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STRESS-PROTECTIVE AND ANTIOXIDANT EFFECT OF THE *Cimicifuga dahurica* (Turcz.) Maxim.

ABSTRACT

The stress-protective and antioxidant effects of *Cimicifuga dahurica* (TURCZ) Maxim tincture were estimated in the experiments on white *Wistar* rats. The stress-induced condition was simulated by immobilization of the animals for 18 hours. There was estimated the evidence of the Selye's triad (hypertrophy of adrenal glands, involution of thymus and spleen, and gastric mucous membrane ulceration, with determination of the Pauls index), the content of malonic dialdehyde (MDA) in the blood serum, activity of catalase in the blood serum and superoxide dismutase (SOD) in erythrocytes and the content of the reduced glutathione in the blood. It has been revealed that the *C. dahurica* tincture has a marked stress-protective effect, preventing hypertrophy of the adrenal glands, involution of thymus and spleen development of ulcerative lesions of the gastric mucosa. The *C.dahurica* tincture inhibits lipid peroxidation and activates the antioxidant system of the body in stress-induced conditions.

Keywords: *Cimicifuga dahurica* (TURCZ) Maxim tincture, immobilization stress, stress-protective and antioxidant effects.

INTRODUCTION

It is known that stress has a negative impact on the body, on mental activity and human behavior, up to their complete disorganization, and also leads to the emergence or aggravation of the course of neurotic, psychosomatic and organic diseases. In this regard, the prevention of stress and its consequences is an extremely urgent task. An important role in the treatment of stress-induced conditions is played by phytotherapy. To the means of vegetable origin, which have sedative effect and can be used to treat and prevent the consequences of stress, include *Valeriana*, *Leonurus*, *Crataegus*, *Passiflora*, *Melissa*, *Organum* and other plants.

In the treatment and prevention of stressful situations a promising plant is *Cimicifuga dahurica* (Turcz.) Maxim. The plant is widely used in the form of powder, tincture and liquid extract in folk medicine for headaches, the initial stages of hypertension, with increased nervous excitability, hysteria, insomnia, etc. [11]. In Tibetan medicine *C. dahurica* is included into the medicinal collections used for the treatment of gza diseases (diseases "inflicted by demons"). These diseases may be identified as insults, paralyses and other functional disturbances of the nervous system [10]. The *C. dahurica* tincture has a sedative effect limiting motility, exploratory activity and reflex excitability of animals and prolonging the narcotic sleep [6]. The anxiolytic, antidepressant and anti-aggressive effects of *C.dahurica* tincture it has been established in the experiments on white rats [2, 3].

The aim of the study was to estimate the stress-protective and antioxidant effects of the *Cimicifuga dahurica* tincture.

MATERIALS AND METHODS OF THE STUDY

The experiments were carried out on 32 white male and female *Wistar* rats weighing 160-180 g. The animals were kept in the standard vivarium conditions observing a similar care, nutrition, and light and temperature regimen according to the GLP rules (Order N. 708H dated 23.08.2010) and the "European Convention for the protection of vertebrate animals used for experimental and other scientific purposes" (Strasbourg, 1986). The test report was approved by the ethics committee of the Institute of General and Experimental Biology SB RAS (Report N. 3 dated 03.09.2012).

The animals were divided into 4 groups: intact, control and 2 experimental ones. The animals of the first experimental group received intragastrically the dealkurized solution of the *C.dahurica* tincture at a dose of 0.5 ml/kg in a volume of 1.0 ml/100 g (1 time per day 30 minutes prior to feeding) respectively for 7 days before simulating a stress-induced condition. The animals of the second experimental group were given a comparative preparation - valerian tincture (JSC Dalkhimpharm) in a dose of 1.0 ml/kg according to a similar scheme. The rats of the intact and control groups received the purified water in the same volume and introduction scheme. The immobilization stress was simulated by the standard method where the animals were fixed in the supine position for 18 hours [8]. The animals of the intact group were subjected no stress testing.

To estimate the anti-stress activity of the tested remedy the evidence of the Selye's triad was determined: hypertrophy of adrenal glands, involution of thymus and spleen, and gastric mucous membrane ulceration, with determination

of the Pauls [1]. The intensity of the lipid peroxidation processes was estimated by the increment of the peroxidation product – malonic dialdehyde (MDA) in the blood serum [9]. The state of the endogen antioxidant system was evaluated by the activity of catalase in the blood serum [4], superoxide dismutase (SOD) in erythrocytes [5], and the content of the reduced glutathione (GSH) in the blood [12].

The statistical processing of the obtained data was made with the use of "Biostat-2006" program pack and Student's t-criterion. The differences between groups compared were significant when $P \leq 0.05$.

RESULTS AND DISCUSSION

The findings have shown that the 18-hour immobilization of the animals causes the complex of pathological alterations characteristic of stress-reactions: hypertrophy of adrenal glands, involution of thymus and spleen, and presence of destruction in the gastric mucosa. Thus, in animals of the control group the relative mass of adrenal glands was 1.7 times higher than this index in animals of the intact group, the mass of the thymus and spleen was lower by 41% and 61% respectively (Table 1).

The *S.dahurica* tincture in dose 0.5 ml/kg has a marked stress-protective effect. Thus, in animals which received the *S.dahurica* tincture the relative mass of adrenal glands was 31% decreased, The relative mass of the spleen and thymus was 200% and 45% higher than this index in the control group. Along with this, the introduction of the *S.dahurica* tincture were decreased the development of deep destruction in the gastric mucosa of white rats. Thus, in animals of the experimental groups, point bleeding in the gastric mucosa were noted in only 5 animals

out of 8, whereas in the control group in 100% of the animals. The amount of data destruction was on average 3.0 times less than in the control, as a result of which the Pauls index for point bleeding in the first and second test groups was 5.7 and 4.8 times lower than in the control. In 50% of the animals receiving *C.dahurica* tincture, there were erosions, at 75% in the control group. In this experimental group the average number of destruction per animal was 3.0 times lower, and the Pauls index was 4.8 times higher than in the control group. Only a quarter of the animals in the experimental groups had banded ulcers; the Pauls index was 7.1 and 5.7 times lower, respectively, in the control group. The stress-protective effect of the *S.dahurica* tincture can be explained by the oxycinnamic acids entering into its composition [13]. Thus, according to the literature [7], ferulic acid has a pronounced stress-protective action, limiting the damage to the gastric mucosa and myocardial damage caused by immobilization-painful stress.

The results of the studies given in the Table 2 demonstrate that the immobilization stress is followed by activation of free radical oxidation as evidenced on the 53% increase in the concentration of this process product - MDA as well as the decrease of the activity in the enzymes of the bodily antioxidant protection i.e. 1.6, 6.6 and 1.2 times decrease of catalase, SOD and GSH respectively as compared with the indices in the intact group of animals. It has been established that the

course administration of the *S. dahurica* tincture and valerian tincture to animals decreases the MDA content on average by 24% and 30%, increases the catalase activity by 1.4 and 1.6 times, the content of GSH – by 30% and 21%, the activity of SOD – by 4.0 and 3.4 times respectively as compared with the indices in the control group of animals.

CONCLUSION

Thus, the data obtained have shown that the *C.dahurica* tincture on the background of the immobilization stress have stress-protective effect; it decreases the pronouncement of stress-induced alterations in animal inner organs. It has been established that the stress-protective effect of the tested remedy is due to its inhibiting influence on the processes of free radical oxidation and its capacity to activate the bodily system of antioxidant protection; it is due to the content of phenolic compounds and saponins exhibiting pronounced antioxidant activity in its composition [14].

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Table 1

The influence of the *Cimicifuga dahurica* tincture on the manifestation of degenerative alterations in inner organs of white rats in stress-induced injury

Indices		Animal groups			
		Intact (H2O), n=8	Control (stress + H2O), n=8	Experimental I (stress + <i>C.dahurica</i>), n=8	Experimental II (stress + valerian), n=8
Mass of organ, mg/100g	thymus	123,5±4,50	72,7±4,29	105,6±3,59*	108,2±1,97*
	spleen	521,0±16,45	204,6±18,63	432,5 ±20,06*	308,3±3,82
	adrenals	21,0±1,54	36,0±3,02	24,7±1,50*	28,3±0,77*
Point bleeding	Percentage of damaged animals	0	100	63	63
	Average number of destructions per animal	0	3,9±0,43	1,1±0,43*	1,3±0,44*
	Pauls index	0	3,90	0,69	0,82
Erosion	Percentage of damaged animals	0	75	50	63
	Average number of destructions per animal	0	1,9±0,55	0,6±0,29	1,0±0,47
	Pauls index	0	1,43	0,30	0,63
banded ulcers	Percentage of damaged animals	0	67	25	25
	Average number of destructions per animal	0	0,9±0,47	0,3±0,18	0,4±0,27
	Pauls index	0	0,57	0,08	0,10

Note: * – differences are significant in comparison with the data in animals of the control and experimental groups when $P \leq 0.05$; n – number of animals in the group.

Table 2

The influence of the *Cimicifuga dahurica* tincture on the lipid peroxidation processes in white rats and the state of their antioxidant system in stress-induced injury

Indices	Animal groups			
	Intact (H ₂ O), n=8	Control (stress +H ₂ O), n=8	Experimental I (stress+ <i>C.dahurica</i>), n=8	Experimental II (stress + valerian), n=8
MDA in the blood serum $\mu\text{mol/l}$	10,7 \pm 1,64	16,4 \pm 1,04	12,5 \pm 0,56*	11,5 \pm 0,43*
SOD in erythrocytes, act. units	13,1 \pm 1,28	2,0 \pm 0,30	8,0 \pm 0,51*	6,7 \pm 0,45*
Catalase in the blood serum, mkat/l	1,4 \pm 0,07	0,9 \pm 0,08	1,3 \pm 0,09*	1,4 \pm 0,11*
GSH in the blood, $\mu\text{mol/l}$	707,0 \pm 30,60	591,0 \pm 24,30	767,2 \pm 58,08*	715,0 \pm 78,77*

Note: * – differences are significant in comparison with the data in animals of the control and experimental groups when $P \leq 0.05$; n – number of animals in the group.

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