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Evaluation of *Serratula coronata* L. extract toxicity for laboratory animals

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Abstract
Acute and subchronic toxicity of lyophilisate of aqueous extract of *Serratula coronata* L. (Asteraceae family) introduced in the Komi Republic (Russia) for laboratory rats has been investigated. Maximal dose of lyophilisate was equal to 6 g/kg. It has been determined that lyophilisate of *Serratula coronata* aqueous extract is less toxic and may be referred to the fourth class of hazard.

Keywords: *Serratula coronata* L.; acute and sub-chronic toxicity; rats; functional and biochemical indices; behavioral response; clinical blood analysis.

Introduction

One of the main research trends at the Institute of Biology (Komi Science Centre, Ural Branch, Russian Academy of Sciences) is the introduction of practically important plant species as a basis for new biologically active substances, herbal teas, veterinary preparations, and feed additives increasing resistance to stress and negative environment. In this respect, ecdysteroid-containing plants are of great interest with *Rhaponticum carthamoides* (Willd.) Iljin being the most extensively studied among them. Preparations on the basis of *Rhaponticum* underground and overground biomass, extracts and individual 20-hydroxyecdysone (20E) isolated from *Rhaponticum* rhizome are already widely used in veterinary medicine, sport, food industry, and stockbreeding [1-3]. Our results of long-term screening of wild and cultivated plants for ecdysteroid content [4] have become a basis for more profound biochemical and pharmacological studies of *Serratula coronata* L. (Asteraceae family) from West Siberian flora. High content of 20-hydroxyecdysone has been detected in the leaves of this plant species [5,6]. *Serratula coronata* has a vast natural habitat. It is spread all over Eurasia in temperate zone and in more southern regions: in the Middle Europe, the European South-West of the former USSR, the Caucasus, Western and Eastern Siberia, Central Asia, Mongolia, the Far East of Russia, China, and Japan [7]. In Russia the northern borders of *Serratula coronata* and *Serratula tinctoria* L. natural habitats lie on the territories of Pskov, Leningrad, Moscow and Kirov Regions. Species of the genus of *Serratula* are not present on the territory of the European North-East of Russia, but *Serratula coronata* has been successfully introduced in the Komi Republic (Russia) and is cultivated now as an important food and medicinal plant [8,9]. A series of biologically active nutritional supplements on the basis of purified ecdysteroids from *Serratula coronata* (Trade mark Serpisten) has been developed by the present moment. Adasten has pronounced actoprotective, immune-stimulating, and radioprotective action. Kardisten possesses anti-ischemic and hematoprotective activity and Diasten has antidiabetic property [10]. The effectiveness of *Serratula* herb use for regulation of reproductive function in cows has also been shown [11]. Thus, for complex use of *Serratula* raw material, it is perspective to create new biologically active

supplements and herbal teas on the basis of crude extracts of *Serratula* leaves and to obtain new feed supplements and veterinary preparations of anabolic, antistress, immune-stimulating, and reproductive function regulating action from byproducts, namely *Serratula* biomass residue after extraction and plant stems that are not used for the production of nutritional supplements. Both substrates contain certain concentration of 20E. Preliminary toxicity assessment of *Serratula coronata* is needed for creation of these products [12].

The aim of this study is to determine acute and subchronic toxicity of aqueous extract of *Serratula coronata* L. grown in the Komi Republic (Russia) for laboratory animals.

Materials and Methods

26 white outbred laboratory male rats with average weight of 270 ± 30 g aged 3-4 months from vivarium of the Ural Scientific Research Veterinary Institute of the Russian Academy of Agricultural Sciences (Yekaterinburg) were used for the experiment. One group of six rats was used to determine LD_{50}, and two groups (control and experimental) with ten rats in each one were used for introduction of subchronic dose of the preparation. Each animal received 25 g of standardized dry feed for laboratory rats (produced by Llc. "Laboratorkorm") daily [13,14]. The animals were kept in standard conditions of the vivarium with free access to water and food. They received drinking water satisfying hygienic requirements to water quality [15]. Temperature, air humidity, and light (natural and artificial, i.e., 12 h of light and 12 h of darkness) were under daily control in each room. The rats were prepared for the experiment according to instructions from general chapter "Toxicity testing" of the USSR State Pharmacopoeia [16]. The feed was taken away from the rats 8 h before dividing into groups, blood sampling, and slaughter. Clinical state of the rats was considered during the experiment. Observation was made during two weeks [13,17,18].

To conduct acute and subchronic experiments (forced drinking of lyophilisate of *Serratula* extract) monthly, a sample was daily weighed on the basis of 6.0 g per kg of animal live weight (dose calculation substantiation is given in the "Results and Discussion" section) and dissolved in the triple amount of distilled water straight before the beginning of the experiment. The extract was given every day before ingestion during 30 days. The animals from the control group received distilled water. The rats had individual markers during the experiment.

Biologic activity of lyophilisate of *Serratula* extract was determined using various indices: functional (body weight dynamics, daily water, and food consumption), behavioral ("Open field" test), clinical blood analysis and hemogram analysis, biochemical (aspartate-aminotransferase and alanine-aminotransferase activity in blood serum, bilirubin, urea and creatinine content, endogenous amylase activity, and glucose level), and morphological (relative changes in weight of visceral organs: heart, liver, spleen, kidneys) indices in animals of the experimental and control groups. All works were carried out on the basis of the Ural Scientific Research Veterinary Institute of the Russian Academy of Agricultural Sciences, Yekaterinburg. Testing Laboratory Accreditation Certificate No. RU.0001.22FV06.

The obtained data were processed using standard methods of variation statistics with parametric and nonparametric techniques. Reliability was estimated using Student's *t*-test [19].

Results and Discussion

Rhaponticum carthamoides is well-known among the plants used in folk, scientific and veterinary medicine as anabolic, stress-protecting and adaptogenic remedies [2,20]. Physiologic activity of preparations on the basis of *Rhaponticum* is caused by the presence of phytoecdysteroids, plant secondary metabolites that are structurally identical or related to insect molting hormones [6,21-23]. Phytoecdysteroids possess a number of favorable physiological properties in mammals. They enhance protein synthesis and increase physical performance [24]. They have stress-protective [25], antidiabetic [26], hypolipidemic and antiatherosclerotic [27] action. They improve nervous [28], renal [29], and hepatic [30] functions, stimulate immune system [31,32]. Ecdysteroids have wound-healing effect, they can be used in cosmetics [33] and slow release liposome form of ecdysteroids is developed [34]. Reviews on pharmacological effects of phytoecdysteroids on mammals and humans are now available in literature [35-38].

In our previous study, it was shown that the content of phytoecdysteroids in *Serratula coronata* is much higher than in *Rhaponticum carthamoides*. In comparison with *Rhaponticum carthamoides* that contains the only major ecdysteroid, *Serratula coronata* contains two major ecdysteroids 20E and its isomer 25S-inokosterone [5,6]. At present *Serratula coronata* is introduced in field culture in the Komi Republic [8] and selection is carried out to create *Serratula* variety [9]. *Serratula coronata* is not an officinal plant, though it is known to be used in Siberian medicine as an astringent, cholagogic, anti-inflammatory, and sedative drug. Its herb infusion is used at treatment of anemia, jaundice, cancerous growths, neuroses, paralyses, and mental diseases. It is known that in nature the overground part of *Serratula coronata* is ate up by wild ungulate animals – dappled deer and marals [39].

Safety of ecdysteroids themselves was studied previously. It has been determined that these substances are low-toxic: in experiments with mice LD_{50} at intraperitoneal introduction of 20E made up 6.4 and 9.0 g/kg, respectively, and at inokosterone introduction it made up 7.8 and 9.0 g/kg, respectively [40]. During studies of acute toxicity of Ecdysten containing individual 20E from *Rhaponticum carthamoides*, rhizomes it has been determined that at endogastric introduction of high dose (500 g/kg) of 20E in the form of aqueous solution reflectory excitability in white mice increases and stomach muscle tension is observed. 20E in 3000-5000 mg/kg dose causes some flabbiness, distensibility of caudal veins, hollowness of sides. These phenomena are observed during 5-6 h, though the animals' death at 5000 mg/kg has been observed neither during the first nor during the following days. At chronic introduction of 0.05, 0.5, and 5 mg/kg doses of 20E two times a day during 6 months, an increase in weight of male rats by 14, 25, and 17%, respectively, has been observed with no changes in general well-being and no behavior disorder. At the doses mentioned above, no structural changes in the morphology of peripheral blood or urea has been found also testifying to extremely low toxicity of 20E [1]. A conclusion about low toxicity and absence of mutagenic effect of preparation Serpisten developed in our laboratory containing mixture of pure ecdysteroids 20E and 25S-inokosterone from *Serratula coronata* leaves has been drawn [41]. At the same time safety of crude aqueous extract of *Serratula coronata* overground part was not previously investigated.

Based on the data, on low toxicity of 20-hydroxyecdysone and guidelines for preclinical studies [13,17,18] regulating the use of only maximally used doses in the cases when low toxicity does not allow to determine LD_{50} or it exceeds 5 g/kg of animal weight we used *Serratula* extract with only the maximal dose, i.e., 6 g/kg.

The study of acute and subchronic action of lyophilisate of *Serratula* aqueous extract has shown that animals receiving it *per os* in the maximal dose mentioned above, have survived, i.e., their death has not been observed. At the same time depression state, i.e., weakening of response to stimulus and feed, and thirst were observed during 2 h after introducing extract. However, in 2-3 h the depression state in rats graded and one day after introducing the extract and during the following period of observation they restored their activity; their feces became darker and dense. The second day after introducing extract and during the following period of observation color and density of the feces were normal.

According to the dynamics of weight change in rats during month's subchronic experiment with lyophilisate of *Serratula* aqueous extract, no valid differences in weight gain dynamics compared to the control group have been identified (Table 1).

A statistically valid difference in increase of water intake by the animals of the experimental group has been determined, probably due to forced introduction of water with the primer and due to caramel structure of lyophilisate possibly leading to the increase of water intake by the experimental group. The difference in food consumption between the groups was insignificant (Table 2).

Central nervous system (CNS) is one of the main stimulating systems at complex impact of phytogenic feed supplements on organism. Behavioral response of animal organism is the most informative criterion of the CNS state. "Open field" test is widely used to investigate behavioral characteristics of laboratory animals, is sufficient for initial screening and appropriate to test toxicity of the preparations. According to the "Open field" test, no valid differences in the indices under study have been found. General decrease of physical activity during the month's experiment testifies to the animals' adaptation to the "Open field" test conditions (Table 3).

Table 1: Dynamics of mean values of body weight in the control and experimental groups of rats in month's subchronic experiment with lyophilisate of *Serratula coronata* aqueous extract.

Time of experiment (days)	MX ± mx		Student's coefficient value (t_{st})	*p*
	Control, *n* = 10	Experiment, *n* = 10		
1 day	274.5 ± 14.6	273.3 ± 7.7	0.073	>005
7 days	296.7 ± 15.5	288.8 ± 10.0	0.428	>005
After 14 days	311.6 ± 16.3	309.2 ± 12.7	0.116	>005
After 21 days	318.3 ± 16.0	321.7 ± 13.0	0.165	>005
After 30 days	325.2 ± 16.0	331.9 ± 13.8	0.317	>005

Note: Here and elsewhere: n – number of animals under study; MX ± mx – mean value with an error of mean value; $p < 0.05$ – validity of differences between the control and experimental groups.

Table 2: Water intake and the amount of the feed residue.

Group	Water (ml) MX ± mx	Feed (g) MX ± mx
Control (10)	299.65 ± 8.77	3.59 ± 1.64
Experimental (10)	332.76 ± 10.01	3.10 ± 1.29
p	<005	>005

Note: Parentheses show the number of animals.

Table 3: Dynamics of the rats' behavioral response in the "Open field" test (MX ± mx) in subchronic experiment with lyophilisate of *Serratula coronata* aqueous extract.

Time (days)	Group	HPA	VPA	SF	Grooming	LD	GPA
7	Control	41.6 ± 3.8	10.9 ± 1.6	2.5 ± 0.4	1.3 ± 0.4	0.9 ± 0.5	57.2 ± 4.8
	Experimental	37.1 ± 6.4	9.1 ± 1.6	1.3 ± 0.5	0.7 ± 0.3	2.1 ± 0.6	50.4 ± 8.1
	p	>005	>005	>005	>005	>005	>005
	Control	13.1 ± 3.9	2.4 ± 1.2	0.6 ± 0.2	0.8 ± 0.3	0.5 ± 0.3	17.4 ± 5.3
14	Experimental	6.4 ± 1.9	1.5 ± 0.7	0.1 ± 0.1	0.5 ± 1.1	0.7 ± 0.2	9.1 ± 2.6
	p	>005	>005	>005	>005	>005	>005
	Control	12.8 ± 2.4	2.8 ± 0.8	1.1 ± 0.5	1.3 ± 0.5	0.4 ± 0.2	18.4 ± 3.3
21	Experimental	11.9 ± 4.9	2.8 ± 1.2	0	0.6 ± 0.34	0.8 ± 0.3	16.1 ± 6.1
	p	>005	>005	>005	>005	>005	>005
	Control	15.4 ± 4.9	3.4 ± 1.1	0.7 ± 0.3	0.7 ± 0.3	0.3 ± 0.2	20.5 ± 6.5
30	Experimental	14.1 ± 4.9	2.9 ± 0.9	0.6 ± 0.3	0.1 ± 0.1	0.3 ± 0.2	18.0 ± 5.9
	p	>005	>005	>005	>005	>005	>005

Note: HPA – horizontal physical activity; VPA – vertical physical activity; Grooming – active animals' behavior to clean body surface, e.g., washing, bathing; SF – sniffing of foramen; LD – level of dejection; GPA – general physical activity.

The results of clinical blood test have shown no significant differences between the control and experimental groups testifying to the absence of inflammatory or allergic reactions to the preparation under study. All indices of clinical blood test in the control and experimental groups did not differ statistically and stayed within the reference values (Tables 4 and 5).

According to biochemical analysis a valid increase of albumin, glucose, bilirubin, and crude protein content in the blood serum of animals from the experimental group compared to the control group has been identified (Table 6).

Other biochemical indices have shown no statistically valid differences between the groups of rats under study. The increase of clinical

Table 4: Leucograms of animals from the control and experimental groups of rats in subchronic experiment with lyophilisate of *Serratula coronata* aqueous extract (%).

Indices: mean ± error of mean value (MX ± mx)						
Group of animals	**Rods**	**Segments**	**Eosinophils**	**Monocytes**	**Lymphocytes**	**Basocytes**
Control	2.20 ± 0.13	23.20 ± 4.19	1.0 ± 0.0	8.50 ± 0.62	65.7 ± 3.77	0.00 ± 0.00
Experiment	2.20 ± 0.13	20.30 ± 4.51	1.3 ± 0.2	8.10 ± 0.62	69.9 ± 3.44	0.20 ± 0.13
t_{st}	0	0.47	1.43	0.11	0.82	1.54
p	>0.05	>0.05	>0.05	>0.05	>0.05	>0.05

Table 5: Indices of clinical blood test of rats from the control and experimental groups in subchronic experiment with lyophilisate of *Serratula coronata* aqueous extract.

Indices: mean ± error of mean value (MX ± mx)					
Group of animals	**Leucocytes, $\times 10^3$/mcL**	**Erythrocytes, $\times 10^6$/mcL**	**Haemoglobin, g/dL**	**Hematocrit, %**	**Platelets, $\times 10^3$/mcL**
Control	13.14 ± 0.88	8.47 ± 0.34	15.32 ± 0.60	45.56 ± 1.84	643.0 ± 38.40
Experiment	11.22 ± 0.98	8.37 ± 0.24	15.20 ± 0.36	45.04 ± 1.00	572.2 ± 27.13
t_{st}	1.46	0.24	0.17	0.25	1.51
p	>0.05	>0.05	>0.05	>0.05	>0.05

Table 6: Biochemical values of blood in rats from the control and experimental groups in the subchronic experiment with lyophilisate of *Serratula coronata* aqueous extract.

Index	**Measurement units**	**Control $n = 10$**	**Experiment $n = 10$**	t_{st}	p
Aspartate aminotransferase	U/L	161.33 ± 11.36	133.60 ± 7.30	2.02	>0.05
Alanine aminotransferase	U/L	56.20 ± 2.96	62.60 ± 6.43	0.90	>0.05
AST/ALT ratio	Relative unit	2.96 ± 0.29	2.40 ± 0.35	1.23	>0.05
Albumin	g/L	34.47 ± 0.65	39.14 ± 0.99	3.94	<0.01*
Glucose	mmol/L	2.09 ± 0.16	3.28 ± 0.19	4.79	<0.001*
Creatinine	mcM/L	41.34 ± 1.63	39.76 ± 2.48	0.53	>0.05
Bilirubin	mcM/L	5.18 ± 1.01	9.37 ± 0.96	3.01	<0.05*
Alkaline phosphatase	U/L	214.50 ± 9.10	217.00 ± 15.07	0.14	>0.05
Amylase total	U/L	1633.19 ± 109.00	1675.00 ± 92.39	0.29	>0.05
Urea	mmol/L	6.25 ± 0.37	6.31 ± 0.31	0.12	>0.05
Crude protein	g/L	70.34 ± 1.38	76.64 ± 1.30	3.32	<0.01*
Phosphorus	mmol/L	2.61 ± 0.09	2.86 ± 0.15	1.43	>0.05
Cholesterol	mmol/L	0.99 ± 0.05	1.06 ± 0.05	0.99	>0.05

Note: t_t – student's coefficient; * – differences between control and experiment are valid.

blood test values in the experimental group taking in lyophilisate of *Serratula coronata* aqueous extract testifies to development of adaptation to this impact. This is proved by the indices of biochemical analysis showing that the experimental animals have the needed range of amino acids and better protein consumption not leading to catabolic processes in the organism. The levels of urea and creatinine have stayed on the control level with no toxic effect on liver, kidneys, or myocardium. This is proved by low levels of aminotransferase and amylase activity. The increase of glucose activity level may be regarded as a positive effect of *Serratula* extract as the values of amylase activity were equal to the values of the control group. The increase of bilirubin level in the rats from the experimental group may be explained by erythrocytes breakup in liver due to probable stimulation of erythropoiesis that may be regarded as a positive effect.

Table 7: Estimation of pathomorphological changes in visceral organs of laboratory rats compared to the control group in subchronic experiment with lyophilisate of *Serratula coronata* L. aqueous extract.

Groups of animals	Ratio of heart weight to body weight, $\times 10^3$				
	Liver	Kidney		Heart	Spleen
		Left	Right		
Control ($n = 10$)	37.9 ± 1.4	3.7 ± 0.1	3.9 ± 0.2	3.7 ± 0.1	3.6 ± 0.2
Experiment ($n = 10$)	36.3 ± 0.6	3.7 ± 0.1	3.8 ± 0.1	3.3 ± 0.1	3.5 ± 0.2
p	>0.05	>0.05	>0.05	<0.05	>0.05

On completion of the month's subchronic experiment with lyophilisate of *Serratula coronata* aqueous extract estimation of pathomorphological changes in external state of visceral organs (liver, kidneys, heart, spleen) showed no differences in the experimental group compared to the control group. Statistically valid differences between the control and experiment were determined by the ratio of heart weight to body weight (Table 7).

Thus, it can be noted that according to hematologic tests no valid differences between the rats from the control and experimental groups during month's subchronic experiment with lyophilisate of *Serratula* aqueous extract have been found. Evidently this proves absence of inflammatory and allergic reactions to the introduced substance. The changes in some biochemical parameters may testify to stimulation of anabolic processes, mostly hemopoiesis and improvement of metabolic processes affecting protein and carbohydrate metabolism. The difference in water consumption by the rats from the experimental and control groups may be explained by the consistence of the extract demanding to be additionally diluted with water for complete assimilation. The conducted functional tests show low toxicity of lyophilisate of *Serratula coronata* aqueous extract. The changes found during pathomorphological studies of the rats' visceral organs may be explained in terms of "response to introduction" of the substance under study. This is proved by glucose level increase in blood of the experimental group.

Conclusions

1. *Per os* investigation of lyophilisate of *Serratula coronata* aqueous extract during month's subchronic experiment using laboratory rats was carried out and functional, biochemical methods, and pathomorphological studies of visceral organs have shown its low toxicity, absence of inflammatory and allergic reactions to the introduced substance, and improvement of metabolic processes. The maximal dose was equal to 6 g/kg.
2. In terms of acute toxicity values lyophilisate of *Serratula coronata*, aqueous extract is a substance with low toxicity according to the All-Union State Standard 7.32-2001 and may be referred to the fourth class of hazard.

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