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Attenuation of Long-Term *Rhodiola rosea* Supplementation on Exhaustive Swimming-Evoked Oxidative Stress in the Rat

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Abstract

Rhodiola rosea **improves exercise endurance and fatigue. We hypothesized that ingredients in** ***Rhodiola rosea*** **may increase antioxidant capability against swimming induced oxidative stress. In this study, we have identified the** ***Rhodiola rosea*** **ingredients, p-tyrosol, salidroside, rosin, rosavin and rosarin by high performance liquid chromatography-mass spectrometer and evaluated their $O_2^{-\cdot}$, H_2O_2, and HOCl scavenging activities by a chemiluminescence analyzer. We next explored the effect and mechanism of** ***Rhodiola rosea*** **on 90-min swimming-induced oxidative stress in male Wistar rats fed with three doses of** ***Rhodiola rosea*** **extracts in drinking water (5, 25, 125 mg/day/rat) for 4 weeks. Our results showed that the 4 major ingredients (salidroside, rosin, rosavin and rosarin) from** ***Rhodiola rosea*** **extracts scavenged $O_2^{-\cdot}$, H_2O_2, and HOCl activity in a dose-dependent manner. The ninety-min swimming exercise increased the $O_2^{-\cdot}$ production in the order: liver > skeletal muscle > blood, indicating that liver is the most sensitive target organ. The level of plasma malonedialdehyde, a lipid peroxidation product, was also increased after exercise. Treatment of 4 weeks of** ***Rhodiola rosea*** **extracts significantly inhibited swimming exercise-enhanced $O_2^{-\cdot}$ production in the blood, liver and skeletal muscle and plasma malonedialdehyde concentration. The expression in Mn-superoxide dismutase Cu/Zn-superoxide dismutase, and catalase in livers were all enhanced after 4 weeks of** ***Rhodiola rosea*** **supplementation especially at the dose of 125 mg/day/rat. Treatment of** ***Rhodiola rosea*** **extracts for 4 weeks significantly increased swimming performance. In conclusion, treatment of** ***Rhodiola rosea*** **extracts for 4 weeks could reduce swimming-enhanced oxidative stress possibly** ***via*** **the reactive oxygen species scavenging capability and the enhancement of the antioxidant defense mechanisms.**

Key Words: ***Rhodiola rosea*****, exercise, oxidative stress, reactive oxygen species, rat**

Introduction

Rhodiola rosea (Golden Root, Roseroot) is a plant in the Crassulaceae family growing in the mountainous and arctic regions of North America, Europe, and Asia. *Rhodiola rosea* can combat fatigue

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by its several unique ingredients (5, 10). Among these, p-tyrosol, salidroside, rosin, rosavin and rosarin are the major active components of *Rhodiola rosea* with adaptogenic characteristics (5) for improvement of cognitive function (35) and endurance performance (1, 12, 35), reduction of mental fatigue (10, 36), anti-diabetic effect (22) and reactive oxygen species (ROS) production (2, 13, 20, 21).

Physical exercise is characterized by an increase in O_2 uptake and consumption and induced stressors such as elevations of body temperature, the formation of reactive oxygen species (ROS), and a decrease in glycogen (3, 25, 26). In extreme conditions such as ischemia/reperfusion or exhaustive exercise, the increased ROS can oxidize macromolecules contributing to abnormal signal transduction or cellular dysfunction, impairment of both enzymic and non-enzymic antioxidant defense systems of target tissues and trigger erythrocyte hemolysis (4) and the cascade of apoptosis, autophagy and necrosis (7, 8, 26, 30-33). Exhaustive exercise enhances xanthine oxidase activities of plasma and skeletal muscle, muscular myeloperoxidase activity and malondialdehyde concentrations of plasma and tissues (25). Exhaustive exercise leads to oxidative damage in the liver including rough endoplasmic reticulum fragmentation and dilatation, glycogen depletion, and mitochondrial enlargement (34, 37). Therefore, exhaustive exercise-enhanced oxidative stress may impair liver, kidney, skeletal muscle and other tissues by different degrees of ROS production. It has been speculated that increased antioxidant/oxidative damage-repairing enzyme activities, increased resistance to oxidative stress and lower levels of oxidative damage may protect oxidative stress-related cardiovascular, kidney, liver and neuronal damages (8, 33, 41). The long-term effects of *Rhodiola rosea* supplementation on exhaustive exercise-induced oxidative stress have not clearly been demonstrated. The purpose of the current study was to identify the active components in the *Rhodiola rosea* extract and to examine the long-term effect and mechanism of *Rhodiola rosea* supplementation on ROS production and oxidized biomarkers in the liver, skeletal muscle and blood after exhaustive exercise.

Materials and Methods

Rhodiola rosea *and High Performance Liquid Chromatography-Mass Spectrometry (HPLC-MS)*

Dry powders from water extraction of roots of *Rhodiola rosea* L. was purchased from Numen Biotech (Taipei, Taiwan, ROC). In brief, fresh original habitats of *Rhodiola rosea* rhizomes from Siberia were thoroughly washed with water, shade-dried for 4 weeks, and powdered using a mixer grinder. A known quantity of the dried powdered material was soaked in distilled water for 24 h at 35-42°C and macerated thoroughly with the help of a mortar and pestle. The mixture was filtered through Whatman filter paper No. 1, condensed using a rotary evaporator and lyophilized.

A model Agilent 1,100 with vacuum degasser, binary pump, autosampler and thermostatic column holder was used. The LC separation was performed using a ZORBAX 300SB-C18 3.5 µm, 1.0 × 150 mm. The temperature of the column oven was 35°C and the injection volume was 1 µl. The eluent flow-rate was 0.08 ml/min. The gradient conditions were initially 90% A (aqueous phase with distilled water produced by Mill-Q, 18.2 MΩ)-5% B (acetonitrile)-5% C (methanol), changed linearly to 76% A-12% B-12% C in 16 min. After gradient elution, the column was washed for 1 min with acetonitrile and equilibrated for 5 min under the initial conditions leading to a total time of 25 min for one analysis. All HPLC-MS experiments were performed using a mass spectrometer (Esquire 3000+, Bruker Daltonik GmbH, Bremen, Germany) equipped with a positive spray ionization source in the full-scan mode over the *m/z* range 100-600. Nitrogen was used as the drying gas at a flow-rate of 8 l/min. The dry air temperature was 310°C at 20 psi pressure. For calibration of the HPLC-MS method with ESI and the eluent system, eight concentration levels of standard were prepared for obtaining the calibration curves. We used salidroside, rosin, rosarin and rosavin for standards (Sigma, St. Louis, MO, USA). Standard solutions were prepared in 6% methanol containing 400 ng/ml internal standard. For all standards, the concentration levels prepared were 0.5, 2, 5, 20, 50, 200, 500 and 2000 ng/ml. Calibration curves were constructed by plotting responses of the standard compounds relative to responses of the internal standard (measured in triplicate for each concentration) against the concentration of standard compounds. Ten mg of the *Rhodiola rosea* extracts was dissolved in methanol and stirred after supersonic vibration for 15 min. The supernatant was filtered after 0.45 µm and was analyzed by HPLC-MS. The data were presented in the Fig. 1. *Rhodiola rosea* extract contains three cinnamyl alcohol-vicianosides, rosavin, rosin, and rosarin, that are specific to this species (14, 15).

Animals and Swimming Model

Male Wistar rats (200-250 g) were housed at the Experimental Animal Center, National Taiwan University, at a constant temperature and with a consistent light cycle (light from 07:00 to 18:00 o'clock). Food and water were provided *ad libitum*. All surgical and experimental procedures were

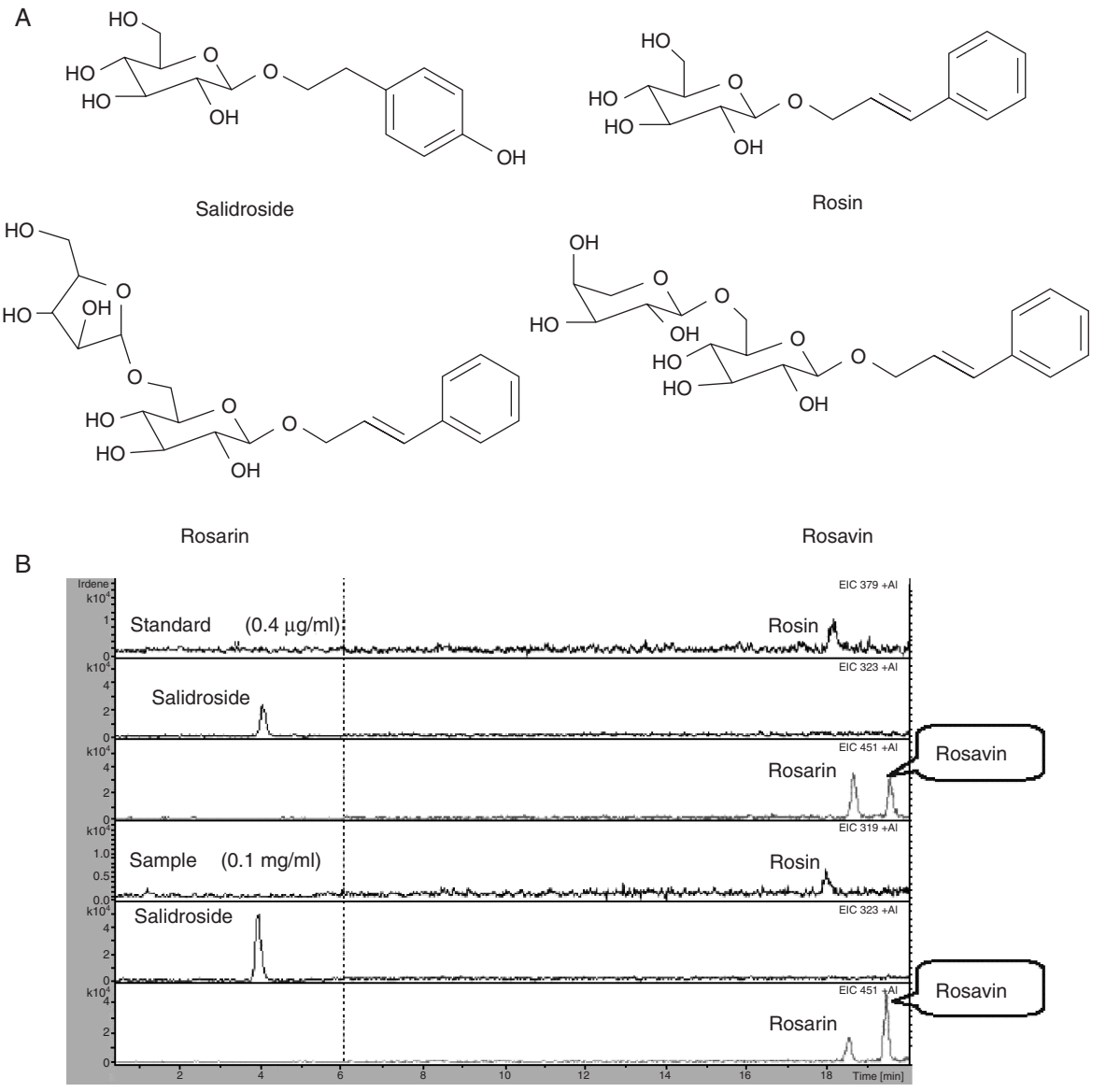

Fig. 1. A. Structures of four compounds. B. Standards and *Rhodiola rosea* extract extracted chromatograms from HPLC-MS experiment (full-scan mode) with positive ESI and eluent system.

approved by the National Taiwan University College of Medicine and College of Public Health Institutional Animal Care and Use Committee in accordance with the guidelines of the National Science Council of Republic of China (NSC 1997).

All the animals were divided into four groups for oral administration of 0, 5, 25 and 125 mg/day *Rhodiola rosea* extracts for 4 weeks. Before the commencement of an experiment, all animals were familiarized to swimming for 10 min/day for 3 days. At the indicated time, rats fasted overnight for 8 h (from 12:00 PM to 8:00 AM) in a 90-min swimming exercise test were measured (6). The swimming exercise in free style was carried out in a circular plastic barrel (diameter, 20 cm; depth, 30 cm) filled with water maintained at a temperature of 24 ± 1°C. The rats swam in the circular plastic barrel for 90 min. After 90 min of swimming challenge, the rats were killed with an overdose of sodium pentobarbital intraperitoneally (90 mg/kg body weight) and the liver, skeletal muscle and blood were removed.

Changes of Lucigenin- and Luminol-Enhanced Chemiluminescence Counts (CL)

The antioxidant activities of 5 major ingredients (tyrosol, salidroside, rosin, rosarin and rosavin) and *Rhodiola rosea* extracts on xanthine (0.75 mg kg^{-1}, dissolved in 0.01 N NaOH) and xanthine oxidase (24.8 mU kg^{-1}) enhanced $O_2^{-\cdot}$, 0.03% H_2O_2 induced H_2O_2

activity, and % HOCl induced HOCl activity were evaluated.

ROS levels were measured using a CL analyzing system (CLD-110, Tohoku Electronic Industrial, Sendai, Japan) as previously described (7). The system contained a photon detector (Model CLD-110), a CL counter (Model CLC-10), a water circulator (Model CH-200) and a 32-bit IBM personal computer system. A cooler circulator was connected to the model CLD-110 photon detector to keep the temperature at 5°C. Under these conditions, radiant energy as low as 10^{-15} W could be detected.

CL was measured in an completely dark chamber of the CL analyzing system. We demonstrated that using the CL-emitting substance lucigenin (*N,N'*-dimethyldiacridinium, Sigma, St. Louis, MO, USA) for $O_2^{-\cdot}$ or luminol (5-amino-2,3-dihydro-1,4-phthalazinedione, Sigma, St. Louis, MO, USA) for H_2O_2 or HOCl to enhance the CL counts provided similar data to those reported in our previous *in vivo* study (7). The lucigenin-enhanced CL method provides a reliable assay for superoxide. After 100 s, 1.0 ml of 0.1 mM lucigenin in PBS (pH = 7.4) was mixed with the tested sample. CL in the tested sample was measured continuously for a total of 600 s. The assay was performed in duplicate for each sample, and the results are expressed as CL counts $(10\ s)^{-1}$. The total amount of CL in 600 s was calculated by integrating the area under the curve. The means ± S.E.M. CL level for each sample was calculated.

We used 1 ml blood samples and 0.2 g homogenized liver and leg skeletal muscle to measure ROS levels. The analysis of lipid peroxidation, malondialdehyde (MDA), concentrations of plasma samples was assessed colorimetrically at 586 nm using a commercial kit (Calbiochem 437634; Calbiochem-Novabiochem, La Jolla, CA, USA) as previously described (40). Concentration was expressed in μM in plasma and in μmol/mg protein in tissue samples.

Effect of Rhodiola rosea *Extract on MnSOD, Cu/Zn SOD, Catalase Protein Expression*

Protein concentration was determined by a BioRad Protein Assay (BioRad Laboratories, Hercules, CA, USA). Ten g of protein was electrophoresed as described below. The expression of MnSOD, Cu/Zn SOD and catalase in liver tissues was evaluated by western immunoblotting and densitometry as described (41). Briefly, total proteins were homogenized with a prechilled mortar and pestle in an extraction buffer of 10 mM Tris-HCl (pH 7.6), 140 mM NaCl, 1 mM phenylmethyl sulfonyl fluoride, 1% Nonidet P-40, 0.5% deoxycholate, 2% β-mercaptoethanol, 10 μg/ml pepstatin A and 10 μg/ml aprotinin. The mixtures were homogenized completely by vortexing and kept at 4°C for 30 min. The homogenate was centrifuged at 12,000 g for 12 min at 4°C, the supernatant was collected, and protein concentrations were determined by the BioRad Protein Assay (BioRad Laboratoriess Hercules, CA, USA).

The polyclonal anti-MnSOD (Stressgen Bioreagents Limited, Victoria, Canada) rabbit anti-human Cu/Zn SOD (Stress Marq Biosciences Inc., Victoria, Canada) and catalase (Chemicon International Inc., Temecular, CA, USA) antibodies, and the monoclonal mouse anti-mouse β-actin (Sigma, Saint Louis, MI, USA) were used at 1:1000 dilutions. All of these antibodies cross-reacted with the respective rat antigens (29).

Statistical Analysis

All values were expressed as means ± standard error mean (SEM). Differences within groups were evaluated by paired *t*-test. One-way analysis of variance was used for establishing differences among groups. Intergroup comparisons were made by Duncan's multiple-range test. A chi-square test was performed in the hepatic antioxidant expression. Differences were regarded as significant if $P < 0.05$ was attained.

Results

Ingredient Analysis of Rhodiola rosea *Extract*

In the present study, the structures of four standard components including salidroside, rosin, rosarin and rosavin are demonstrated in Fig. 1A. The original diagram obtained from the four standard components (upper panel) and the samples of *Rhodiola rosea* extract (lower panel) was analyzed by HPLC-MS (Fig. 1B). *Rhodiola rosea* ingredient was analyzed by HPLC-MS as shown in Table 1. By the use of our techniques, *Rhodiola rosea* extract was shown to contain four major components including salidroside, rosin, rosarin and rosavin. Among these four components, the salidroside content was the highest (13128 ppm). Our data verified that the *Rhodiola rosea* extract in this study contains approximately 1.3% salidroside, 0.4% rosin, 0.4% rosarin and 1% rosavin, but did not contain p-tyrosol in our HPLC-MS analysis. *Rhodiola rosea* extract supplementation was provided to the rat in drinking water in a dose-dependent manner. Table 1 shows the level of the four components in the three dosages of *Rhodiola rosea* extract supplementation. The salidroside, rosin, rosarin and rosavin contents were dose-dependently increased with the *Rhodiola rosea* extract dose.

Table 1. Aqueous concentrations of *Rhodiola rosea* extracts as determined by HPLC-MS

	Salidroside (μg)	Rosin (μg)	Rosarin (μg)	Rosavin (μg)
R0 (0 mg)	0 ± 0	0 ± 0	0 ± 0	0 ± 0
R5 (5 mg)	71 ± 5	20 ± 2	21 ± 3	52 ± 6
R25 (25 mg)	334 ± 39	87 ± 10	85 ± 9	247 ± 32
R125 (125 mg)	1,645 ± 169	416 ± 61	382 ± 42	1,187 ± 155

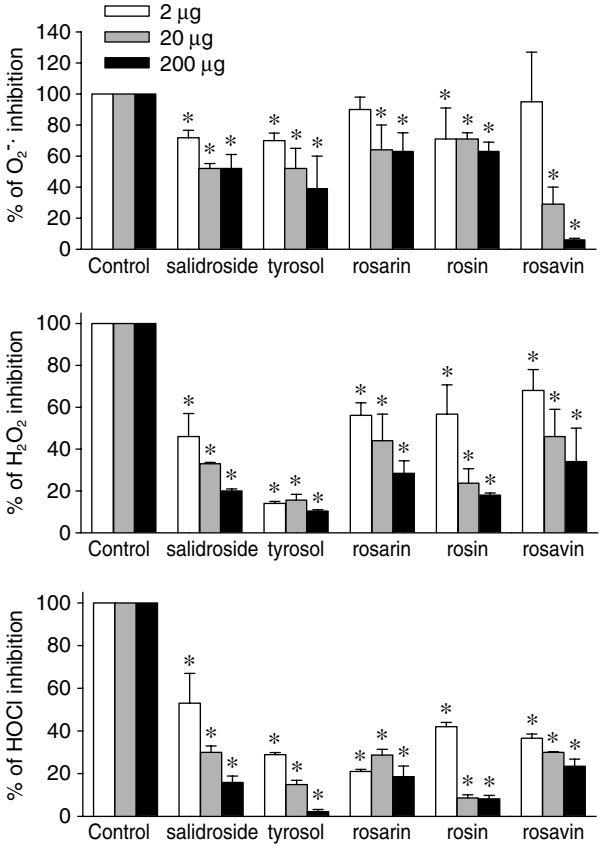

Fig. 2. Different dosages of five standards displaying $O_2^{-\cdot}$, H_2O_2 and HOCl scavenging activities in a dose-dependent manner. The sample concentration was 2-200 μg/ml. Each data point was tested for four times. *P < 0.05 when compared to the control value.

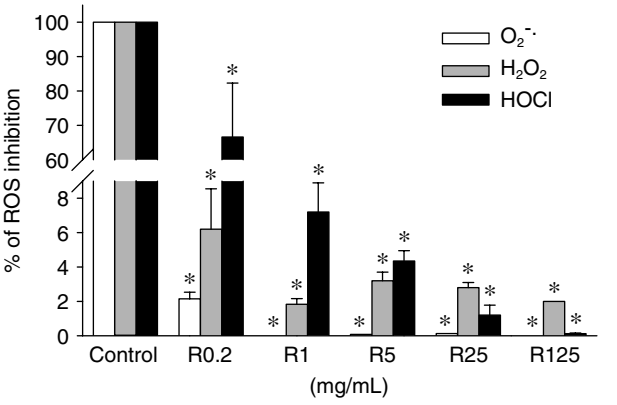

Fig. 3. The antioxidant activity against $O_2^{-\cdot}$, H_2O_2 and HOCl in different dosages of *Rhodiola rosea* extract with serial dilutions at 0 (R0), 0.2 (R0.2), 1 (R1), 5 (R5), 25 (R25) and 125 (R125) mg/ml. *P < 0.05 when compared to the control value.

Antioxidant Activity of the Rhodiola rosea *Extract*

We first compared the antioxidant activities of $O_2^{-\cdot}$, H_2O_2, HOCl of five ingredients, p-tyrosol, salidroside, rosin, rosavin and rosarin, of the *Rhodiola rosea* extract in 2, 20 and 200 μg/ml. As shown in Fig. 2, p-tyrosol, salidroside, rosin, rosavin and rosarin significantly (P < 0.05) inhibited xanthine- and xanthine oxidase-induced $O_2^{-\cdot}$ levels at 2, 20 and 200 μg/ml. The inhibited $O_2^{-\cdot}$ ability was most prominent in p-tyrosol and rosavin at 200 μg/ml. Also, p-tyrosol, salidroside, rosin, rosavin and rosarin significantly (P < 0.05) inhibited H_2O_2 and HOCl activities (Fig. 2) at 2, 20 and 200 μg/ml. The inhibited H_2O_2 ability was most prominent in p-tyrosol and rosin at 200 μg/ml. The inhibited HOCl ability was most prominent in p-tyrosol at 200 μg/ml.

The second part of study was to explore the antioxidant activity in different dosages of *Rhodiola rosea* extract with serial dilutions at 0, 0.2, 1, 5, 25 and 125 mg/mL. *Rhodiola rosea* extract significantly reduced xanthine- and xanthine oxidase-induced $O_2^{-\cdot}$, H_2O_2, and HOCl activities in a dose-dependent manner (Fig. 3).

To explore the effect of 90-min exhaustive swimming exercise on blood and tissue ROS production, we investigated lucigenin-dependent $O_2^{-\cdot}$ chemiluminescence counts in the blood and in homogenized liver and skeletal muscle. As shown in Fig. 4, after 90-min swimming, the level of lucigenin-dependent $O_2^{-\cdot}$ chemiluminescence counts was significantly (P < 0.05) increased in blood, liver and skeletal muscle. The enhancement in $O_2^{-\cdot}$ production was in an order of liver > skeletal muscle > blood. The plasma level of malonedialdehyde, a lipid peroxidation product, was also significantly (P < 0.05) increased.

Long-term *Rhodiola rosea* extract supplementation at different dosages for 4 weeks significantly decreased the swimming exercise-enhanced $O_2^{-\cdot}$ chemiluminescence counts in blood, liver and skeletal

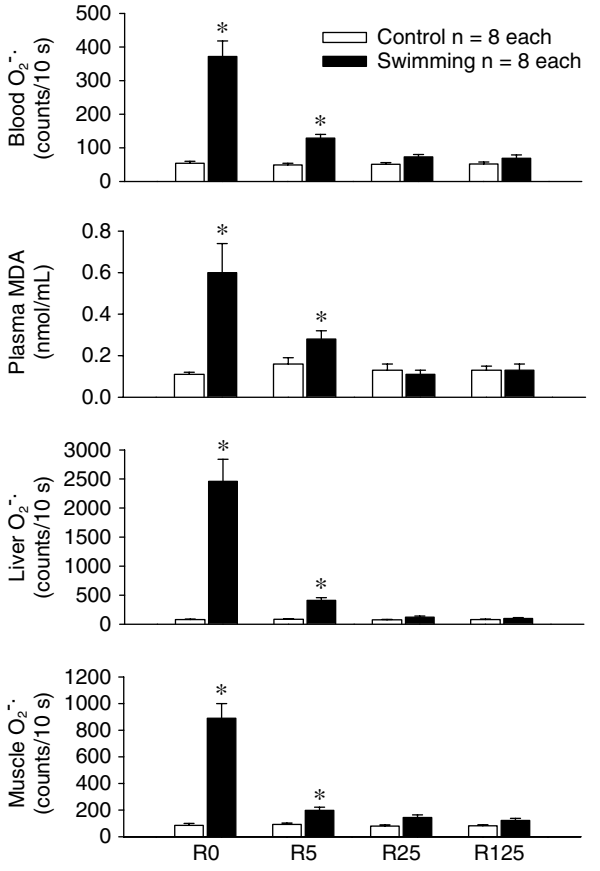

Fig. 4. Antioxidant effects by different dosages of *Rhodiola rosea* extract against the $O_2^{\cdot-}$ level in blood, liver and muscle, and plasma MDA in the rats subjected to 90-min swimming exercise that significantly increased the oxidative stress. The animals were fed *Rhodiola rosea* extract at 0 (R0), 5 (R5), 25 (R25) and 125 (R125) mg/ml in the drinking water for 4 weeks. **P* < 0.05 when compared to the control value.

muscle. The increased plasma malonedialdehyde level induced by swimming was also depressed by long-term *Rhodiola rosea* extract supplementation (Fig. 4).

Chronic Rhodiola rosea *Treatment Enhanced Hepatic Antioxidant Enzymes Expression*

We explored whether 4 weeks of *Rhodiola rosea* extract supplementation affected expression of antioxidant enzymes in the rat liver. As shown in Fig. 5, Mn SOD, Cu/Zn SOD and catalase were all expressed in the liver of R0 group. After 90 min of swimming exercise, the expression of Cu/Zn SOD, but not Mn SOD and catalase, was significantly decreased in the R0 group. Four weeks of administration of the *Rhodiola rosea* extract at 25 and 125 mg enhanced hepatic Mn SOD and Cu/Zn SOD expression before the exhaustive swimming exercise. Catalase

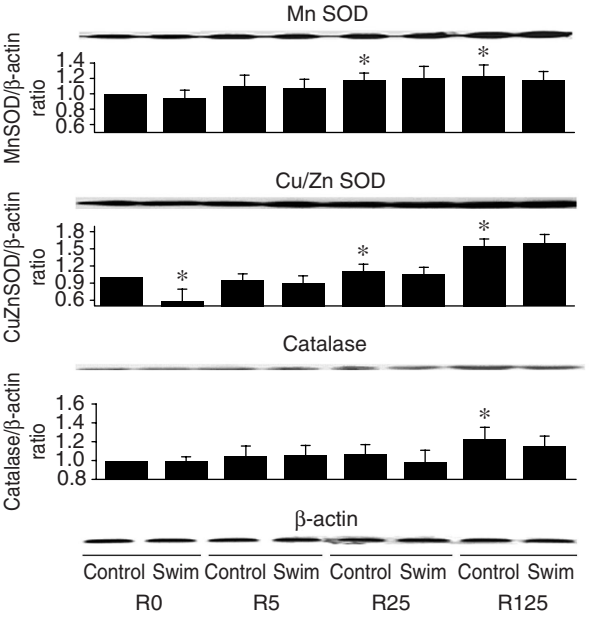

Fig. 5. Effects of different dosages of *Rhodiola rosea* extract supplement and swimming test on MnSOD, Cu/Zn SOD and catalase expression in the rat livers. R0, no *Rhodiola rosea* extract supplement; R5, 5 mg/ml; R25, 25 mg/ml; R125, 125 mg/ml. **P* < 0.05 when compared to the control value of R0 group.

expression was mildly enhanced at the dosage of 125 mg of *Rhodiola rosea* extract for four weeks. Four weeks of *Rhodiola rosea* extract supplementation attenuated the depression of Cu/Zn SOD after exhaustive swimming.

Rhodiola rosea *Extract Supplementation Increased Exercise Performance*

After 4 weeks of *Rhodiola rosea* extract supplementation at different dosages, 5% weight-loaded swimming was used to evaluate the exercise performance by the swimming time to fatigue. Treatment of four weeks of the *Rhodiola rosea* extract at 5, 25 and 125 mg significantly ($P < 0.05$) increased the swimming performance by 18.8%, 46.8% and 59.3% (n = 5 each).

Discussion

In the present study, we described a HPLC-MS technique to identify and quantify 4 major components, salidroside, rosin, rosarin and rosavin, in the *Rhodiola rosea* extract. The application of HPLC for determining hydrophilic extracts from *Rhodiola rosea* and Rodiola quadrifida has led to the identification of cinnamic alcohol, chlorogenic acid, rhodiooctanoside, rosiridin, rosavin and the phenolic compounds salidroside, rhodiolin and viridoside (20, 24, 38).

Our technique did not detect p-tyrosol in our *Rhodiola rosea* extract.

It is frequently stated, but poorly demonstrated, that exercise could have adverse effects related to inflammatory response, ROS production and accumulation of oxidative damage in several organs (3, 11, 16, 19, 33). Unlike the skeletal muscle, liver contains high levels of xanthine dehydrogenase; during exercise, xanthine dehydrogenase is converted to xanthine oxidase generating ROS and oxidative damage (31). In the present study, we found that 90-min exhaustive swimming exercise increased $O_2^{-\cdot}$ production in an order liver > skeletal muscle > blood indicating that liver is the most sensitive target organ. This result is agreement with a previous report with nuclear 8-hydroxydeoxyguanosine as the oxidative stress; the report showed that the nuclear 8-hydroxydeoxyguanosine content increased in liver, not in skeletal muscle and brain (33). The increased blood ROS after exhaustive exercise may contribute to the enhanced level of plasma malonedialdehyde, a lipid peroxidation product, found in our analysis. Increased blood ROS including $O_2^{-\cdot}$, H_2O_2 or HOCl may induce oxidation of phospholipid bilayers in the erythrocytes, increase phosphotidylcholine hydroperoxide and malonedialdehyde accumulation in the erythrocyte membrane and consequently contributes to hemolysis (13, 17, 18).

Therefore, an increased activity in the antioxidant defense mechanism or a decrease in oxidative stress may protect organs against oxidative damages. Exhaustive exercise on the treadmill resulted in significant increases in lipid peroxidation of skeletal muscle, liver and kidney inrats, and this was prevented by superoxide dismutase derivatives (30, 31). Previous studies have indicated that *Rhodiola rosea* extracts containing specific ingredients may have beneficial effects in enhancing exercise performance (1, 12, 23, 35) and in reducing ROS levels (2, 13, 21), but the major active component has not clearly been demonstrated. It has been reported that some *Rhodiola species* did not have antioxidant effects on hypoxemia and oxidative stress (39). The chemical composition and physiological properties of *Rhodiola species* are to a degree species-dependent although overlaps in constituents and physiological properties do exist in many *Rhodiola* species (20, 27, 28). *Rhodiola rosea* contains a range of biologically active substances including organic acids, flavonoids, tannins and phenolic glycosides. The stimulating and adaptogenic properties of *Rhodiola rosea* were originally attributed to two compounds isolated from its roots identified as p-tyrosol and the phenolic glycoside salidroside found in all studied species of *Rhodiola* (9). However, other active glycosides, including rosavin, rosin and rosarin, have not been found in the *Rhodiola* species examined (20). Because of this variation within the *Rhodiola* genus, verification of *Rhodiola rosea* by HPLC is dependent on the content of the rosavin, rosin and rosarin rather than salidroside and p-tyrosol (9, 14, 15, 20). Based on a comparative analysis, the most uniquely active chemical constituents are rosavin (the most active), rosin, rosarin, salidroside and its aglycon, p-tyrosol. In our data and in a previous study, p-tyrosol, salidroside, rosavin, rosin, and rosarin are all antioxidant substances (13, 20, 21, 42, 43), especially in p-tyrosol. In the present study, we have clearly identified that *Rhodiola rosea* extracts used in our study contained rosavin, rosin and rosarin, but did not contain p-tyrosol as analyzed by our HPLC-MS technique. A previous study has indicated that *Rhodiola rosea* roots contain 1.3 to 11.1 mg/g salidroside and 0.3 to 2.2 mg/g p-tyrosol (20). Our HPLC-MS data show that 13 mg/g salidroside, 3.3 mg/g rosin, 3.1 mg/g rosarin and 9.5 mg/g rosavin are found in our *Rhodiola rosea* extracts. We therefore suggest that high contents of rosin, rosarin and rosavin may exert a more efficient potential than p-tyrosol (not detected or too low in our *Rhodiola rosea* extract) in the reduction of swimming-induced oxidative stress.

Our results also showed that four weeks of *Rhodiola rosea* extract supplementation can up-regulate Mn SOD and Cu/Zn SOD protein expression in the rat liver. Although we did not know the detailed mechanisms involving *Rhodiola rosea*-enhanced antioxidant protein expression, direct scavenging ROS activity and enhancement of several antioxidant proteins of *Rhodiola rosea* extract may have provided hepatic protection against exhaustive exercise- induced oxidative stress in the liver. In the present study, we have clearly indicated that p-tyrosol, salidroside, rosin, rosarin and rosavin or the *Rhodiola rosea* extract can significantly and dose-dependently decreased $O_2^{-\cdot}$, H_2O_2 and HOCl activity *in vitro*. In addition, chronic *Rhodiola rosea* extract supplement significantly and dose-dependently reduced swimming exercise-enhanced plasma malonedialdehyde concentrations and $O_2^{-\cdot}$ levels in the liver, skeletal muscle and blood. In the present study, we evaluated all the responses after *Rhodiola rosea* extract supplementation. We did not determine the responses after terminating the supplementation. However, we suggest that upregulation of several antioxidant proteins in the liver may persist for several days until these proteins are degraded. This potential effect requires further investigation for the mode and kinetics of possible medicinal applications of the *Rhodiola rosea* extract.

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