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Effect of Extracts from *Rhodiola Rosea* and *Rhodiola Crenulata* (*Crassulaceae*) Roots on ATP Content in Mitochondria of Skeletal Muscles

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We studied the effects of oral treatment with extracts from *Rhodiola rosea* (50 mg/kg) and *Rhodiola crenulata* (50 mg/kg) roots on the duration of exhaustive swimming and ATP content in mitochondria of skeletal muscles in rats. Treatment with *R. rosea* extract significantly (by 24.6%) prolonged the duration of exhaustive swimming in comparison with control rats and rats treated with *R. crenulata*. *R. rosea* extract activated the synthesis or resynthesis of ATP in mitochondria and stimulated reparative energy processes after intense exercise. Experiments proved different pharmacological characteristics of *R. rosea* and *R. crenulata*: *R. rosea* is most effective for improving physical working capacity.

Key Words: *ATP; mitochondria; Rhodiola rosea; Rhodiola crenulata; rosavines; salidroside*

Rhodiola rosea (*Crassulaceae*) or golden root grows in Arctic highlands and is used for phytotherapy in Russia, Scandinavia, and Asia [5]. Extract of *Rhodiola rosea* root is characterized by stress-protective and antidepressive action. It alleviates emotional, mental, and physical disorders [10,11], reduces the severity of exhaustion after intensive physical exercise [1,4], elevates concentrations of norepinephrine, dopamine, and serotonin in the brain, and acts as a nicotinic cholinergic agonist in the CNS [7]. Professional athletes use *R. rosea* for increasing physical activity, stimulating anabolic processes in skeletal muscles, increasing endurance during maximum physical exercise, and promoting subsequent recovery of the cardiovascular system [1,4].

The main components determining the phytochemical and pharmacological characteristics of *R. rosea* are rosavine (cinnamic alcohol vicyanoside), rosine, rosarine (common name rosavines, which seem to be components of *R. rosea* alone), and hydroxyphenylethanol-2-D-glucopyranoside (salidroside) [3]. On the other hand, all plants of the *Rhodiola* genus contain salidroside [13,14].

Medicinal plant *R. crenulata* grows in Uzbekistan, China, and other Asian countries. It seems to possess the effects of "informing" or "preventing" which are due to salidroside. However, this hypothesis was not confirmed clinically.

We studied the effects of extracts from *R. rosea* roots and *R. crenulata* root on ATP content in the muscle mitochondria of rats before and after the exhaustive swimming test.

MATERIALS AND METHODS

The underground parts of *R. rosea* were collected in East Siberia. *R. crenulata* Fish et Mey roots were received from the Academy of Sciences of China. The plants were collected during the late blooming period;

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the soil was removed, the roots were carefully cut into 1-3-cm fragments and dried in chambers at 30°C at constant air flow.

Plant materials were extracted with water-ethanol mixture (60:40 volume ratio) in extraction vessels for 12 h. The extracts were purified from fragments of cell walls, ethanol was removed by distillation, and ethanol-free extract was lyophilized.

Rosavines and salidroside were measured by high-performance liquid chromatography (HPLC) using Water System HPLC with a sensitive matrix photodiode 996 Photodiode Array Detector. The separation was carried out in an analytical column RP-C18, 3.9×150 mm (Symmetry, WATO27324, Water Associates Inc.). UV radiation wavelength was 254 nm for rosavine and 280 nm for salidroside [2].

Reference samples of rosavine and salidroside for HPLC were received from Russian Institute of Medical Plants (VILAR, Moscow). Calibration curve for each reference sample was linear in the studied concentration range. The correlation coefficient was 0.99.

Experiments were carried out on 24 adult Sprague-Dawley rats (250±20 g). The animals were kept in cages at 20±2°C with free access to water and food (light from 8.00 to 20.00). The animals were divided into 3 groups: control (n=8) and two experimental. Rats of experimental group 1 (n=8) received *R. rosea* extract (50 mg/kg), those of group 2 *R. crenulata* extract (50 mg/kg).

The effects of plant extracts (administered through a tube 30 min before each session) on ATP content in the muscle mitochondria of rats were studied in forced swimming test. The animals swam until exhaustion in water at room temperature. Normally, the rats can swim for 10-14 h without rest. After weight load the animals are exhausted within 5 h. Two sessions of exhaustive swimming with 30-min intervals were carried out every day. The maximum duration of swimming was estimated on the basis of data recorded for 6 days. Plant extracts were administered through a tube 30 min before each session. The period until exhaustion was considered as the period from the start of the test until the moment when the animals could swim no more. Four rats from each group were sacrificed by intraperitoneal injection of sodium pentobarbital directly after estimating the duration of exhaustive swimming. Other animals were sacrificed 24 h after the experiment.

Muscle mitochondria were isolated by the method of Tonkonogi and Sahlin [12]. ATP content in isolated mitochondria was measured by the bioluminescence method using commercial ATP kit (Drew and Leeuwenburgh). Experiments with mitochondria were carried out 3 times and the mean ATP content was estimated.

The data were expressed as means and errors. The differences were detected using ANOVA monofactorial analysis of dispersions with repeated measurements and were considered significant at $p<0.05$.

RESULTS

R. rosea extract contained a complex of rosavines and salidroside (3.02% and 0.89% estimated for dry weight). The ratio of rosavines and salidroside in *R. rosea* root extract was 3:1, which is in line with published data [3]. *R. crenulata* extract contained only salidroside (2.05%).

The mean duration of exhaustive swimming of control rats and rats receiving *R. crenulata* and *R. rosea* was 34.2±2.5, 35.5±3.1, and 44.9±2.1 min, respectively. The mean time until exhaustion was virtually the same in rats treated with *R. crenulata* and controls. Hence, *R. crenulata* root extract (in contrast to the *R. rosea* root extract) did not increase physical working capacity. Importantly, salidroside content in *R. crenulata* extract was 2.5 times higher than in *R. rosea* extract. Hence, the effects of *R. rosea* extract are due to the presence of rosavines and/or their complex with other compounds.

Changes in ATP content in mitochondria under the effects of extracts from these *Rhodiola* plants confirmed their different pharmacological characteristics (Table 1). ATP content in the mitochondria decreased after swimming exercise in controls and animals of both experimental groups. However, the decrease in ATP content in rats receiving *R. rosea* extract was less pronounced in comparison with not only the control, but also *R. crenulata* group. It can be hypothesized that *R. rosea* root extract more actively stimulated

TABLE 1. Effects of *R. rosea* and *R. crenulata* Extracts on ATP Content in Mitochondria of Spraque-Dawley Rats (μmol/g protein; *M±m*)

Group	Before test	After 6 days	After 24-h rest
Control	5.38±0.30	3.86±0.40*	4.69±0.50*
R. crenulata	5.48.0±0.5	3.81±0.50*	4.63±0.20*
R. rosea	5.41±0.40	4.85±0.30*, **	5.22±0.40*, **

Note. $p<0.05$: *compared to previous value; **compared to controls and rats treated with *R. crenulata*.

ATP synthesis or resynthesis in muscles during exercise.

Our results are in line with the data published in the 1970s and 1980s, asserting that *R. rosea* root extract increased the content of high-energetic phosphates in the mitochondria.

Anabolic effects of *R. rosea* root extract were studied for the first time in mouse experiments.

Acidosis caused by accumulation of lactic acid in muscles and the presence of ammonia are the metabolic factors causing fatigue during exercise and decreasing the rate of ATP and cyclophosphate synthesis in mitochondria [4,6]. Accumulation of lactate during intensive muscle work impairs the respiratory function of mitochondria in skeletal muscles and causes reorganization of their ultrastructure. Treatment with *R. rosea* promoted the decrease in ammonia concentration in mouse muscles.

The increase in working capacity during intense physical and intellectual work was observed in athletes and other humans receiving *R. rosea*.

Based on numerous studies carried out in Russia over recent 35 years, Russian scientists and trainers recommend *R. rosea* as the means improving strength and endurance and replenishing the energy resources of the body [4].

Our findings indicate that *R. rosea* extract is characterized by unique pharmacological properties and produces a positive effect on ATP synthesis in mitochondria. *R. rosea* extract prolonged the duration of exhaustive swimming in rats and accelerated recovery after intensive exercise. The effects of *R. rosea* can be explained by the presence of rosavine complex, although the role of other bioactive compounds of this plant cannot be excluded.

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