Anti-Fatigue Effects of Fermented *Rhodiola rosea* Extract in Mice

Dong-Zhou Kang1, Hee-Do Hong2, Kyung-Im Kim3, and Sang Yoon Choi2,4

1College of Pharmacy, Yanbian University, Yanji 133-002, China
2Korea Food Research Institute, Gyeonggi 463-746, Korea
3Department of Hotel Culinary Arts & Food Service, Hyejeon College, Chungnam 350-702, Korea
4Korea University of Science and Technology, Daejeon 305-350, Korea

**ABSTRACT:** *Rhodiola rosea* is a perennial plant which grows in the alpine regions of Europe and Asia. Although the protective effects of *R. rosea* extract from fatigue due to exercise stress have been reported, studies on fermented *R. rosea* extract remain insufficient to date. Therefore, this study was conducted to examine the protective effects of fermented *R. rosea* extract against fatigue and exercise stress. As a result, fermented *R. rosea* extract was found to significantly increase swimming time, hepatic superoxide dismutase content, and serum lactate dehydrogenase in mice, while decreasing serum blood urea nitrogen content compared to *R. rosea* extract. Given the above results, it is considered that fermented *R. rosea* extract effectively protects against fatigue caused by strenuous exercise.

**Keywords:** anti-fatigue, exercise, fermentation, *Rhodiola rosea*

**INTRODUCTION**

Regular exercise is known to help protect and alleviate hypertension, stroke, cardiovascular disease, diabetes, hyperlipidemia, and cancer (1,2). However, strenuous exercise causes excessive production of reactive oxygen, lipid peroxides, and lactic acid, which can damage muscle tissues (3-5).

*Rhodiola rosea* (*Rhodiola sachalinensis* A. Bor) is a perennial plant of the genus *Rhodiola*, of the family Crassulaceae, and Angiospermae families, which grows in the alpine regions of Europe and Asia (6). *R. rosea*, whose ingredients include salidroside and tyrosol, has been used as an anti-pyretic, sedative, and astringent agent in folk remedies, and it has been reported as having anti-oxidative, anti-carcinogenic, antibacterial, anti-diabetic, and anti-hepatotoxic effects (7-10). Recently, ingestion of fermented products was identified as a healthy part of a functional diet. During fermentation, hydrolysis of glycosidic precursors occurred in ingredients of diet (11). Although several studies reported that *R. rosea* extract protects against fatigue and inhibit immediate-early gene expression in the hypothalamus of rats after forced swimming (12-14), there have been no studies on the anti-fatigue effects of fermented *R. rosea* extract.

In this study, the protective effects of *R. rosea* extract and fermented *R. rosea* extract on exercise-induced fatigue in mice were investigated by measuring their swimming time after oral administration of the extract. This is the first study to investigate the anti-fatigue activity of fermented *R. rosea* extract. The anti-fatigue effects were also identified by measuring the concentrations of superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), malondialdehyde (MDA), and hepatic glycogen in the liver, and serum blood urea nitrogen (BUN), lactate dehydrogenase (LDH), and lactic acid (LA) in the blood.

**MATERIALS AND METHODS**

**Sample preparation**

*R. rosea*, native to Baekdusan, was provided by Yanbian University (Yanbian, Jilin, China) in 2011. *R. rosea* was used in the experiment after being finely pulverized using a grinder (Cyclotec™ 1093, FOSS, Hillerød, Denmark). Briefly, 100 g of *R. rosea* powder was mixed with 1 L of distilled water and extracted at 90°C for 3 h, chilled at room temperature, and then centrifuged at 6,500 g for 20 min to obtain the supernatant. Acid-clay was added to the supernatant and stirred for 24 h at room temperature, and centrifuged again at 6,500 g for 20 min to...
obtain the supernatant for fermentation. The strain, Lactobacillus acidophilus KFRI 128, used for the fermentation of R. rosea, was obtained from the Korea Food Research Institute collection of food microorganisms (15). R. rosea extract was sterilized at 121°C for 15 min, and inoculated with 1% (1.0×10⁶ CFU/mL) activated L. acidophilus KFRI 128 and incubated at 37°C for 48 h. After fermentation, the fermented liquid was sterilized at 101°C for 20 min and freeze dried to obtain fermented R. rosea extract samples used in the experiments to evaluate R. rosea’s anti-fatigue activity.

HPLC analysis
For the analysis of p-tirosol, the major ingredient of R. rosea, 0.1 g of each sample was dissolved in 10 mL of methanol, and then filtered through a membrane filter (PP, 0.45 μm, Whatman International Ltd., Maidstone, UK) for HPLC (Jasco Co., Tokyo, Japan) analysis. The column was a Waters Sunfire™ C18 (4.6×250 mm i.d., 5 μL; Waters, Milford, MA, USA). The mobile phase was 20% methanol (v/v), at a flow rate of 1 mL/min and an absorption wavelength of 278 nm.

Grouping of animals
All animal experiments were conducted according to protocols approved by the Animal Ethics Committee in Yanbian University. One hundred twenty three-month-old male Kunming mice were used in this study. The animals were obtained from the laboratory animal center of Yanbian University. They were housed at 20~22°C, 40~60% humidity, with a 12 h light-dark cycle. The mice were selected after 3 swimming training sessions and divided into 3 groups of 36 mice: C (control group), RE (R. rosea extract group), and FRE (fermented R. rosea extract group). R. rosea extract was dissolved in normal saline. Mice in the treatment groups were orally administered 1.5 g/kg of R. rosea extract or fermented R. rosea extract daily for 15 days.

Swimming test
After 15 days of being orally administered 1.5 g/kg of R. rosea extract or fermented R. rosea extract, 12 mice per group were randomly selected for a swimming test. Thirty minutes after the final dose, a tin wire (7% of the mouse’s bodyweight) was attached to the tail of each mouse and its swimming time was measured in a 30 cm-deep swimming pool at 25±1°C. The swimming time was the point at which the mouse’s physical strength was exhausted and it could not float on the surface for more than 10 s after entering the swimming pool. After the times were collected, the mice were taken out of the water and pat dried using a paper towel before collecting their livers. The liver was rinsed with pre-cooled normal saline (0.86%), and then the samples were made into a 10% homogenate using a tissue grinder. The homogenate was centrifuged at 3,500 rpm for 15 min. The supernatant was collected to analyze SOD, GSH-Px activities and MDA content using a reagent kit from Nanjing Jiancheng Biotechnology Institute Co., Ltd. (Nanjing, China).

Blood and liver analysis
R. rosea extract (1.5 g/kg) or fermented R. rosea extract (1.5 g/kg) were orally administered for 15 days. Thirty minutes after the final oral administration, 24 mice per group were placed in a swimming pool for 90 min of forced swimming. Blood and liver samples were collected from 12 mice per group to analyze the serum LA, and liver glycogen, and LDH concentrations using a reagent kit from Nanjing Jiancheng Biotechnology Institute Co., Ltd. The remaining 12 mice in each group were rested for 60 min before blood samples were collected from the retro orbital sinus. Blood was centrifuged at 3,500 rpm for 8 min, and plasma was collected to measure BUN.

Statistical analysis
The results of the study were analyzed using SPSS18.0 software (SPSS Inc., Chicago, IL, USA). Data were expressed as the mean±standard deviation, and their statistical significance was tested by ANOVA analysis.

Fig. 1. HPLC chromatogram of R. rosea extract (A) and fermented R. rosea extract (B).
Differences were regarded as significant if $P<0.05$ or $P<0.01$ was attained.

**RESULTS AND DISCUSSION**

**Tyrosol content analysis**

It has been reported that the active ingredient of $R$. *rosea* is p-tirosyl, which exhibits excellent anti-oxidative properties (16,17). The p-tirosyl content in $R$. *rosea* extract and fermented $R$. *rosea* extract revealed that it was 190.5 mg% in the $R$. *rosea* extract and 714.0 mg% in the fermented $R$. *rosea* extract (Fig. 1). This result suggests that fermentation increased the p-tirosyl content. Therefore, fermented $R$. *rosea* showed anti-fatigue effects possibly via the anti-oxidative effect of p-tirosyl.

**Effects on bodyweight**

The effects of $R$. *rosea* extract and fermented $R$. *rosea* extract on the bodyweight of mice during the experiment are shown in Table 1. The results show that there were no statistical differences among the groups and that no notable adverse effects were observed.

**Effects on swimming time**

The effects of $R$. *rosea* extract and fermented $R$. *rosea* extract on the swimming time of mice are shown in Table 2. The groups treated with $R$. *rosea* extract and fermented $R$. *rosea* extract showed a statistically significant increase in their swimming time compared with the untreated group ($P<0.01$), with the increased swimming time effect being markedly higher in the group administrated the fermented $R$. *rosea* extract.

**Effects on hepatic SOD, GSH-Px activities, and MDA content after swimming**

Although reactive oxygen species are produced during normal metabolic processes and perform various physiological functions, excessive production of reactive oxygen by strenuous exercise causes peroxidation of the membrane lipids, inducing tissue damage and DNA damage in cells (18). Defensive systems for oxidative damage include the anti-oxidative enzymes SOD and GSH-Px (19,20). The effects of $R$. *rosea* extract and fermented $R$. *rosea* extract on hepatic SOD, GSH-Px activities and MDA content after swimming are shown in Table 3. $R$. *rosea* extract and fermented $R$. *rosea* extract increased the activities of SOD and GSH-Px while decreasing MDA content. In particular, the fermented $R$. *rosea* extract significantly increased SOD activity compared to the $R$. *rosea* extract.

**Effects on BUN and hepatic glycogen content**

BUN is protein metabolites. In the high intensity exercise for a long time, protein metabolism and amino acid decomposition are increased. There was a positive correlation between BUN level and fatigue degree (21). In addition, glycogen is an important energy material for movement. A large number of liver glycogen storage provides enough energy for muscle contraction. Increase glycogen content will increase exercise endurance (22, 23).

The effects of $R$. *rosea* extract and fermented $R$. *rosea* extract on hepatic glycogen content are shown in Table 4.

### Table 1. Effects on bodyweight (n=36)

| Groups | Doses (g/kg·d) | Bodyweight (g) |
|--------|----------------|----------------|
|        | Initial stage (0 d) | Intermediate stage (7 d) | Terminal stage (15 d) |
| C      | -               | 23.95±2.19       | 31.17±2.67          | 37.83±2.63        |
| RE     | 1.5             | 23.86±1.73       | 30.04±3.42          | 37.25±2.89        |
| FRE    | 1.5             | 23.78±2.43       | 30.95±3.90          | 35.50±3.87        |

*1C, control group; RE, $R$. *rosea* extract group; FRE, fermented $R$. *rosea* extract group.

### Table 2. Effects on swimming time exhaustion (n=12)

| Groups | Doses (g/kg·d) | Swimming time (s) |
|--------|----------------|-------------------|
| C      | -               | 98.2±23.20        |
| RE     | 1.5             | 160.1±19.63**     |
| FRE    | 1.5             | 197.2±41.85**     |

*1C, control group; RE, $R$. *rosea* extract group; FRE, fermented $R$. *rosea* extract group.

**P<0.01 compared with the control group; *P<0.05 compared with the RE group.

### Table 3. Effects on SOD, GSH-Px activities and MDA content (n=12)

| Groups | Doses (g/kg·d) | SOD (U/mgprot) | GSH-Px (U/mgprot) | MDA (nmol/mgprot) |
|--------|----------------|----------------|-------------------|-------------------|
| C      | -              | 49.85±9.53     | 39.97±12.57       | 0.50±0.13         |
| RE     | 1.5            | 65.30±19.70*   | 58.74±20.79*      | 0.28±0.73**       |
| FRE    | 1.5            | 81.38±13.73**  | 62.99±13.16**     | 0.22±0.06**       |

*1C, control group; RE, $R$. *rosea* extract group; FRE, fermented $R$. *rosea* extract group.

**SOD, superoxide dismutase; GSH-Px, glutathione peroxidase; MDA, malondialdehyde.

**P<0.05, ***P<0.01 compared with the control group; **P<0.05 compared with the RE group.

### Table 4. Effects on BUN and hepatic glycogen content (n=12)

| Groups | Doses (g/kg·d) | BUN (mmol/L) | Hepatic glycogen (mg/g) |
|--------|----------------|--------------|-------------------------|
| C      | -              | 8.56±1.35    | 5.71±2.39               |
| RE     | 1.5            | 7.78±0.76    | 5.76±1.78               |
| FRE    | 1.5            | 6.86±0.53*** | 6.87±2.64               |

*1C, control group; RE, $R$. *rosea* extract group; FRE, fermented $R$. *rosea* extract group.

**P<0.01 compared with the control group; **P<0.05 compared with the RE group.

**BUN, blood urea nitrogen.

**P<0.01 compared with the control group; **P<0.05 compared with the RE group.
extract on BUN and hepatic glycogen content after swimming are shown in Table 4. The BUN in the group administered fermented *R. rosea* extract was significantly lower than that in the control group and the *R. rosea* extract group. Meanwhile, no significant differences were observed in the hepatic glycogen content between the three groups, although it tended to be higher in the group administered fermented *R. rosea* extract.

### Effects on LDH activity and LA content

In the process of vigorous exercise for a long time, excess lactic acid accumulates in the body. Lactic acid can be used as an index of strenuous exercise, and fatigue (24). The effects of *R. rosea* extract and fermented *R. rosea* extract on LDH activity and LA content after swimming are shown in Table 5. Fermented *R. rosea* extract significantly increased LDH activity and LA content. In particular, LDH activity was significantly higher compared to the group administered *R. rosea* extract.

The biological effects of *R. rosea* have been widely investigated. However, no previous studies have investigated the biological effects of fermented *R. rosea* except for the report on tyrosinase inhibitory effect (25). The significance of this study is the potent anti-fatigue effects of fermented *R. rosea* in mice being reported for the first time. Treatment of fermented *R. rosea* extract significantly increased swimming time, SOD, GSH-Px activities, and LDH content. In addition, MDA, BUN, and LA content were reduced by fermented *R. rosea* extract treatment. The results indicated that fermentation can be considered as an effective process for increasing anti-fatigue effects of *R. rosea*. Our further study will investigate toxicity tests and clinical trials based on the results of this study.

### References

1. Bassuk SS, Manson JE. 2005. Epidemiological evidence for the role of physical activity in reducing risk of type 2 diabetes and cardiovascular disease. *J Appl Physiol* 99: 1193-1204.
2. Roberts CK, Barnard RJ. 2005. Effects of exercise and diet on chronic disease. *J Appl Physiol* 98: 3-30.
3. Jenkins RR. 1993. Exercise, oxidative stress, and antioxidants. *Int J Sport Nutr* 3: 356-375.
4. Hyun KY. 2009. An association of changed levels of inflammatory markers with hematological factors during one-time aerobic exercise in twenty-aged young men. *J Life Sci* 19: 1658-1665.
5. Moxnes JF, Sandbak Ø. 2012. The kinetics of lactate production and removal during whole-body exercise. *Theor Biol Med Model* 9: 7.
6. Chan SW. 2012. Panax ginseng, *Rhodiola rosea* and *Schisandra chinensis*. *Int J Food Sci Nutr* 63: 75-81.
7. Bae SJ. 2005. Anticarcinogenic and antioxidant effects of *Rhodiola sachalinsensis*. *J Korean Soc Food Sci Nutr* 34: 1302-1307.
8. Lee EJ, Im JS, Park CK, Jeon BS, Kyung JS. 2005. Anti-hepatotoxic activity of *Rhodiola sachalinsensis* roots. *Food Industry and Nutrition* 10(3): 37-42.
9. Park KU, Yoon JH, Kim YJ, Jeong CH, Park CK, Song WS, Seo KI. 2005. Biological activity of the fractions extracted from *Rhodiola damalosa*. *Korean J Food Preserv* 12: 496-500.
10. Li HB, Ge YK, Zheng XX, Zhang L. 2008. Salidroside stimulated glucose uptake in skeletal muscle cells by activating AMP-activated protein kinase. *Eur J Pharmacol* 588: 165-169.
11. Ugiano M, Bartsowky EJ, McCarthy J, Moio L, Henschke PA. 2006. Hydrolysis and transformation of grape glycosidically bound volatile compounds during fermentation with three *Saccharomyces* yeast strains. *J Agric Food Chem* 54: 6322-6331.
12. Ryu SH, Kim SY, Jung HS, Sohn NW, Sohn YJ. 2008. Effects of *Rhodiola rosea* on anti-fatigue and hypothalamic IEGs expressions of forced swimming rats. *Kor J Herbolology* 23: 9-19.
13. Jung HS, Kim EY, Shim ES, Lee HS, Moon EJ, Jin ZH. 2008. Effects of *Rhodiola rosea* (KH101) on anti-fatigue in forced swimming rats. *Korean J Orient Int Med* 29: 922-938.
14. Lee FT, Kuo TY, Liou SY, Chien CT. 2009. Chronic *Rhodiola rosea* extract supplementation enforces exhaustive swimming tolerance. *Am J Chin Med* 37: 557-572.
15. Yang Hj, Weon Jb, Lee Bh, Ma Cj. 2011. The alteration of components in the fermented *Hwangryunhaedok-tang* and its neuroprotective activity. *Pharmacogn Mag* 7: 207-212.
16. Huang Sc, Lee Ft, Kuo Ty, Yang Jh, Chien Ct. 2009. Attenuation of long-term *Rhodiola rosea* supplementation on exhaustive swimming-evoked oxidative stress in the rat. *Chin J Physiol* 52: 316-324.
17. García-Padial M, Martínez-Ohárriz MC, Navarro-Blasco I, Zornoza A. 2013. The role of cyclodextrins in ORAC-fluorescence assays. Antioxidant capacity of tyrosol and caffeic acid with hydroxypropyl-β-cyclodextrin. *J Agric Food Chem* 61: 12260-12264.
18. Djordjević VB. 2004. Free radicals in cell biology. *Int Rev Cytol* 237: 57-89.
19. Irshad M, Chaudhuri PS. 2002. Oxidant-antioxidant system: role and significance in human body. *Indian J Exp Biol* 40: 1233-1239.
20. Urso ML, Clarkson PM. 2003. Oxidative stress, exercise, and antioxidant supplementation. *Toxicology* 15: 41-54.
21. Huang WC, Chiu WC, Chuang HL, Tang DW, Lee ZM, Wei L, Chen FA, Huang CC. 2015. Effect of curcumin supplementation on physiological fatigue and physical performance in mice. *Nutrients* 7: 905-921.

### Table 5. Effects on LDH and LH content  

(n=12)

| Groups | Doses (g/kg·d) | LDH (U/gprot) | LA (mmol/L) |
|--------|---------------|--------------|-------------|
| C      | -             | 1,107.6±84.83| 10.32±2.74  |
| RE     | 1.5           | 1,249.5±137.88| 8.65±2.99  |
| FRE    | 1.5           | 1,430.7±201.20***| 6.88±2.31***|

**P<0.01 compared with the control group; #P<0.05 compared with the RE group.

### Author Disclosure Statement

The authors declare no conflict of interest.
22. Liu J, Du C, Wang Y, Yu Z. 2015. Anti-fatigue activities of polysaccharides extracted from Hericium erinaceus. *Exp Ther Med* 9: 483-487.

23. Anand T, Phani Kumar G, Pandareesh MD, Swamy MS, Khanum F, Bawa AS. 2012. Effect of bacoside extract from *Bacopa monniera* on physical fatigue induced by forced swimming. *Phytother Res* 26: 587-593.

24. Gibson H, Edwards RH. 1985. Muscular exercise and fatigue. *Sports Med* 2: 120-132.

25. Chen YS, Liou HC, Chan CF. 2013. Tyrosinase inhibitory effect and antioxidative activities of fermented and ethanol extracts of *Rhodiola rosea* and *Lonicera japonica*. *Sci World J* 2013: 612739.