

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

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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 antioxidative, 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

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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 culture collection of food microorganisms (15). *R. rosea* extract was sterilized at 121°C for 15 min, and inoculated with 1% (1.0×10^6 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*-tyrosol, 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 μ m, Whatman International Ltd., Maidstone, UK) for HPLC (Jasco Co., Tokyo, Japan) analysis. The column was a Waters Sunfire™ C₁₈ (4.6 \times 250 mm i.d., 5 μ L; 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 \pm 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 \pm standard deviation, and their statistical significance was tested by ANOVA analysis.

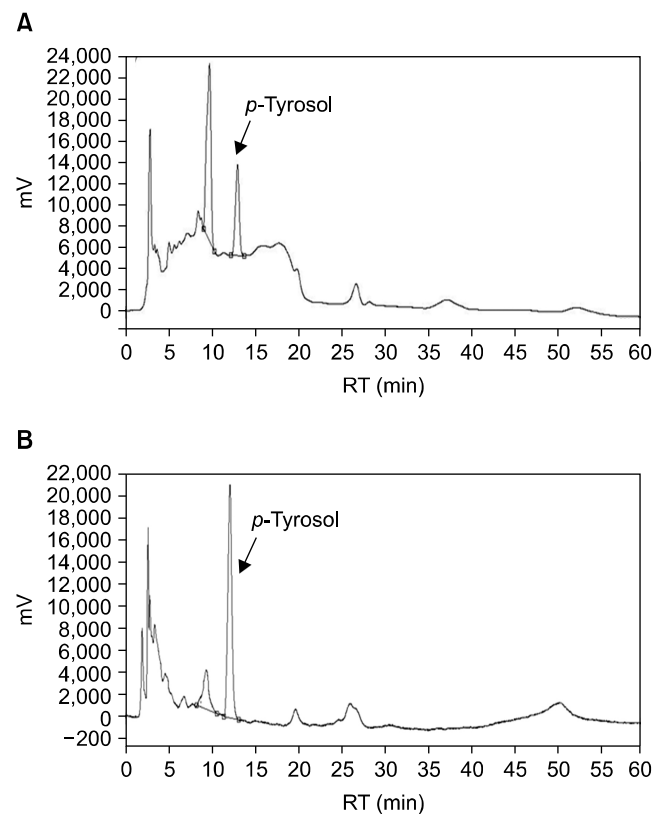


Fig. 1. HPLC chromatogram of *R. rosea* extract (A) and fermented *R. rosea* extract (B).

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*-tyrosol, which exhibits excellent anti-oxidative properties (16,17). The *p*-tyrosol 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*-tyrosol content. Therefore, fermented *R. rosea* showed anti-fatigue effects possibly via the anti-oxidative effect of *p*-tyrosol.

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 administered the fermented *R. rosea* extract.

Table 1. Effects on bodyweight (n=36)

Groups ¹⁾	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

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

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

Groups ¹⁾	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***

¹⁾C, 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.

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*

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

Groups ¹⁾	Doses (g/kg·d)	SOD ²⁾ (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**

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

²⁾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 ¹⁾	Doses (g/kg·d)	BUN ²⁾ (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

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

²⁾BUN, blood urea nitrogen.

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

Table 5. Effects on LDH and LA content (n=12)

Groups ¹⁾	Doses (g/kg·d)	LDH (U/gprot)	LA (mmol/L)
C	-	1,107.64±84.83	10.32±2.74
RE	1.5	1,249.53±137.88	8.65±2.99
FRE	1.5	1,430.78±201.20 ^{***#}	6.88±2.31 ^{**}

¹⁾C, 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.

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.

AUTHOR DISCLOSURE STATEMENT

The authors declare no conflict of interest.

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