Study of the effect of in vivo extract of Serpukha (Serratula coronata L.) on biomarkers general adaptation syndrome

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In vivo study of Serratula coronata L. extract on biomarkers of general adaptation syndrome

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SUMMARY

The effect of 30-day consumption of the phytoecdysteroid-containing extract of Serratula coronata L on some indicators of hormonal status and apoptosis activity in various organs of growing male Wistar rats after stress exposure was studied. There were no significant differences in the mean values of the determined biochemical parameters for both groups of animals. The average values of the relative mass of the thymus of the animals of the control and experimental groups at the end of the experiment differed statistically significantly and amounted to 0.163 ± 0.012% and 0.193 ± 0.010%, respectively. The inhibition of the decrease in the thymus mass in stressed rats of the experimental group, which received the extract, was combined with a significant decrease in apoptosis activity in this organ, as compared with animals of the control group, characterized by the level of DNA damage (7.61 ± 0.14% and 8.14 ± 0, 16%, respectively) and the number of apoptotic cells (1.08 ± 0.10% and 1.34 ± 0.07%, respectively). Determination of the activity of apoptosis of thymus cells by the DNA comet method made it possible to reveal the inhibitory effect of the ecdysteroid-containing extract on the intensity of the general adaptation syndrome.

Key words: adaptogen, phytoecdysteroids, corticosterone, beta-endorphin, prostaglandin E2, norepinephrine, apoptosis index, degree of DNA fragmentation.

RESUME

The impact of consumption (30 days) of phytoecdysteroids containing Serratula coronata L extract on some parameters of hormonal activity and apoptosis in various organs of growing male Wistar rats after stress was studied. No significant differences were determined by the mean values of biochemical parameters for both groups of animals. Average values of the relative thymus weight of control and experimental animals differed significantly at the end of the experiment and figure to 0.163 ± 0.012% and 0.193 ± 0.010%, respectively. Significant decrease in apoptosis activity in thymus cells is determined in compared to control animals characterized in the level of DNA-damages (7.61 ± 0.14% and 8.14 ± 0.16% respectively) and the amount of apoptotic cells (1.08 ± 0.10% and 1.34 ± 0.07% respectively).

Keywords: adaptogen, phytoecdysteroids, corticosterone, beta-endorphin, prostaglandin E2, norepinephrine, apoptosis index, DNA fragmentation.

Phytoecdysteroids are biologically active compounds structurally identical or close to arthropod molting hormones, belong to the class of plant metabolism regulators, which are of significant interest for multifaceted use [1]. Phytoecdysteroids, acting on herbivorous invertebrates and thus participating in the regulation of the number of phytophages, do not have a hormonal effect on mammals and have low toxicity [2].

The pharmacological effects of ecdysteroids have been studied for more than 40 years and indicate their multifaceted effect on mammals and the prospects for use in preventive, therapeutic and sports medicine [3–7]. In vivo studies and with the use of cell cultures indicate the corrective effect of ecdysteroids on the processes of carbohydrate and fat metabolism, as well as their anabolic effect [8–12]. Research is actively underway to identify

molecular targets of ecdysteroids in mammals [10].

In 1996 and 2010 in Russia in the city of Syktyvkar on the basis of the Institute of Biology of the Komi Scientific Center of the Ural Branch of the Russian Academy of Sciences, the First and Second International Meetings on Phytoecdysteroids were held, in which leading experts in this field from Russia, the CIS and Western Europe took part [13]. In recent years, great progress has been made in the study of phytoecdysteroids: plant species have been identified in which new ecdysteroids have been discovered, the patterns of distribution of phytoecdysteroids in the plant kingdom have been revealed, highly productive cell lines of ecdysteroid-containing plants have been obtained, new methods of chemical modification of ecdysteroids have been developed and the scientific basis for the production of phytoecdysteroids from plant raw materials [2,14,15,16]. Natural sources of phytoecdysteroids are various types of medicinal plants. A promising plant in terms of obtaining extracts with a high content of one of the most widely studied phytoecdysteroids - 20-hydroxyecdysone (20E) - is the crowned sickle (Seratulla coronata L.), in the aerial part of which the content of 20E can reach 2% (in terms of dry weight) [eight]. Another major ecdysteroid in this plant is 25S-inocosterone (11% of the total ecdysteroid). "Crowned serpukha leaves" are registered as raw materials for the production of biologically active additives (certificate number 77.99.23.3.У.1922.3.08 dated 11.03.2008, TU 9371-001-15092611-2008). The in vivo study of the effect of the extract of this plant on biomarkers of the central stress system and central stress-limiting systems is of interest to substantiate the possible expansion of the scope of its application as part of specialized anti-stress food products. Such biomarkers can include stress mediators characterizing the course of the General Adaptation Syndrome, as well as indicators of cell apoptosis activity (the degree of DNA fragmentation and apoptosis index) of various organs, primarily the thymus [17, 18].

The aim of this study is to study the effect of phytoecdysteroid-containing serum extract for the content of corticosterone, beta-endorphin, prostaglandin E2 and norepinephrine in blood plasma and the activity of apoptosis in some organs of male Wistar rats stressed by electrocutaneous stimulation.

The studies were carried out in accordance with the program of the Presidium of the Russian Academy of Sciences "Fundamental Sciences - Medicine" (project No. 12-P-4-1023: "Scientific foundations for the creation of adaptogenic and geroprotective agents of plant origin").

MATERIALS AND METHODS

Growing male Wistar rats obtained from the Stolbovaya nursery, after a seven-day quarantine, were placed in separate cages, one individual in each, and received a standard general food ration [19]. The animals were divided into two groups of 8 animals each. The average values of the initial body weight of the animals of the control group and the experimental group before the beginning of the experiment were 111.0 ± 2.1 and 109.8 ± 1.8 g, respectively. The animals of the experimental group were daily added to the water a dry extract from the leaves of Serpukha Vencenosnaya. The phytoecdysteroid concentrations in the extract were determined by HPLC on an Agilent 1100 Series instrument (Agilent Technology, United States) [20]. The sample was applied to an Atlantis C18 column 4.6 * 250 mm, 5 μm, elution rate 0.9 ml / min, mobile phase: water (A) - acetonitrile (B), analytical wavelength 247 nm, sample volume 10 μl. The total content of phytoecdysteroids was 61.5 mg / g, while the concentrations of 20 hydroxyecdysone (20E) and 25S-inocosterone were 40.6 and 14.2 mg / g, respectively. The rats of the experimental group received daily an aqueous solution of this extract at the rate of 2 mg of phytoecdysteroids (in total) per kg of body weight. The animals of the control group received water throughout the experiment. The rats were examined daily, the volume of liquid drunk was recorded and weighed regularly every other day. The experiment lasted 30 days. The animals of the control group received water throughout the experiment. The rats were examined daily, the volume of liquid drunk was recorded and weighed regularly every other day. The experiment lasted 30 days. The animals of the control group received water throughout the experiment. The rats were examined daily, the volume of liquid drunk was recorded and weighed regularly every other day. The experiment lasted 30 days.

On the penultimate day of the experiment, the animals were subjected to stress using an installation (PanLab, Spain), which is a large illuminated white compartment and a small black compartment, separated by a motorized sliding gate. The lattice floor of the small black compartment is electrified (output 0–2mA). The rat was placed in the light compartment of the chamber with its back to the dark compartment. As soon as the rat entered the dark compartment of the chamber, it received an electrocutaneous stimulation on the paws (current 0.4 mA for 8 seconds). Then the animal was returned to the cage and after 16 hours was removed from the experiment by decapitation under light ether anesthesia and subjected to postmortem dissection to extract samples of internal organs: thymus, brain and heart; the thymus was weighed.

Blood was collected in tubes with pre-added Trilon B solution (1.25%, 400 μl) and

blood plasma was collected by centrifugation at T = 4 ° C for 25 minutes at 3000 rpm. on a J-6B centrifuge (Beckman, Austria).

In blood plasma, the content of corticosterone (Corticosterone EIA kit, Immunodiagnostic System, Great Britain), prostaglandin E2 (Rat Prostaglandin E2 (PGE2) ELISA Kit, CUSABIO, China) and beta-endorphin (Peptide Enzyme immunoassay kit) was determined using commercial kits. (EIA) kit, Peninsula lab.immunoassay, USA) according to the manufacturer's procedures.

The content of norepinephrine in blood plasma was determined by HPLC according to [21] with some modifications. Sample preparation of the tested samples was carried out as follows: blood plasma (1–2 ml) was filtered through a syringe filter (0.2 μm), then the pH of the sample was adjusted to 8.5 using a 1.0 M Tris-HCl buffer (pH 8.6). indicator, 30 μL of an internal standard solution - 3,4-dihydrobenzylamine hydrobromide (ALDRICH, USA) was added and quantitatively applied to a glass microcolumn (0.5 * 1.0 cm) with alumina. The sorbent was washed with distilled water (2x2 ml) and norepinephrine and 3,4-dihydrobenzylamine hydrobromide were eluted with 1.0 M acetic acid solution. The resulting wash was applied to a chromatographic column (Nucleodur C18, 5 μm, 250 * 5 mm), pre-calibrated against an internal standard and norepinephrine (ALDRICH, USA). Composition of the mobile phase: 0.1M phosphate-citrate buffer, pH 4.0, containing 50 mg / L of ion-pair reagent (1-octane sulfonic acid sodium salt for HPLC, Dudley Chemical, USA) and 2.5% acetonitrile (qualification "For HPLC"). The volume of the injected sample was 100 μL. Elution rate 1.0 ml / min. An amperometric detector (NPO Khimavtomatika, Russia, software Multikhrom 3, Russia) with a glassy carbon electrode and an operating voltage of +1.0 V was used as a detector.

In isolated cells of the thymus, heart, and brain, the level of DNA damage and the percentage of apoptotic cells (apoptosis index) were determined by alkaline gel electrophoresis (DNA comet method) [22–24]. Microscopic analysis was performed on a Zeiss Axio Imager Z1 microscope at 400x magnification. The obtained images of DNA comets (SYBR Green I dye) were analyzed using the Comet Imager system software, Metasystems GmbH. The percentage of DNA in the tail of DNA comets (% DNA in the tail) was used as an indicator of DNA damage. Cells with a DNA content in the DNA comet tail of ≥75% were considered apoptotic.

The research results are presented in the form M ± m, where M is the sample mean of the measured values, m is the standard error. The results were statistically processed using the SPSS Statistics 20 software package using the nonparametric Mann-Whitney rank test and the Student test. The critical level of significance of the null statistical hypothesis (p) was taken equal to 0.05.

RESULTS AND DISCUSSION

The general condition of all animals during daily examination was satisfactory: in appearance, quality of coat, food and water consumption, behavior and growth rate, the animals of the experimental groups did not differ from the animals of the control group. The gain in body weight of rats of all groups corresponded to the level of gain characteristic of animals of a given species and age. The average values of the relative increase in the body weight of animals for 30 days of the experiment for the control and experimental groups were 158.3 ± 5.8 and 167.6 ± 9.9%, respectively (the differences are insignificant). Table 1 shows the average values of some biochemical parameters used to characterize the course of the General Adaptation Syndrome in response to distress caused by electrocutaneous irritation.

Table 1

Results of determination of biochemical parameters - biomarkers of stress in rats

Группа животных	Показатель			
	Кортикостерон, нг/мл	β-эндорфин, нг/мл	Норадреналин, нг/мл	Простагландин Е2, пг/мл
Контрольная группа	5,7 ± 2,9	0,739 ± 0,155	7,4 ± 1,0	8,76 ± 0,76
Опытная группа	9,3 ± 3,9	0,516 ± 0,191	6,1 ± 1,6	8,95 ± 0,67

There were no significant differences in the average values of biochemical biomarkers of the central stress system and stress-limiting systems in animals of the control and experimental groups that experienced forced relatively short-term electrocutaneous stimulation. Dosage

ecdysteroid-containing extract of Seratulla coronata L. (2 mg of phytoecdysteroids / kg of animal body weight) in this study was significantly less than the dosages of 20 and 50 mg / kg of body weight, which were used in one of the previous studies, which showed that intragastric intake of the extract limited the increase in the blood of rats corticosterone [18]. The content in the blood of beta-endorphin, a neuropeptide considered by some authors as a “possible informative indicator of the adaptability of the organism” [17, 25], and the content of prostaglandin E2, dosages of 20 and 50 mg / kg body weight did not affect [18].

Determination of thymus mass and apoptosis activity in stressed animals turned out to be more informative in our study. The average values of the absolute and relative mass of the thymus of the animals of the compared groups at the end of the experiment differed statistically significantly, as follows from the data presented in table. 2.

The obtained result can be qualitatively compared with the data of [8], in which it was noted that oral administration of ecdysterone to immature intact rats itself led to an increase in thymus mass by more than 20%. It is known that a decrease in thymus mass is a generally recognized consequence of distress of sufficient intensity [29, 30]. Inhibition of this process in stressed rats receiving the extract was combined with a decrease in apoptosis activity in this organ, characterized by the level of DNA damage and the number of apoptotic cells (Table 3).

The relative decrease in the rate of apoptosis in the thymus when taking the extract can most likely be explained by the similarity of the structures of 20-hydroxyecdysone and 25S - inocosterone, with corticosterone, one of the main stress mediators that protects the rat's body from an excessive (exhausting) reaction to distress. It has been suggested that an adaptogen as a “mild prostressor” is capable of itself influencing the ratio of the most important activators and inhibitors of stress and thus reducing their “excess” increase during subsequent stress exposure [26]. By interacting with cell membrane receptors, phytoecdysteroids, like glucocorticosteroids, can serve as mediators of signaling pathways, promoting through secondary messengers the release of calcium ions from the sarcoplasmic reticulum, increasing the concentration of these ions in the cytoplasm by opening Ca2 + channels and ultimately leading to gene expression and synthesis of the corresponding proteins [27]. Concerning 20-hydroxyecdysone, there is experimental evidence that this compound (or its metabolites) triggers the activation of phosphatidylinositol-3-kinase via the PI3K signaling pathway, which leads to the activation of protein kinase B (PKB / Act), a signaling macromolecule that is key in the regulation of cellular activity [10, 28].

table 2

The relative and absolute weight of the thymus in animals of the control and experimental groups stressful effects of electrocutaneous irritation

Параметр	Группа животных	
	Контрольная группа	Опытная группа (2 мг/кг)
Масса тела по окончании эксперимента, г	286 ± 4,2	292 ± 7,2
Масса тимуса, г	0,46 ± 0,03	0,56 ± 0,03*
Относительная масса тимуса, % от массы крысы	0,163 ± 0,012	0,193 ± 0,010*

*Различия по сравнению с контрольной группой достоверны согласно критерию Стъюдента при P < 0,05 и согласно непараметрическому ранговому критерию Мана-Уитни.

Table 3

Effect of an ecdysteroid-containing extract from the leaves of Serpukha crown on activity apoptosis in some organs of rats subjected to stress

Орган	Группа животных			
	Степень фрагментации ДНК (% ДНК)		Индекс апоптоза, %	
	Контрольная группа	Опытная группа (2 мг/кг)	Контрольная группа	Опытная группа (2 мг/кг)
Тимус	8,14 ± 0,16	7,61 ± 0,14*	1,34 ± 0,07	1,08 ± 0,10*
Мозг	4,08 ± 0,14	4,12 ± 0,13	0,41 ± 0,07	0,42 ± 0,05
Сердце	7,53 ± 0,15	7,23 ± 0,13	1,23 ± 0,10	1,02 ± 0,14

*Различия по сравнению с контрольной группой достоверны согласно критерию Стъюдента при P < 0,05 и согласно непараметрическому ранговому критерию Мана-Уитни.

CONCLUSIONS

The study of the phytoecdysteroid-containing extract of Serratula coronata L. allowed

to establish that a relatively low dosage of phytoecdysteroids (2 mg / kg body weight) does not affect such important stress mediators as corticosterone, norepinephrine, beta-endorphin and prostaglandin E2.

It was found that the ecdysteroid-containing extract of the Crowned Herb has an inhibitory effect on the intensity of the general adaptation syndrome, which is accompanied by a decrease in the thymus mass and a decrease in the intensity of apoptosis in thymus cells of stressed animals receiving the extract.

The results obtained allow us to consider it promising to carry out further research with the aim of the subsequent use of this extract in the composition of dietary supplements for food and specialized food products.

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