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PHARMACO-TOXICOLOGICAL ACTIONS OF LIQUID EXTRACT "HEMOSTAT".

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Abstract:
According to literary data, in folk medicine, medicinal herbs of the mountaineer, the bird's mountaineer, pepper and nettle, have long been widely used. They have astringent, diuretic, anti-inflammatory and antimicrobial effects[1]

Keywords: literary data, folk medicine, medicinal herbs, anti-inflammatory

According to literary data, in folk medicine, medicinal herbs of the mountaineer, the bird's mountaineer, pepper and nettle, have long been widely used. They have astringent, diuretic, anti-inflammatory and antimicrobial effects[1]

In particular, folk medicine herbal preparations from the herbs of the mountaineer bird, mountaineer pepper and nettle in the form of infusion, decoction are used for diseases of the liver, kidneys, bladder, uterus, stomach ulcers and hemorrhoids as an anti-inflammatory, diuretic and hemostatic agent.[2] Preparations of knotweed, pepper and nettle due to the content of flavonoid compounds (avicularin, quercetin, isorhamnetin, myricetin, kaempferol, luteolin), flavonol derivatives (rhamnasine, hyperoside), tannins, phylloquinone, vitamins B2, B6, C, E , K, carotene, pantothenic acid, coumarins, chlorophyll, as well as essential oils, mucus, fats, sugars, silicic acid compounds, a significant amount of trace elements - iron, copper, vanadium, calcium, magnesium and silver have astringent, diuretic, anti-inflammatory and antimicrobial action [3,4]

Composition of the drug Liquid extract "Hemostat" per 1 liter.
Grass mountaineer bird ((Polygonum aviculare L.) - 300 g
Grass mountaineer pepper (Polygonum hydropiper L) - 300 g
Nettle leaves (Urtica dioica L.) - 300 g
Ethyl alcohol 70% - a sufficient amount to obtain 1 liter.

The study of acute toxicity of the liquid extract "Hemostat"

The general effect and acute toxicity of the drug are necessary on 30 mice weighing 17-21g and on 30 rats weighing 136-165g, of both sexes. The liquid extract studied was administered orally at a dose of 0.1 ml to 1 ml per body weight of mice (i.e., 1.5 ml to 2 ml/kg) [5,6] weeks under vivarium conditions. The results of the experiments showed that when administered orally, the studied liquid extract at a dose of 0.1 ml to 0.25 ml per animal weight does not change. At a dose of 0.25 ml to 1 ml per animal weight in mice, a pronounced, pronounced excited state is quickly detected, after 7-10 minutes there is a notice of mobility, a pronounced calm, and in rare mice a drowsy state is observed. With the introduction of the study drug in large doses (from 0.5 ml in mice to 3 ml in rats), the indicated semi-drowsy state in animals lasted up to 1.5-2 hours, in animals the initial state. No deaths were observed during the observation period. In a separate group of animals, experiments with a dealcoholized liquid extract were carried out on 12 mice and 12 rats. The studied liquid extract was dealcoholized by evaporation to a two-fold decrease in volume and administered orally at doses of 0.75–1 ml per mouse weight and at a dose of 4–8 ml per rat weight. At the same time, pronounced sedation and drowsiness were not observed. At the same time, there was a limitation of mobility, shallow and clear breathing, which passed on its own within 45-60 minutes.

Table No. 1

The results of indicators of acute toxicity of hemostat

Doses, ml/kg	Number of mice dead/total	LD50 ml/kg
0,1	0/6	≥ 100,0
0,25	0/6	
0,5	0/6	
0,75	0/6	
1,0	0/6	

Therefore, it can be said that the studied liquid extract from herbs Knotweed, Knotweed and nettle is relatively low toxic.

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DISCUSSIONS:

When studying the acute toxicity of the hemostat, it was noted that the general behavior of the experimental animals did not differ from that of the control animals. The animals were active, took water and food well, and responded to external stimuli. During the observation period, all animals remained alive. It was not possible to install LD50.

Macroscopic examination revealed that the drug in the studied doses with a single injection on the mucous membrane of the oral cavity and gastrointestinal tract, as well as on the morphology of parenchymal organs, does not have any negative effect.

CONCLUSIONS:

According to the classification of the toxicity of substances, the hemostat belongs to the IV class of low-toxic compounds.

The study of the allergenic and cumulative action of the liquid extract "Hemostat"

To study the effect of drugs on the course of allergic reactions, the animals were sensitized and after 21 days they were intraperitoneally injected with native chicken egg protein (1 ml/kg). In the control group of animals treated with distilled water, signs of anaphylaxis were noted: the frequency of respiratory movements became more frequent, breathing became shallow, the tone of skeletal muscles relaxed, there was a violation of coordination of movements, and the animals became restless. And against the background of a hemostat and tincture of the highlander bird, these changes in animals decreased and proceeded less pronouncedly than in the control.

Therefore, the drugs under study have a weak hyposensitizing effect.

Study of the locally irritating effect of the liquid extract "Hemostat"

In the first series of experiments, the conjunctival test was performed on mice, rats, guinea pigs and rabbits. [5] Each experimental group had 3-5 animals.

The studied liquid extract "Hemostat" in various concentrations and in the form of a dealcoholized form, 1-3 drops were applied to the conjunctival sac of the right eye of guinea pigs and rabbits. The left eye was the control. The drug solvent was applied to it in the appropriate volume and concentration. The reaction of conjunctiva to the drug at a concentration of 0.25 -1% solution and the dealcoholized form at a dilution of 1:10 was evaluated after 5, 15, 30, 60 minutes and after 6 and 24 hours. Observation of the state of the conjunctiva showed that the studied drug in the studied concentrations and forms does not significantly irritate the conjunctiva of the eyes of experimental animals.

In another series of experiments, the studied drug at a concentration of 10, 20, 40% solution was applied to the oral mucosa of rats, guinea pigs and rabbits in a volume of 2-5 drops, as well as by oral administration of the drug in doses of 2.5-5 ml/kg and 10 ml/kg per gastrointestinal mucosa. It was found that after applying the liquid extract in the studied doses of the oral mucosa and gastrointestinal tract, the animals were normal and without signs of irritation and changes.

In another series of experiments on mice and rats, the irritating effect of the liquid extract on the skin was studied. At the same time, the pre-shorn skin of animals was smeared with the drug in the studied concentrations and in the dealcoholized form for two weeks. Experiments have shown that the studied liquid extract and its dealcoholized form do not have a locally irritating effect, since macroscopic examination of the skin of animals did not reveal any special changes compared to the control ones.

DISCUSSIONS:

To study the local irritating effect, the preparations were applied to pre-shorn skin areas. It was found that the hemostat and the tincture of the highlander bird do not have an irritating effect. There were no signs of inflammation (erythema, edema and redness) on the scarified area.

When the studied substances were instilled into the conjunctiva of the eyes of guinea pigs, it was determined that the preparations in the indicated concentrations did not cause any reaction from the conjunctiva after 24 and 48 hours. The state of the conjunctiva of the right eye did not differ from the state of the conjunctiva of the left eye, where water was injected.

CONCLUSIONS:

Therefore, we can say that the studied drug in the studied concentrations does not have a local irritant effect.

The study of the cumulative properties of the liquid extract "Hemostat"

Experiments on the study of cumulative properties [7] were carried out on 30 rats weighing 137-153 g of both sexes. Animals were divided into 3 (10 laboratory animals) group. The studied liquid extract Hemostat was administered to the first group of animals, and the dealcoholized form of the drug was administered to the second group of animals. The third group was the control. In the first 5 days, the studied

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Volume-8, March 2022
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preparations were administered, respectively, 1 ml and 1.5 ml per animal weight.

In the next 5 days, respectively, 2 ml and 2.5 ml per weight of animals, and on days 11-15 of the experiment, 2.5 ml and 4 ml per weight, and in the next 5 days, respectively, 3 ml and 6 ml per weight of animals.

The control group received the drug diluent with distilled water according to the same scheme and volume dose.

The condition of the animals was monitored visually, with the main attention being paid to the general condition, reactions to external stimuli, appetite, and weight of the animals.

The experiments showed that no significant differences in weight were noted in both the experimental and control groups of animals. Mucous membranes and wool covering of all animals were without any special changes. Animals ate food and water with pleasure. The respiratory rate in all groups of animals were the same, digestive disorders (such as diarrhea or constipation) were not observed. At autopsy of the animals on the 20th day of the experiment, a normal morphological picture of the internal organs was observed. In all animals, no visual changes were found in the vital internal organs.

Conclusions: Therefore, the liquid extract "Hemostat" in the studied concentrations and in the dealcoholized form does not have a cumulative effect.

The study of chronic toxicity of the liquid extract "Hemostat".

In order to introduce the study drug, the chronic toxicity of the liquid extract was studied in rats. [7,8]

In a separate series of experiments, we studied the effect of long-term administration of the studied drug on the animal body. The experiments were carried out on 30 rats weighing 135-147 g, of both sexes. The liquid extract was administered daily, orally, during the first month at a dose of 0.15 ml/kg and 1.5 ml/kg. Control groups of rats received 35% ethanol at a dose of 1.5 ml/kg. In the course of the study, special attention was paid to the dynamics of changes in the weight of animals, the picture of peripheral blood and the composition of urine. In parallel, the state of the cardiovascular system, lungs, liver and central nervous system was monitored.

In all animals at the beginning, in the middle and at the end of the experiment, blood and urine tests were performed.

It was noted that the animals tolerated long-term oral administration of the study drug well: the behavior, appearance, appetite, body weight of rats and the reaction of animals treated with the studied liquid extract to external stimuli did not differ from control animals. Under the influence of the studied liquid extract of the mountaineer, the mountaineer and the nettle, the amount of hemoglobin, erythrocytes was within the physiological norm, pH-urine and specific gravity also did not change (Table No. 2). There were slight tendencies to accelerate the time of blood clotting.

To study the chronic effect on the organism of animals, the drugs were administered for three months. It was noted that the animals tolerated long-term oral administration of the study drug well. Behavior, appearance, appetite, body weight and reaction to external stimuli, animals of the experimental groups did not differ from the control. Peripheral blood parameters were monitored on dynamics.[7,8]

The results are shown in table. 2

Table number 2

Influence of hemostat on the picture of peripheral blood of rats during its long-term administration

Blood parameters	Initial data	Picture of peripheral blood after the introduction of liquid extract through:					
		45 days			90 days		
		Gemo-stat 1 ml/kg	Gemo-stat 4 ml/kg	Tincture 5 ml/k	Gemo-stat 1 ml/kg	Gemo-stat 4 ml/kg	Tincture 5 ml/kg
Hemoglobin, g%	14,1±0,3	14,5±0,6	15,1±0,5	14,9±0,4	14,8±0,6	15,6±0,5*	15,9±0,2** *
Erythrocytes, 10^{12} g/l	5,2±0,25	5,45±0,5	5,7±0,3	6,0±0,5	6,2±0,45	6,5±0,50*	6,9±0,53*
Leukocytes, 10^{9} g/l	13,2±2,3	13,5±1,8	14,0±2,1*	12,8±1,3	13,7±2,1	13,5±1,6	13,4±2,4
Platelets	365+4,8	390+6,5	425+5,0*	450+3,5	410+7,5*	485+6,0	525+8,0

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10^9 g/l							
Leukoformula:							
Young	-	-	-	-	-	1	-
p / nuclear	2	2	1,5	2	1,5	1,5	2
Eosinophyla	2,5	2	2,5	2	2,5	2,0	2,5
Basophils	2	2,5	2,5	3	2,5	2,5	2,0
c/nuclear	19	23	24	23,5	24,0	22,5	22,0
Lymphocytes	72	68,5	67,7	68,5	66,6	68,5	69,0
Monocytes	2	1	2,0	1,0	2,0	2,0	1,0

Note: * - differences relative to outcome data are significant (* - P<0.05)

DISCUSSION:

Analysis of peripheral blood showed that with the introduction of a hemostat at 1 and 4 ml / kg for 45 days, the amount of hemoglobin increased by 3.0%; 7.1% and 5.7% from the initial level. At the end of the experiment, hemoglobin indicators increased by 4.9%, respectively; 10.6% and 12.7% from the baseline. With the introduction of drugs for 45 days, the number of erythrocytes increased by 4.8%, respectively; 9.6% and by 15.4%, and by the end of the experiment - by 9.2%; 25.0% and 32.7% of the original. The number of leukocytes and leukocyte formula during the observation period were within the physiological norm.

It was noted that morphological changes in erythrocytes and leukocytes were not detected. Peripheral blood parameters in control animals remained unchanged.

CONCLUSION:

Therefore, hemostat in the studied doses has a beneficial effect on peripheral blood parameters.

When studying the chronic toxicity of drugs, the dynamics of changes in body weight of animals was taken into account. The experiments showed that the body weight of the control animals did not change significantly during the observation period.

The results of biochemical studies in table 3

Table #3

Some biochemical parameters of blood in rats after prolonged administration of hemostat (M+m; n=6)

Doses, ml/kg	45 days			
	Total protein, g/l	Glucose mmol/l	AST, mkat/l	ALT, mkat/l
Control	65,0±2,0	5,3±0,50	0,26±0,02	0,21±0,03
Hemostat 1 ml/kg	68,6±3,5	6,9±0,30*	0,31±0,015	0,24±0,02
Hemostat 4 ml/kg	73,5±5,0	7,6±0,45*	0,35±0,04	0,27±0,015
doses, ml/kg	90 days			
	Total protein, g/l	Glucose mmol/l	AST, mkat/l	ALT, mkat/l
Control	60,5±1,5	4,5±0,20	0,27±0,03	0,24±0,01
Hemostat 1 ml/kg	64,9±2,5	5,1±0,35	0,30±0,015	0,29±0,015*
Hemostat 1 ml/kg	78,8±4,5**	5,6±0,45	0,35±0,01*	0,32±0,025*

Note: * - differences relative to the data of the control group are significant (* - P<0.05, ** - P<0.01, *** - P<0.001)

After the completion of the experiments, rats were slaughtered by decapitation, pieces were removed from vital internal organs for histomorphological studies.

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Histomorphological studies were carried out at the Department of Histology of TMA, Doctor of Medical Sciences, Professor A. Yu. Yuldashev. At the same time, the following were noted in the experimental groups:

Stomach - the gastric pits are well defined, the lumens of the fundic glands are somewhat dilated. There are small lymphoid-histocytic infiltrates under the glands. Muscle layer of normal thickness (Fig. 1).

Small and large intestine - there are small infiltrations around the vessels of the submucosal layer. Crypts are well expressed, there is mucilage of epithelial cells. The blood vessels are markedly dilated.

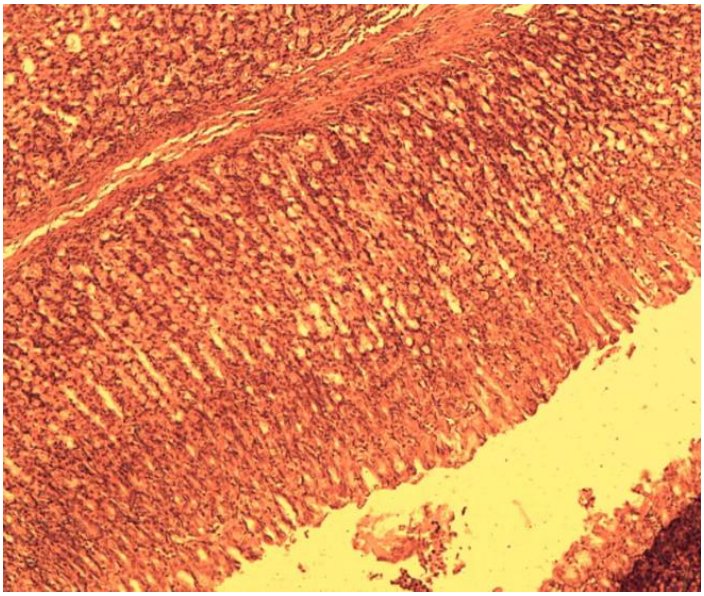

Fig. 1 Histostructure of the stomach with chronic administration of a hemostat
Chief and parietal cells of the fundic glands of the stomach
Staining: hematoxylin-eosin. SW. 10×20

Liver - the structure of the liver cells is preserved, large vessels are dilated and filled with blood. In the area of triads, there is a slight lymphoid-histocytic infiltration.

Kidneys - there is a well-distinguishable cortical and medulla substance. In the glomeruli, there is a slight expansion of the lumen of the capillaries. The walls of arterial vessels are hypertrophied. There are no signs of alteration and inflammation (Fig. 2).

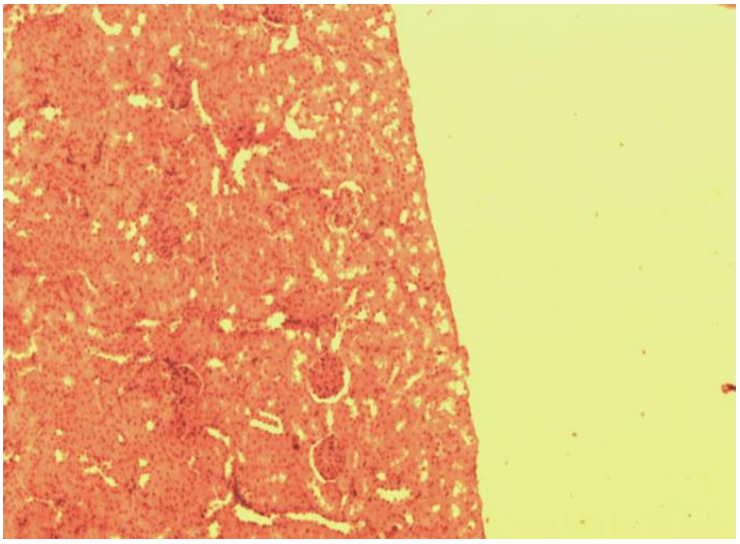

Fig. 2. Morphological picture of the kidneys in chronic administration
hemostat. Renal corpuscle and nephron tubules.
Staining: hematoxylin-eosin. Magnification 10x20

Spleen - well differentiated white and red pulp. The red pulp occupies most of the parenchyma. It is noted that in the islets of the white pulp, the lumen of the thrombocular vessels is somewhat dilated.

Heart - a normal morphological picture is preserved. There is a transverse and longitudinal striation of cardiomyocytes (Fig. 3)

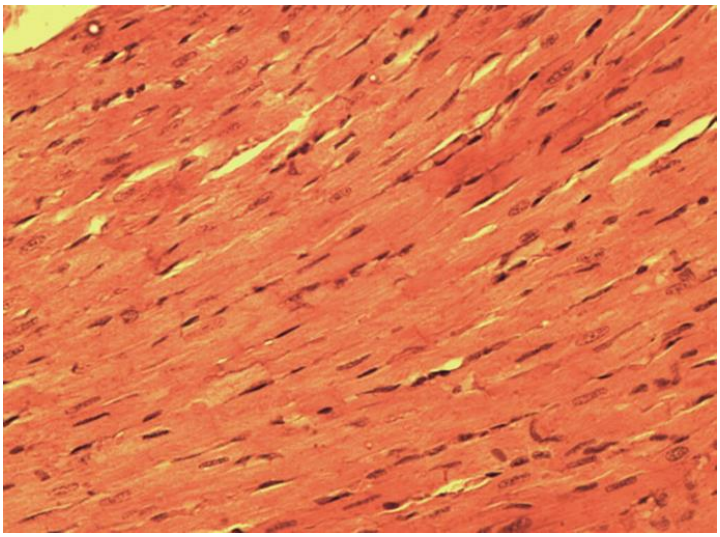

Fig. 3 Morphological picture of the heart in chronic administration
hemostat. Cardiomyocytes without structural changes.
Staining: hematoxylin-eosin. SW. 10×20

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Lungs - the lung tissue is airy, the blood vessels are dilated, there are lymphoid-histocytic infiltrates around the vessels. There is a slight swelling of the epithelial cells of the bronchi.

Conclusions: Thus, based on the results of histological studies, we can conclude that the studied preparations do not lead to pathomorphological changes in the internal organs. Hemostat does not have a toxic effect with prolonged use.

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