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ANTI-INFLAMMATORY ACTIVITY OF THE COMPLEX HERBAL REMEDY

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ABSTRACT

During experiments on white rats of the Wistar line, anti-inflammatory activity of the extract of dry complex herbal remedy (*Juglans regia L.* Rich ex Kimth., *Corylus avellana L., Agrimonia eupatória L., Bidens tripartita L., Xanthium strumarium L., Urtica dioica L. Lemna minor L., Cichorium intybus L., Onopordum acanthium L.*) was examined on models of acute exudative (carrageenan and formalin), chronic alterative and proliferative inflammation. It is found that phytoextract in doses of 100-300 mg/kg has antiexudative activity, reducing the exudation caused by phlogogenic agents. The tested extract has an anti-alterative effect, limiting the alteration of tissues with acetic acid and enhancing regenerative processes.

Keywords: complex remedy (*Juglans regia* L. Rich ex Kimth., *Corylus avellana* L., *Agrimónia eupatória* L., *Bidens tripartita* L., *Xanthium strumarium* L., *Urtica dioica* L. *Lemna minor* L., *Cichorium intybus* L., *Onopordum acanthium* L.), anti-inflammatory activity.

Introduction

Currently, hypothyroidism is one of the most common diseases of the endocrine system. According to epidemiological studies, the incidence of this disease in the population is about 2%; while in the group of women over 74 years old it reaches 21% [3]. Patients with hypothyroidism, against the decrease in the level of basal metabolism and anabolic processes in general, as well as the activation of free radical oxidation and the weakening of the body's antioxidant defense, show a slowdown in the regeneration of damaged tissues and recovery processes. Posttraumatic intoxication in turn aggravates disorders of thyroid regulation of intracellular metabolism [11].

To treat hypothyroidism, thyroid hormone preparations, drugs containing iodine, and preparations that affect the immune system (immunosuppressors and immunomodulators), as well as efferent therapy are used. Drug treatment methods for hypothyroidism, which are part of the scope of evidence-based medicine, allow for achieving clinical results, while not always achieving the proper life quality for a particular patient, require compulsory medical supervision and often have side effects [5].

Of particular interest in the treatment of hypothyroidism are herbal remedies that, due to the synergism of biologically active substances, have systemic exposure on the body: normalize the level of hormones, manifest antioxidant, anti-inflammatory, psychotropic, cardioprotective and other actions, and thus contribute to delaying the administration of hormone replacement therapy or reducing the dose of hormones during its administration [4]. In the light of the development of personalized medicine, a complex herbal remedy consisting of: *Juglans regia L. Rich ex Kimth., Corylus avellana L., Agrimonia eupatória Ldb, Bidens tripartita L., Xanthium strumarium L., Urtica dioica L. Lemna , L., Onopordum aconthium* L is of interest. Earlier in animal experiments, it was found that this complex remedy exerts a pronounced pharmacotherapeutic efficacy in experimental hypothyroidism, increasing the synthesis of thyroid hormones, peripheral conversion of fT4 to fT3, normalizing the cardiovascular parameters, increasing animal resistance to hypoxia [14].

The **study objective** was to evaluate the anti-inflammatory activity of the dry complex herbal remedy extract with thyroid-stimulating activity.

Study materials and methods

the experiments were performed on Wistar rats of both sexes with an initial mass of 180-190 g. The animals were kept in standard vivarium conditions with the same care and nutrition, light and temperature conditions in accordance with Order No. 708H of the Ministry of Health of the Russian Federation of August 23, 2010 "On Approval of Laboratory Practice Regulations". Experimental studies were carried out in accordance with the "Work code for using experimental animals" (Appendix to the Order of the Ministry of Health of the USSR No. 755 of 12.08.77) and "Rules adopted in the European Convention for the Protection

of Vertebrates" (Strasbourg, 1986). Experimental studies are coordinated with the ethics committee of the Institute of General and Experimental Biology of the SB RAS (Protocol № 6 12.10.2016).

Animals were divided into 5 groups: one control and four experimental groups. Each group consisted of 8 animals. Animals of I - III experimental groups received an aqueous solution (10 ml / kg) of an extract of a dry complex herbal remedy, respectively (hereinafter phytoextract) in doses of 100, 200 and 300 mg / kg, animals of the IV group received referential preparation caleflon ("Vifitekh" Russia) in a dose of 100 mg / kg, the animals of the control group received water purified in an equivalent volume. The anti-inflammatory activity of the phytoextract was assessed in the setting of modeling the aseptic inflammation according to the Guidelines [9].

The antiexudative effect of the tested remedy was studied on two models: formalin and carrageenan edema [9]. In the first series of experiments, the tested phytoextract and referential preparation were administered 3 hours prior to subplantation into the hindlimb of the animal with a 3% formalin aqueous solution, and also at 5 and 18 hours after initiating the inflammation. In the second series of experiments, phytoextract and caleflon were administered to the animals of the test groups 1 hour before the administration of the phlogogenic agent (0.1 ml of a 1% aqueous solution of carrageenan). The severity of formalin and carrageenan edema was assessed 24 and 3 hours after initiating the inflammatory process by the oncomometric method, according to the difference between the volume of the healthy and edematous legs. The antiexudative activity of the remedy tested was judged by the degree of suppression of the edema:

% edema suppression sdegree = $\Delta V_к$ - $\Delta V_о$ / $\Delta V_к$;

where: $\Delta V_к$ is the difference in the volume of the legs with edema and without edema in the animals of the control group; $\Delta V_о$ is the difference in mass of the legs with edema and without edema in the animals of the experimental group.

The alterative phase of the inflammatory reaction in white rats was reproduced by subcutaneous injection of 0.5 ml of a 9% solution of acetic acid into the back region [9]. The remedy tested and the reference preparation were administered 1 hour prior to the administration of the acetic acid solution and then daily once a day for 21 days. The antiallergic effect was assessed by the planimetric method according to the development degree of a necrosis and tissue regeneration on the 7th, 14th, 21st and 28th days of the experiment.

To assess the proliferative activity of rats under ether anesthesia, a sterile cotton pad weighing 25 mg was implanted in the back region under aseptic conditions, after which the wound was closed layer-by-layer [9]. Phytoextract and caleflon were administered once a day for 7 days. The proliferative reaction was assessed by the difference between the weight of the dried granuloma and the initial mass of the cotton pad. The effect of the remedy tested on the proliferative component of chronic inflammation was expressed as a percentage of control.

The experimental material was processed using the methods of variation statistics with software like Microsoft Excel 2003 and Statistica 10. The study results are presented as the mean value (M) and the mean error (m). The reliability of the differences found between the mean values (M) of the groups was evaluated using the Student's t-test. Differences were considered statistically significant at $p < 0.05$.

Results and discussion

The results of the study showed that phytoextract and caleflon at a dose of 100 mg / kg statistically significantly inhibited the leg edema of white rats by 24% compared to the control (Table 1). The most pronounced antiexudative effect of the test extract was in doses of 200 and 300 mg / kg: edema volume was 32% lower than in control group. In the model of carrageenan edema, the tested phytoextract reduced the degree of edema by 24-31% compared to the control (Table 1). The most pronounced antiexudative effect of the phytoextract was in a dose of 200 mg / kg.

As the data presented in Table 2 show, the extract of a complex herbal remedy reduces the alteration of tissues and enhances regenerative processes in the inflammatory focus (Table 2). For example, on the 7th day of observation, administration of phytoextract to the animals at a dose of 100 mg / kg reduced the wound injury area by 27% compared to that of the control group animals, whereas the use of phytoextract in doses of 200 and 300 mg / kg reduced this index by only 21% . At this starting from 14 days the most pronounced anti-alterative effect, exceeding one of the reference preparation (caleflon), was shown by the phytoextract in doses of 200 and 300 mg / kg. Thus, in the animals of the first and third experimental groups, on the 14th day of observation, the area of the wound defect was lower by 13%, 26% and 25%, on the 21th day by 35%, 29% and 50%, and on 28 day by 30%, 36% and 51% respectively. Against the administration of caleflon to animals, the necrosis area was lower than that of control animals by 25%, 20%, 40% and 35% according to the observation time, which is comparable with the average indices of the experimental groups, but lower than

Table 1

Effect of the dry complex herbal remedy extract on the exudation degree with formalin and carrageenan edema in white rats

Group of animals	Formaline edema		Carrageenan edema	
	Leg volume difference, ml	Degree of edema suppression, %	Leg volume difference, ml	Degree of edema suppression, %
Control (H2O), n=8	0,41±0,032	-	0,48±0,026	
Experimental I (phytoextract, 100 мг/кг), n=8	0,31±0,009*	24	0,35±0,024*	27
Experimental II (phytoextract, 200 mg/kg), n=8	0,28±0,034*	32	0,33±0,028*	31
Experimental III (phytoextract, 300 mg/kg), n=8	0,28±0,017*	32	0,34±0,034*	29
Experimental IV (caleflon, 100 mg/kg), n=8	0,31±0,018*	24	0,37±0,031*	23

Note. In the Tbl. 1-3 * the differences are statistically significant between the control and experimental groups at $P \leq 0.05$; n is the number of animals in the group.

Table 2

Effect of the dry complex herbal remedy extract on the alteration degree in white rats

Group of animals	Alteration ares, , mm^2			
	7th day	14 th day	21 th day	28 th day
Control (H2O), n=8	409,3±33,62	306,3±12,95	124,7±14,57	74,7±10,8
Experimental I (phytoextract, 100 mg/kg), n=8	297,3±23,85*	266,8±29,61	81,2±9,43*	52,7±7,47
Experimental II (phytoextract, 200 mg/kg), n=8	325,0±30,17	225,8±26,99*	88,3±11,89	47,5±4,95*
Experimental III (phytoextract, 300 mg/kg), n=8	324,3±21,13*	230,5±33,21*	61,8±5,51*	37,0±7,10*
Experimental IV (caleflon, 100 mg/kg), n=8	314,4±28,5*	240,2±23,2*	78,6±6,21*	50,3±3,20*

Table 3

Effect of the dry complex herbal remedy extract on the proliferation processes in white rats

Group of animals	Dry granuloma weight, мг	Granuloma formation degree, %
Control (H2O), n=8	62,8±4,53	
Experimental I (phytoextract, 100 mg/kg), n=8	68,0±5,21	8
Experimental II (phytoextract, 200 mg/kg), n=8	69,1±5,51	10
Experimental III (phytoextract, 300 mg/kg), n=8	66,3±5,33	6
Experimental IV (caleflon, 100 mg/kg), n=8	67,1±5,86	7

in the group of animals treated with the phytoextract in a dose of 300 mg / kg.

It was found that a moderate effect on the proliferative component of chronic inflammation is exerted by a phytoextract in a dose of 200 mg / kg (Table 3): the weight of the connective tissue capsule in the inflammatory focus increased by 10% compared to the control. The use of phytoextract in doses of 100 and 300 mg / kg exhibited a proliferative effect similar to the reference preparation (caleflon).

The ascertained pharmacological effect of the tested phytoextract results from the anti-inflammatory activity of its components. Thus, according to the literature, anti-inflammatory properties were detected in *C. avellana* L. [2, 15], *A. eupatória* L. [8, 17], *X. strumarium* L. [1], *U. dioica L*. [7], *C.intybus [10], O. acanthium [6], L. minor [13].* Some species of the genus *Juglans* L. are widely used as anti-inflammatory drugs in traditional medicine of many countries [19].

The anti-inflammatory activity of medicinal plants is implemented due to a wide range of biologically active substances: flavonoids (kaempferol, quercetin, luteolin, apegenin, etc.), hydroxycinnamic acids, phenol carboxylic acids, triterpenoids, vitamins (ascorbic acid, carotenoids, etc.) and others [12, 16, 18, 20, 21].

According to some authors [7], the anti-inflammatory effect of plants is also exerted due to the antioxidant effect of flavonoids, carotenoids, ascorbic acid and α-tocopherol. Plant antioxidants directly neutralize the free radicals of neutrophils and macrophages and endoperoxides afforded in the cyclooxygenase reaction, and also potentiate anti-peroxide protection. Inhibition of peroxidation is accompanied by a decrease in the production of anti-inflammatory and algogenic factors - prostaglandins, kinins, IL-1, IL-6, IL-8, INF-γ, TNF-α, complement and cell adhesion molecules, weakening the synthesis of collagen and glycosaminoglycans in fibroblasts.

Conclusion

thus, the results obtained indicate that the extract of dry complex herbal remedy possesses antiexudative activity, reducing the exudation induced by phlogogenic agents. The test extract has an antialterative effect, limiting the alteration of tissues with acetic acid and enhancing regenerative processes. Moderate proliferative activity was manifested by the phytoextract in a dose of 200 mg / kg. The pronounced anti-inflammatory activity of the complex agent is caused by a wide range of biologically active substances like flavonoids, terpenoids, carotenoids, essential oils, vitamins, organic acids and other compounds included in its formulation.

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INFLUENCE OF LOW TEMPERATURES ON LIPID PEROXIDATION IN TISSUE OF EXPERIMENTAL ANIMALS DEPENDING ON EXPOSURE TIME

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ABSTRACT

The article reports the study on the influence of low temperatures on the intensity of free radical lipid oxidation and antioxidant protection in the tissues of internal organs (liver, kidneys, lungs, heart) in experimental animals, depending on the exposure time.

The few data available in the literature indicate that the effect of low temperatures on the organism of experimental animals is accompanied by the activation of free radical processes. We noted an increase in the concentration of low-molecular antioxidants in the organs of animals, whose exposure time in the cold lasted 1 hour. An increase in exposure time of rats in the cold to 3 hours is associated with an increase in catalase activity.

The purpose of this study was to study the effect of experimental animals on the processes of lipid peroxidation in tissues of internal organs (liver, kidneys, lungs, heart) during one-hour and three-hour exposure to low temperatures for 14 days.

The effect of cold on the processes of lipid peroxidation in the tissues of rats was investigated at a temperature (10 ± 20C below zero). The processes of lipid peroxidation and antioxidant protection parameters were quantitatively studied by spectrophotometric method, using SPECORD 40 spectrophotometer, determining the content of dienic conjugates and malonic dialdehyde in the tissues of the internal organs (liver, kidneys, lungs and heart), the total content of low molecular weight antioxidants and catalase activity.

Conclusion. The ecological and biochemical reaction of the rat organism to the effect of cold is the activation of antioxidant protection, due to the increase in the rate of lipid peroxidation. In the first group of animals, whose exposure time in the cold lasted one hour, biochemical mechanisms of antioxidant protection are realized by increasing the concentration of low-molecular antioxidants in organs. An increase in the exposure time to three hours of rats in the cold is associated with an increase in the activity of the antioxidant enzyme catalase.

Keywords: low-temperature effect, lipid peroxidation, free radical lipid oxidation, lipoperoxidation, active oxygen species, malonic dialdehyde, diene conjugates, experimental animals, spectrophotometric methods, electrothermometer with needle sensor, adaptation.

Introduction

One of the fundamental problems of biology at the present time is the study of the state of the organism under the influence of various negative factors of the external environment, as well as ways and means of increasing the resistance of the living organism to them. Such a factor in the extreme climatic and natural