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Note

Fatigue-Alleviating Effect on Mice of an Ethanolic Extract from *Rubus coreanus*

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The fatigue-alleviating effects on mice of *Rubus coreanus* were investigated by using an adjustable-current water pool. The mice were exhaustively exercised for 2 consecutive days, and those administered with the 80% ethanol extract (RCE) of *R. coreanus* displayed a lower reduction (20%) in swimming time on day 2 than the control group (41% reduction). RCE significantly prevented the depletion of hepatic antioxidants during exercise-induced fatigue. These results suggest that RCE alleviated fatigue by elevating the antioxidative potential.

Key words: *Rubus coreanus*; fatigue; exercise; blood lactate; antioxidant

Rubus coreanus is a type of red raspberry that grows wild in Korea, Japan, and China. The fruit, known as 'Bokbunja' in Korea, has long been used in traditional alternative medicines. The fruit, which is rich in sugars, organic acids and several vitamins, and includes various antioxidants, has shown promise in reducing the risk of such diseases as asthma and allergy, and is effective in dampening inflammation and oxidation.[1,2)] However, data supporting the antioxidative effect of *R. coreanus* based its anti-fatigue function are lacking.

The excessive production of reactive oxygen species (ROS) causes muscle fatigue, suggesting that a high level of oxidative damage increases fatigue.[3)] The superoxide anion radical ($O_2^{\cdot -}$) and hydrogen peroxide (H_2O_2) are generated as metabolic intermediates in the presence of oxygen. These can lead to a disturbance in the homeostasis of the endogenous antioxidative defense systems in the body, resulting in the development of fatigue.[4)] Antioxidants provided as a nutritional regime, which are substances delaying or preventing the oxidation of inter- or intra-cellular oxidizable substrates, may help reduce the degree of fatigue by protecting against oxidative stress.[5)]

Prolonged exercise leads to oxidative stress and fatigue. The present study, which was undertaken to investigate the fatigue alleviating effect of an extract derived from *R. coreanus* together with changes in the antioxidative defense systems of the liver, used mice which were forced to exhaustively exercise in an adjustable-current swimming pool to determine the relevant factors for fatigue and antioxidation.

Ripe fruit of *R. coreanus* (1 kg) was collected from Kwangyang city (Jeollanamdo, Korea) in 2008. The fruit was extracted twice with a total of 20 L of 80% ethanol or water by Soxhlet apparatus for 3 h at standard atmospheric pressure. Each extracted solution was then filtered, concentrated and lyophilized, respectively yielding an organosoluble extract (RCE, 434 g) and hydrosoluble extract (RCW, 348 g). Both extracts contained a similar level of carbohydrate (RCE: $34.3 \pm 4.1\%$, RCW: $31.9 \pm 1.4\%$). These extracts were stored at $-20\,^{\circ}C$ until needed.

Four-week-old male ICR mice (16 ± 2 g of body weight, b.w.) were purchased from Orient Bio (Seongnam, Korea) and housed in cages under automatically controlled environmental conditions of temperature ($22 \pm 2\,^{\circ}C$), humidity (about 60%), and lighting (12:12-h light–dark cycle). The mice were provided with a commercial pelleted feed (AIN-76A rodent purified diet, Orient Bio) and water *ad libitum*. The Institutional Animal Care and Use Committee of Chonnam National University approved the protocol for the animal study, and the animals were cared for in accordance with the "Guidelines for Animal Experiments" established by the university.

The anti-fatigue test was conducted on the mice that had been assigned to three groups ($n = 10$ per group) with similar mean swimming capacity, as determined in an adjustable-current water pool ($90 \times 45 \times 45$ cm with water filled to a depth of 38 cm) at a flow rate of 8 L/min:[4,6)] the control group (saline), RCE-administered group (1 g/kg b.w.), and RCW-administered group

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Abbreviations: CAT, catalase; DPPH, 1,1-diphenyl-2-picrylhydrazyl; GST, glutathione-*S*-transferase; ROS, reactive oxygen species; GSH, reduced glutathione

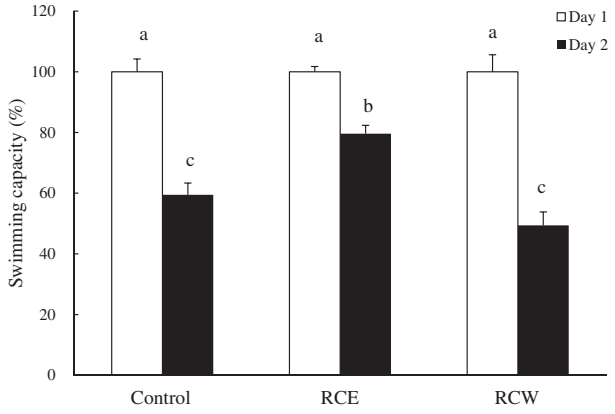

Fig. 1. Anti-Fatigue Effects of *Rubus coreanus* Extracts on Consecutive Swimming Exercise in Mice.

RCE is the 80% ethanol extract of *R. coreanus*, and RCW is the hot water extract of *R. coreanus*. The mice were given the vehicle (control) or sample (1 g/kg of body weight) before exhaustive exercise. Data express the mean ± SE for 10 mice in each group. Data were analyzed by a two-way analysis of variance (ANOVA) and subsequent Duncan's multiple-range test for pairwise comparisons ($p < 0.05$). Different letters above the bar are statistically different.

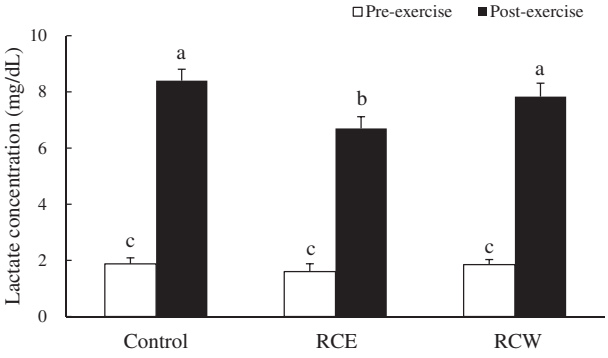

Fig. 2. Effects of the *Rubus coreanus* Extracts on the Change in Blood Lactate Level between the Pre- and Post-Exercise Mice.

RCE is the 80% ethanol extract of *R. coreanus*, and RCW is the hot water extract of *R. coreanus*. The mice were given the vehicle (control) or sample (1 g/kg of body weight) before exhaustive exercise. The lactate levels in the mice were measured before and after exercise. Data express the mean ± SE for 10 mice in each group. Data were analyzed by a two-way analysis of variance (ANOVA) and subsequent Duncan's multiple-range test for pairwise comparisons ($p < 0.05$). Different letters above the bar are statistically different.

(1 g/kg b.w.). The mice were forced to swim for 30 min after the vehicle or sample treatment by gastric intubation on both days 1 and 2, and the swimming time to exhaustion was determined. The mice were assessed to be exhausted when they failed to rise to the surface of the water to breathe within a 7-s period. A period of longer than 7 s frequently resulted in drowning, while a period of less than 5 s reduced the reproducibility of the test.[6] The difference between the maximum swimming times on the days 1 and 2 was measured to evaluate the anti-fatigue effect on consecutive swimming exercise. The animals were fasted for 12 h before the experiment, and water was provided *ad libitum*.

Thirty minutes after administering RCE and RCW, the levels of liver glycogen and blood glucose were determined to investigate the influence of the extract on the energy deficit during prolonged exercise. Additional ICR male mice were randomly assigned to two groups (n = 10) with similar body weight for these experiments. The mice treated with RCE and RCW respectively possessed 3.86 ± 0.37 and 3.46 ± 0.17 mg glycogen/g tissue, suggesting no statistical difference. The blood glucose levels of mice treated with RCE and RCW were also similar (133.3 ± 3.1 mg/dL and 136.6 ± 10.6 mg/dL). As shown in Fig. 1, the RCE-treated mice exhibited a smaller decrease in swimming time (20%) than the control and RCW groups (41% and 51%, respectively) on day 2. Continuous exercise usually causes fatigue which can be inhibited by nutritional supplements to suppress fatigue relevant factors.[7,8] The relatively small decrease in swimming capacity after consecutive exercise that occurred after the provision of RCE indicates that the prolonged time to fatigue was due mainly to the effect of organosolubles in the *R. coreanus* extract. RCE was therefore identified as possessing a fatigue-alleviating effect.

The blood lactate level is one of fatigue-relevant factors measured before and after exercise.[8] Many organs, especially the liver and skeletal muscle, help remove lactate from the blood.[9,10] However, intense exercise can increase lactate production to a point that exceeds the rate of lactate removal, this condition resulting in fatigue. The RCE-administered mice showed a significantly smaller increase in blood lactate level than the control and RCW-administered mice, as measured before and after exercise (Fig. 2). These results indicate that the RCE-treated mice experienced a reduction in lactate production and/or an increased rate of lactate removal.

The hepatic antioxidative activity assays were conducted on liver collected after sacrificing the mice at the end of the swimming test that was homogenated in a 50 mM phosphate buffer. The suspension was centrifuged at 13,000 × *g* for 15 min at 4 °C, and the supernatant was used for the measurements. The activity of catalase (CAT) was determined as described by Aebi,[11] and the hepatic glutathione-*S*-transferase (GST) activity was assayed by the method of Habig and Jakoby.[12] The level of reduced glutathione (GSH), a key intracellular antioxidant, was measured by the method of Akerboom and Sies,[13] and the amount of protein was measured by using the Bradford assay.[14] The antioxidative effects of the extracts from *R. coreanus* on the fatigue-induced depletion of hepatic antioxidants are summarized in Table 1. CAT activity in the exercised control group was significantly lower than that in the pre-exercised control group. However, the pretreatment with RCE prevented the decrease in CAT activity. In comparison with the exercised control group, the CAT activity of the mice administered RCE was higher by 16%, while no statistical difference was apparent in the mice pretreated with RCW. Consistent with the CAT activity, the administration of RCE significantly protected against the reduction in GST activity and GSH depletion. The difference in intracellular antioxidative activities among the groups suggested the role of oxidative stress in fatigue. Fatigue can develop due to an imbalance between ROS production and the antioxidative defense system.[5] The latter plays an important role in the body's protection against oxidative stress. Inactivation of

Table 1. Changes in the Hepatic Antioxidative Activities Due to the Administration of *Rubus coreanus* Extracts after Consecutive Swimming Exercise[1]

Group[2]	CAT[3] (U/mg protein)	GST[3] (U/mg protein)	GSH[3] (μmoles/mg protein)
Pre-exercised control	66.09 ± 1.94^{a5}	50.05 ± 1.08^{a}	3.32 ± 0.07^{a}
Exercised control	56.70 ± 1.33^{b}	26.07 ± 0.67^{b}	3.20 ± 0.06^{b}
RCE-administered[4]	67.30 ± 1.42^{a}	49.07 ± 0.84^{a}	3.37 ± 0.08^{a}
RCW-administered[4]	58.02 ± 0.72^{b}	33.54 ± 1.22^{c}	3.10 ± 0.03^{c}

[1]Data are expressed by the mean ± SE for 10 mice.
[2]The mice were given vehicle (control) or sample (1 g/kg of body weight) before exhaustive exercise. The antioxidative activities of pre-exercised control group were measured before exercise.
[3]CAT, catalase; GST, glutathione-*S*-transferase; GSH, reduced glutathione.
[4]RCE, 80% ethanol extract of *R. coreanus*; RCW, hot water extract of *R. coreanus*.
[5]Values with different letters in a column are statistically different by Duncan's multiple-range test ($p < 0.05$).

hepatic antioxidants is caused by exposure to ROS.[15] In the present study, the level of GSH and the activities of CAT and GST were higher after pretreating with RCE in the fatigue-induced mice than in the exercised control and RCW-administered groups. When the antioxidative potential of RCE and RCW was assessed by the 1,1-diphenyl-2-picrylhydrazyl (DPPH)- and ABTs-radical scavenging assays, RCE showed relatively higher DPPH- and ABTs-radical scavenging activities than RCW (data not shown), suggesting RCE to be a potentially useful radical scavenger. These combined findings indicate that ROS produced by β-oxidation during the utilization of energy[16] and the increased oxygen consumption in the exhaustive exercise model could be scavenged by RCE supplementation to subsequently protect against the inactivation of hepatic antioxidants. RCE might therefore provide the necessary protection against muscle damage and thereby alleviate exercise-induced fatigue.

In summary, oxidative stress causes fatigue which can be ameliorated by antioxidant supplementation. The administration of an ethanolic extract from *R. coreanus*, which possesses antioxidative potential, could stabilize the intracellular antioxidative defense system in mice, and thereby reduce fatigue. This suggests that the alleviation of fatigue by RCE might have been due, in part, to its protective effect against oxidative stress. To the best of our knowledge, the present study is the first to investigate the effect of the antioxidative activity of *R. coreanus* on fatigue. The results indicate that the development of dietary supplementation using *R. coreanus* could be helpful in protecting against fatigue mediated by oxidative stress. Further research is currently underway to isolate and identify the structure of the anti-fatigue compound in *R. coreanus*.

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