi, Fructus Rosae, Glycyrrhiza glabra and Flores chamomillae at 0.5; 2.5 and 5.0% concentrations do not have an irritating effect on the conjunctiva of the eyes of rabbits.

The local irritant effect of Dorusim, also studied in another series of experiments on rats, showed that solutions containing various concentrations of Dorusim do not cause irritation, redness, swelling or other visible changes on the skin and the effect of the drug Dorusim is rated 0 points (Table 2). The obtained results allow us to conclude that the medicine Dorusim in the studied concentrations does not have an irritating effect on the skin.

It is common for lipophilic medicines to have a systemic effect when they are administered cutaneously. This property allows them to be used in the form of medicines for external applying (ointment, gel, etc.). However, good absorption of drugs through intact skin can lead to dysfunction of organs and systems as a result of toxic effects. Based on these positions, we studied the skin-resorptive effect of Dorusim in a separate series of experiments.

An analysis of the obtained experimental studies on the skin-resorptive effect showed that the experimental animals showed there were no signs of intoxication and death of rats, which may indicate the absence of the skin-resorptive effect of the Dorusim.

Slowing the excretion of biologically active substances or their biotransformation can lead to the development of a cumulative effect.

In connection with the repeated use of various medicines is arisen the problem of assessing them from the point of view of safety of use in practice for excluding possible

long-term effects on animals and humans. This problem can be solved by conducting a toxicological assessment taking into account the cumulative activity of new pharmacological agents and especially long-term administrated drugs. The drug can accumulate in the body with the repeated daily use (cumulation of the substance).

Cumulative properties – accumulation of effect upon repeated administration of toxic substances; determined on the basis of a quantitative indicator – the cumulation coefficient [12].

In the first eight days after the daily administration of the Dorusim orally, some decrease in motor activity was observed during the observation of the general conditions of the animals within 10-20 minutes after administration of the medicine; all animals became active and freely consumed food and water after 50-60 minutes. During the entire study period, administration of the medicine did not cause clinical signs of disturbance in the general condition of the animals, such as cyanosis of the face, ears, body and limbs. Some rats showed changes in stool consistency (mushy). During the entire period of the experiment, the animals' fur was smooth and shiny; there were decreased activity and limited consumption of food and water in single animals. However, all these changes disappeared after 2-3 hours, and the animals returned to their normal state. A design of the study and the results of studies for establishing the cumulative properties of the test drug is shown in Table 3.

When calculating the cumulation coefficient of the drug, it was equal to K <0,9.

The observed changes in clinical manifestations and behavioral reactions during the experimental period were reversible in many animals (1-28 days).

Therefore, based on the experimental results, it can be concluded that the medicine Dorusim does not have cumulative properties.

4. Conclusions

1. The phytocompositionDorusim has a distinct diuretic activity.
2. The pharmacological activity of Dorusim is not inferior to the phytodrugcanephron in experimental aseptic cystitis.
3. Dorusim does not have a local irritant, skin-resorptive effect or cumulative properties.
4. Dorusim can be recommended as a pathogenetic agent in the treatment of acute cystitis.

Table 2. Skin test scale and assessment of the local irritant effect of Dorusim in various concentrations in points

Description of the reaction	Score, in points	Dorusim		
		0,5%	2,5%	5,0%
Erythema and eschar formation				
Absence of erythema	0	0/0	0/0	0/0
Very slight erythema (slightly noticeable)	1	0/0	0/0	0/0
Marked erythema	2	0/0	0/0	0/0
Moderate erythema	3	0/0	0/0	0/0
Severe erythema (bright red with eschar formation)	4	0/0	0/0	0/0
Edema formation				
No swelling	0	0/0	0/0	0/0
Very mild swelling (slightly noticeable)	1	0/0	0/0	0/0
Noticeable swelling	2	0/0	0/0	0/0
Moderate swelling	3	0/0	0/0	0/0
Severe swelling	4	0/0	0/0	0/0
Maximum points	8	0/0	0/0	0/0

Table 3. Cumulative properties of Dorusim

Days of study	Daily dose, mg/kg	Total dose, mg/kg	dead/alive		Animal death, %	
			experiment	control	experiment	control
1-4	50	200	0/10	0/10	0	0
5-8	75	300	0/10	0/10	0	0
9-12	112,5	450	0/10	0/10	0	0
13-16	168,8	675,2	0/10	0/10	0	0
17-20	253,2	1012,8	0/10	0/10	0	0
21-24	379,9	1519,6	0/10	0/10	0	0
25-28	569,8	2279,2	0/10	0/10	0	0
Total per day	1609,2	6436,8	K_{cum}= 6436,8 : 8000 = 0,8			

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