Research Article

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Study of The Acute Toxicity and Specific Activity of Veninorm

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ABSTRACT

This paper presents the results of a comparative study of the pharmacological and toxicological properties of Veninorm, a drug based on Aesculus hippocastanum extract, and Heparin Ointment against varicose veins, as well as venous stasis, thrombophlebitis and trophic ulcers of the lower extremities. It has been established that "LD50" for Veninorm is 5g/kg. In the study of specific activity for comparison with the drug "*Aesculus hippocastanum* extract". It has been established that Veninorm ointment effectively (at different stages) inhibits the process of transudation and enhances the process of transudate resorption during experimental venostasis.

KEYWORDS: Veninorm ointment; Acute toxicity; Specific activity; LD50

INTRODUCTION

One of the urgent problems of modern resorption is the complex use of medicinal plants based on resource-saving technologies. Promising in terms of integrated plant used is *Aesculus hippocastanum L. Aesculus hippocastanum* is quite widespread in many countries of the world; it has antioxidant, vascular-strengthening, anti-edematous, anti-inflammatory effects [1]. It is also known as antimicrobial, antifungal, antitumor effect of extracts of various organs of *Aesculus hippocastanum* nut [2]. For the manufacture of herbal medicines, plant seeds are used in the production of cosmetics and in folk medicine leaves, bark and flowers of *Aesculus hippocastanum* nut. The use of plant phytomass is associated with an insufficient degree of knowledge of the chemical composition. The pharmacological properties of horse *Aesculus hippocastanum* fruits are associated with their content of coumarins and oxycoumarins. Thus, the fruits contain coumarins and oxycoumarins, an important component of the seeds is the triterpene saponin glycoside escin, which is a mixture of α-escin, β-escin and cryptoescin (also known as escin Ia, Ib, IIa, IIb and IIIa) [3-6], and their aglycones (escigenin, protoescigenin, baringtogenins C and D) [6,7]. The biological activity of escin is due to β-escin. *Aesculus hippocastanum* fruits contain about 0.13% flavonoid glycosides, about 0.9% tannins, 5-7% fatty oils, 11% proteins, pectin's, starch (up to 49.5%). Flowers and leaves of *Aesculus hippocastanum* nut contain substances of flavonoid nature, derivatives of quercetin and kaempferol [8]. In addition to flavonoids, *Aesculus hippocastanum* flowers contain polysaccharides and tannins; leaves also contain polysaccharides (pectin's) and carotenoids [6]. Horse chestnut bark contains coumarins, oxycoumarins, tannins, phytosterols, fatty oils (2.5-7%), polysaccharides (9%), and vitamins. Horse chestnut drugs are used in therapy for chronic venous insufficiency [9,10], they have an angioprotective (capillary-strengthening) effect and are used for hemorrhoids [1], varicose veins, as well as venous stasis, thrombophlebitis and trophic ulcers of the lower extremities. Chronic venous insufficiency is the most common disease of the cardiovascular system [11]. With venous insufficiency, in the form of stagnation of blood in the lower extremities and a violation of its outflow. This syndrome often occurs as a consequence of varicose veins or deep vein thrombosis. Also, the cause of development [12,13]. The purpose of this study is to determine the acute toxicity and specific activity of the Veninorm drug developed by us based on the local *Aesculus Hippocastanum*.

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MATERIALS AND METHODS

To determine the parameters of acute toxicity, the Noakes D.N method was used and Sanderson et al. [14], with some modifications, determination of dermal toxicity of pesticides. A method for measuring the transdermal "LD50" of a pesticide using a laboratory rat as a test animal is described. The technique involves removing the hair from the torso and applying a chemical to the exposed skin, which is covered with an impervious bandage for the required contact time; after decontamination, the animal is examined for toxic effects. All pharmacological studies were carried out on healthy adult white outbred rats weighing 180-210 g of both sexes, quarantined for 10-14 days. Rats were kept in standard plastic cages on sawdust bedding. The temperature was maintained at 20-25°C, relative humidity 40-70%, fed and watered ad libitum throughout the trials. The day before the experimental studies, hair was removed from the body with 4.5x7cm electric veterinary clippers. Only animals whose epidermis appeared intact and healthy at the time were used. Veninorm is applied to the cut area of the skin of rats at a dose of 5g/kg. Animals were observed hourly during the first day of the experiment. Every day, for 2 weeks, groups of diseases were observed, causing a general condition and activity, and their behavioral reactions were recorded. All experimental animals were kept on a general diet with free access to water and food. Acute toxicity was assessed by changes in body weight, behavioral characteristics, the nature of motor activity, the presence of seizures, coordination of movements, reactions to tactile, pain and sound stimuli, the condition of hair and skin, as well as microscopic changes in the skin [15,16].

The venotonic effect of 4% Veninorm gel was studied on white rats weighing 180-200 g of both sexes using the method of venostasis followed by the development of edema. Healthy quarantined animals were selected for the experiment. The animals were divided into three groups of six individuals each:

Group № 1 control group; Group № 2 - experimental, Veninorm ointment + venostasis was used; Group № 3 - experimental, heparin ointment + venostasis was used.

In rats starving for 6 hours, the initial volume of the tail was measured with an oncometer up to the mark at its base. 1 - hour after application of the studied 4% Veninorm gel and reference preparations, the base of the tail in the region of the mark was squeezed with a ligature with a force of 200.0g, and the dynamics of the increase in the volume of the tail was recorded at 1,2,3,4 hours after the ligation was applied. 1- hour before the removal of the ligature, the preparations in the indicated doses were applied repeatedly. The control animals were in equal conditions, the registration time was the same. The ligature was removed, and the dynamics of edema involution was recorded at 1,2,3,4 and 24 hours. The experiment involved 4 groups of animals; each group consisted of 6 animals.

RESULTS AND DISCUSSION

The acute toxicity experiment showed that after a single application of the test drug at a dose of 5g/kg, no visible changes in the behavior and functional state of the animals were observed, food and water intake was normal. All rats were active, and no signs of intoxication were observed. The rats responded adequately to tactile, pain, sound, and light stimuli. The frequency and depth of respiratory movements were normal. Macroscopic changes in the skin and pathological changes in the hairline of the animals were not observed. Within 2 weeks, the death of rats was not observed. "LD50" of the drug was more than 5g/kg (Table 1).

Table 1: Determination of acute toxicity of Veninorm 4 % ointment.

No. of Animal	Veninorm» 4 % ointment			
	Weight, g	Dose	The Way of Introduction	Results
1	185	5 g/kg	topically	These is no death
2	194			These is no death
3	180			These is no death
4	198			These is no death
5	193			These is no death
6	200			These is no death
LD_{50}	>5 g/kg			

Table 2: Effect of Veninorm and Heparin ointment on the dynamics of tail volume tail in rats after ligation (M ± m, n=6).

Groups	Volume Tail in Rats								
	Exodus	After 1 hour		After 2 hours		After 3 hours		After 4 hours	
		Abs. Volume	Swelling	Abs. Volume	Swelling	Abs. Volume	Swelling	Abs. Volume	Swelling
Control	2,06 ± 0,12	2,43 ± 0,08	0,37 ± 0,11	2,54 ± 0,11	0,47 ± 0,12	2,99 ± 0,09	0,94 ± 0,08	3,17 ± 0,08	1,11 ± 0,06
Veninorm	1,98 ± 13	2,28 ± 0,17	0,33 ± 0,05	2,42 ± 0,16	0,46 ± 0,05	2,44 ± 0,19	0,48 ± 0,09	2,48 ± 0,09	0,52 ± 0,11
Heparin Ointment	2,03 ± 0,12	2,59 ± 0,10	0,56 ± 0,08	2,54 ± 0,14	0,50 ± 0,13	2,83 ± 0,10	0,79 ± 0,09	2,85 ± 0,11	0,82 ± 0,10

The results of the specific activity experiment showed that in the control animals, the maximum edema caused by venostasis developed by the 4th hour after compression and did not completely disappear within 24 hours. In the control group, 1 hour after the ligation, the edema increased statistically significantly by 17.9%, after 2 hours - by 23.3%, after 3 hours - by 45.1%, after 4 hours -

by 53.8% compared to with original data. In the group of animals treated with Veninorm, 1- hour after dressing, the edema was 10.8% lower, after 2 hours it was 2.1% lower than in control animals, and after 3 hours it was 48.9% lower than in control animals, and after 4 hours it was 53.1% lower than in control animals. Only 3 hours after dressing, a 15.9% decrease in edema was observed, and after 4 hours, a 26.1% decrease in edema was observed compared with the control data (Table 2).

In the control group, after removal of the ligatures, there was a slight decrease in edema compared to the initial result. In the group of animals treated with Veninorm, tail edema after 1 hour, after 3 hours and after 4 hours was significantly less than in the control group after removal of the ligature. This shows that Veninorm helps to accelerate the involution of edema in venostasis. In the group of animals on which the reference drug heparin ointment was used, a significant decrease in edema was observed after 2 hours, 3 hours and 4 hours after removal of the ligature. Thus, the results obtained during the experiment showed that the drugs effectively (at different stages) inhibit the process of extravasation and enhance the process of resorption of transudate during experimental venostasis (Table 3).

Table 3: Effect of Veninorm and Heparin ointment on edema involution in the dynamics after ligature removal (M ± m, n=6).

Groups	Volume Tail in Rats									
	After 1 hour		After 2 hours		After 3 hours		After 3 hours		After 24 hours	
	Abs. volume	swelling	Abs. volume	swelling	Abs. volume	swelling	Abs. volume	swelling	Abs. volume	swelling
Control	2,67 ± 0,11	0,61 ± 0,05	2,52 ± 0,13	0,46 ± 0,08	2,59 ± 0,15	0,53 ± 0,10	2,57 ± 0,15	0,51 ± 0,10	2,25 ± 0,18	0,11 ± 0,09
Veninorm	2,09 ± 0,18	0,13 ± 0,12	2,14 ± 0,17	0,46 ± 0,05	2,11 ± 0,16	0,14 ± 0,10	2,06 ± 0,17	0,10 ± 0,01	2,042, 25 ± 0,13	0,08 ± 0,07
Heparin Ointment	2,42 ± 0,09	0,38 ± 0,08	2,13 ± 0,09	0,10 ± 0,03	2,10 ± 0,09	0,06 ± 0,03	2,18 ± 0,10	0,14 ± 0,08	2,14 ± 0,13	0,11 ± 0,08

CONCLUSION

Varicose veins are not only unpleasant, but also painful. Therefore, it certainly needs to be treated using all available means. Most often we are talking about complex therapy, which includes, among other things, the use of various creams, ointments and gels. We offer "Veninorm" 4% ointment in comparison with the similar drug "Heparin ointment", prove that the drug effectively (at different stages) inhibits the process of transudation and enhances the process of resorption of transudate during experimental venostasis. "LD50" of the drug was more than 5 g/kg. The preparation belongs to practically non-toxic substances.

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