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STRESS-PROTECTIVE ACTIVITY OF THE DRY EXTRACTS FROM RHIZOMES AND ABOVE-GROUND PART OF RHAPONTICUM UNIFLORUM L.

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*The article presents the results of a study of the stress-protective activity of extracts obtained from the rhizomes and above-ground part of *Rhaponticum uniflorum* L., in which phytoecdysteroids and flavonoids are the main active ingredients. The developed extracts are practically non-toxic substances. Their course administration in an experimental therapeutic dose of 100 mg/kg has the stress-protective effect in acute emotional stress, decreasing the manifestation of the Selye's triad signs due to inhibition of hyperactivity of central stress-realizing systems of the body. Peripheral mechanisms of adaptogenic action of *R. uniflorum* extracts are associated with inhibition of free radical oxidation processes.*

Keywords: medicinal herbs, *Rhaponticum uniflorum*, phytoecdysteroids, dry extracts, stress-protective activity

Currently, there is an interest in ecdysteroid-containing herbs as sources of new adaptogenic medicines that increase the body's non-specific resistance. Phytoecdysteroids have a wide range of pharmacological properties: they regulate metabolism, have anti-inflammatory, antioxidant, antitumor, immunomodulatory, nootropic, stress-protective, and anabolic effects [1,2]. At the same time, protein synthesis under their influence is not associated with the hormonal effect of animal-derived and synthetic anabolics, which provides that there are no life-threatening side effects when taking them [3]. In this regard, they are used to correct body weight during the training process and achieve high performance in professional sports. In Russia, the only ecdysteroid-containing herb included in the State Pharmacopoeia of the Russian Federation and the State register of medicinal products is a maral root (*Rhaponticum carthamoides* (Willd.) Iljin.).

In recent years, active study of another species of *Rhaponticum* – *Rhaponticum uniflorum* (L.) DC has been provided. Unlike *R. carthamoides*, this species is more widely distributed, growing in the steppe and mountain regions of Eastern Siberia and the Russian Far East. The content of ecdysteroids in the underground organs of the herb varies from 0.023 to 0.85% according to various sources [4]. Preparations of *R. uniflorum* are widely used in traditional medicine of the East: Tibetan, Mongolian and Chinese [5].

The purpose of this work is to determine the stress-protective activity of dry extracts made of the aboveground and underground parts of *R. uniflorum* in case of emotional stress.

MATERIALS AND METHODS

Dry extracts are obtained from underground (rhizomes with roots) and aboveground (herb) parts of *R. uniflorum*. Plant raw materials were harvested during the period of mass flowering in 2015–2016 in the Republic of Buryatia and the Trans-Baikal territory. The method for obtaining the extracts consists of three-time extraction with 60% ethanol and purified water, followed by filtration, evaporation and drying in a vacuum-drying apparatus. A method for obtaining the dry extract from rhizomes with the roots of *R. uniflorum* is patented [6]. Biologically active substances of the obtained extracts are represented by ecdysteroids, flavonoids, phenolcarboxylic acids, triterpene saponins, amino acids, etc. The content of the sum of ecdysteroids in terms of ecdysterone in dry extract is 3.9% [7].

Experimental work was performed on white male and female Wistar rats weighing 180–200 g. The model of psychoemotional stress was reproduced by immobilization of animals in metal cases submerged in water (25°C) for 4 hours [8]. To the rats of the experimental groups the intragastric extracts in doses of 100 mg/kg in a volume of 10 ml/kg of aqueous

solution were administered prophylactically for 7 days prior to stress exposure. A de-alcoholized extract of *R. carthamoides* at a dose of 5.0 ml/kg was used as a comparator. On the 7th day of the experiment, the animals were subjected to psychoemotional stress and the severity of stress injuries was assessed. To do this, the indicators of the Selye's triad were determined: adrenal hypertrophy, thymus and spleen involution, the number of injuries in the gastric mucosa with the calculation of the Pauls index. In serum, the intensity of free radical oxidation processes and the activity of endogenous AOS were determined by the content of malondialdehyde [9], catalase activity [10] and superoxide dismutase [11], as well as by the concentration of reduced glutathione [12]. The plasma and serum levels of epinephrine, norepinephrine, adrenocorticotropic hormone (ACTH), corticosterone and aldosterone were determined using standard Tri Cat ELISA immunoassay kits and a DSX analyzer (USA). Statistical processing of the obtained data was performed using the Student's t-test.

RESULTS AND DISCUSSION

Determination of acute toxicity showed that extracts of rhizomes and herbs of *R. uniflorum* are practically non-toxic substances in accordance with the current classification [13].

It was found that course prophylactic administration of rhizome and herb extracts of *R. uniflorum* to animals in doses of 100 mg/kg against the background of 4-hour emotional stress had a pronounced antistress effect, as evidenced by a significant decrease in the severity of signs of the Selye's triad (Table 1).

According to Table 1, the course administration of *R. uniflorum* extracts was accompanied by decrease in the severity of signs of stress reaction: adrenal hypertrophy in rats receiving *R. uniflorum* root and herb extracts was by 27 and 20% less,

respectively, than in the control group; the weight of the thymus by 35 and 42% and the spleen – by 14 and 18%, respectively, more than in the control group of rats. Along with this, the products to be examined had a gastroprotective effect, reducing the severity of ulcerative lesions of the animal mucosa, as evidenced by decrease in the Pauls index for spot hemorrhages and erosions. In rats receiving extract of *R. uniflorum* roots, the stripe-like ulcers were not observed; in animals receiving extracts of *R. uniflorum* and *R. carthamoides* the stripe-like ulcers were observed in one rat in the group, while in the control group, these ulcers were observed in 80% of animals. In general, according to the Selye's triad, the effectiveness of *R. uniflorum* extracts was comparable to that of the comparator, i.e. *R. carthamoides* extract, and exceeded that in a number of parameters.

It was found that the stress-protective activity of *R. uniflorum* extracts is due to the restriction of hyperactivation of the central stress-relieving systems such as sympathetic-adrenal and hypothalamic-pituitary-adrenal systems (Table 2).

According to the data presented in Table 2, administration of extracts of *R. uniflorum* is accompanied by decreased activity of a trigger of the stress response – sympathetic-adrenal system, as evidenced by reduction in concentration of catecholamines in blood of animals in the experimental groups: with administration of extracts of *R. uniflorum* roots and herbs, the concentration of adrenaline is reduced respectively by 23 and 30%; the content of norepinephrine is reduced by 20 and 25% compared with those of rats in the control group. Along with this, due to administration of the phyto-medicines to

Table 1

EFFECT OF R. UNIFLORUM EXTRACTS ON THE DEGREE OF ADRENAL HYPERTROPHY, INVOLUTION OF IMMUNE-COMPETENT ORGANS, AND THE PAULS INDEX IN WHITE RATS UNDER EMOTIONAL STRESS

Parameters	Groups of animals				
	Intact, n=8	Control (stress + H ₂ O), n=10	Experimental 1 (stress + <i>R. uniflorum</i> roots), n=10	Experimental 2 (stress + <i>R. uniflorum</i> herb), n=10	Experimental 3 (stress + <i>R. carthamoides</i>), n=10
Weight (mg/100 g)					
atr biliary capsules	16,0±1,08	25,0±2,51	18,3±1,34*	20,2±1,95*	16,3±1,62*
thymus	57,3±2,23	33,5±3,16	45,4±3,47*	47,6±4,03*	49,5±2,53*
spleens	458,0 ±14,5	359,2±20,5	408,5±23,4	425,3±16,8*	430±26,2*
PI for hemorrhagic diseases		6	2,8	3,2	4,3
PI for erosion		3	0,65	0,55	0,75
PI for chancres		1,25	0	0,01	0,01

Note: * – Hereinafter these are values that differ significantly from the data for animals in the control groups at $p \leq 0.05$.

be examined the decrease in the activity of the hypothalamic-pituitary-adrenal system is observed, as indicated by the decreased concentration of adrenocorticotrophic hormone by 30 and 40%, respectively, corticosterone by 22 and 24%; aldosterone – by 12 and 24% in comparison with the similar data for rats of the control group. It was shown that the stress-protective activity of the extract of *R. uniflorum* herb was slightly higher than that of the extract of *R. uniflorum* roots, as well as the activity of the comparator, i.e. the extract of *Rhaponticum carthamoides*.

The data presented in Table 3 show that the course administration of *R. uniflorum* extracts is accompanied by decrease in induction of free radical oxidation (FRO) processes, which are the universal leading molecular-cellular mechanism of cell membrane damage in stress injuries. In particular, this is indicated by decrease in the concentration of MDA in the blood of rats in experimental groups 1 and 2 on average by 30%

compared to the data of the control group. It was found that restriction of FRO processes is due to increase in the activity of the endogenous antioxidant system (AOS) of the body, as evidenced by increase in the concentration of reduced glutathione by 2.6 times – with administration of *R. uniflorum* root extract and by 3 times – with administration of *R. uniflorum* herb extract. Also, against the background of administration of test extracts, the activity of enzymes of antioxidant protection such as catalase and SOD, increases with administration of the extract of *R. uniflorum* roots – by 18 and 82%, respectively, and with administration of the extract of *R. uniflorum* herb – by 24 and 57% compared to similar data of control group rats. At the same time, the antioxidant activity of the tested products was similar to that of the comparator such as of *Rhaponticum carthamoides*.

It can be assumed that the stress-protective activity of these extracts is due to the high content

Table 2

EFFECT OF R. UNIFLORUM EXTRACTS ON THE CONTENT OF PITUITARY AND ADRENAL HORMONES IN THE BLOOD OF WHITE RATS UNDER EMOTIONAL STRESS

Parameters	Groups of animals				
	Intact, n=8	Control (stress + H ₂ O), n=10	Experimental 1 (stress + <i>R. uniflorum</i> roots), n=10	Experimental 2 (stress + <i>R. uniflorum</i> herb), n=10	Experimental 3 (stress + <i>R. carthamoides</i>), n=10
Adrenaline, nM/l	8,5±0,59	37,8±0,35	29,3±0,85*	26,5±1,24*	31,6±0,51*
Noradrenaline, nM/L	64,1±0,27	120,6±4,71	96,3±4,21*	87,7±5,35*	111,3±3,77
Adrenocorticotrophic hormone, pg/mL	15,8±1,69	51,0±4,27	35,7±2,06*	31,2±0,86*	42,6±2,10*
Corticosterone, nM/L	44,3±3,74	65,7±3,80	51,6±1,83*	50,5±2,45*	54,7±4,38
Aldosterone, pg/mL	271,8±10,45	296,1±11,74	263,0±15,8	226,0±12,4*	257,3±16,62

Table 3

EFFECT OF *R. UNIFLORUM* EXTRACTS ON FREE RADICAL OXIDATION PROCESSES AND THE STATE OF THE ANTIOXIDANT SYSTEM OF WHITE RATS UNDER EMOTIONAL STRESS

Groups	Parameters			
	MDA, nM/mL	VH, mmol/L	Catalase, mcat/L	Total radiation dose (TRD), activity unit
Intact, n=8	12,2±1,03	3,1±0,16	8,3±0,61	15,6±1,08
Control (stress + H ₂ O), n=8	24,7±1,41	0,8±0,12	5,9±0,48	6,2±0,57
Experimental 1 (stress + <i>R. uniflorum</i> roots), n=8	15,3±1,04*	2,1±0,04*	7,0±0,22*	11,3±0,94*
Experimental 2 (stress + <i>R. uniflorum</i> трава), n=8	14,8±1,05*	2,3±0,17*	7,3±0,46*	9,74±0,14*
Experimental 3 (stress + <i>R. carthamoides</i>), n=8	14,3±0,92*	1,5±0,09*	6,7±0,72*	10,9±0,86*

of ecdysteroids, as well as such compounds as flavonoids, amino acids, etc., enhancing their biological effects, which ultimately provides inactivation of free radicals, leading to a violation of the functional and structural consistency of biological membranes under emotional stress. Thus, the data obtained indicate that *R. uniflorum* is a promising ecdysteroid-containing plant raw material for obtaining new adaptogenic agents. Taking into consideration that the content of ecdysteroids in the herb of the plant is 1.3 times higher than in underground organs, and the pharmacological activity of the extract of the herb is similar to that of the extract of rhizomes, the use of the aboveground part of *R. uniflorum* is important for the rational use of medicinal plant raw materials.

CONCLUSION:

1. Extracts of *R. uniflorum* roots and herbs with course administration in doses of 100 mg / kg have a stress-protective effect in emotional

stress, preventing the development of signs of the Selye's triad.

2. Stress-protective activity of *R. uniflorum* extracts is caused by restriction of hyperactivation of central stress-realizing systems such as sympathetic-adrenal and hypothalamic-pituitary-adrenal ones.

3. Peripheral effects of adaptogenic action of *R. uniflorum* extracts are associated with inhibition of free radical oxidation processes and increased activity of the endogenous antioxidant system of the body.

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