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Research Note

Oral Administration of a Hot Water Extract of the Softshell Turtle (*Trionyx sinensis*) Improves Exercise Performance

Dicky Harwanto[1,2], Gong-Hyeon Lee[1], Sun-Mee Park[3], Jae-Suk Choi[4], Mi-Ryung Kim[4], and Yong-Ki Hong[1]

[1]Department of Biotechnology, [3]Institute of Marine Biotechnology, Pukyong National University, Busan 608-737, Korea
[2]Faculty of Fisheries and Marine Science, Diponegoro University, Semarang 50275, Indonesia
[4]Department of Bio-Food Materials, Silla University, Busan 617-736, Korea

ABSTRACT: Freshwater softshell turtle (*Trionyx sinensis*) extract has been used traditionally as a tonic soup, and to recover from physical fatigue. To support these claims, the forelimb grip strength of mice was measured after feeding a softshell turtle extract for 7 days. The *T. sinensis* extract significantly increased the grip strength to 1.25±0.07 N ($P<0.01$), which is 16.8% higher than the force on day 0. After exercising, the blood glucose levels in extract-fed mice were 202% higher and urea levels were 73% lower, which were both significantly different than the levels observed after control treatment. Lactate dehydrogenase was significantly higher by 314%, and glutathione peroxidase increased by 165%. In addition, the obesity markers, serum triglyceride and cholesterol, decreased to 62% and 49%, respectively, after mice were fed the extract. These data show that the *T. sinensis* extract provided more energy for forelimb exercise, prevented protein catabolism and muscle fatigue, and decreased the oxidative stress caused by an exhaustive workout.

Keywords: anti-fatigue, force strength grip, softshell turtle, *Trionyx sinensis*

INTRODUCTION

The effectiveness of various natural food products in improving exercise performance is of interest to sports and healthcare industries. During physical exercise, the contracting muscles generate force or power, metabolites, and heat, which ultimately induce fatigue and exhaustion (1). Fatigue is categorized as physical or emotional exhaustion resulting in negative effects on physical endurance capacity, work performance, and exercise intensity. Hard work or intense exercise can lead to the production and accumulation of excess reactive free radicals, which results in oxidative stress injury to the body (2). Several natural food resources have been studied as supplements to improve exercise performance and recovery from physical fatigue (3-5). Most of these are polysaccharides or terpenoids from herbal sources. Nevertheless, few studies have examined agents from aquatic organisms that can improve exercise performance. Traditionally, the water-soluble extract of the freshwater softshell turtle, *Trionyx sinensis*, has been used as a tonic soup, and to recover from physical fatigue (6). *T. sinensis* is the most common turtle species raised in Asia, and has immense aquaculture potential. In 2012, the production of softshell turtles by inland water fisheries in Korea was 250 metric tons (wet weight), worth 12 million US dollars (7). To evaluate the tonic activity of *T. sinensis*, changes in forelimb grip strength and anti-fatigue activity were measured in mice fed the hot water extract prepared in the traditional way.

MATERIALS AND METHODS

Extract preparation and reagents

Fresh softshell turtle (*T. sinensis*) bodies were obtained from the Gunwi Zara Aquaculture Farm (Ubo, Gunwi, Korea). A voucher specimen is deposited in the laboratory of Dr. Mi-Ryung Kim (Silla University, Busan, Korea). Whole bodies (2 kg) were extracted with boiling water (20 L) for 25 h. After removing the residue and oily layer by centrifugation at 3,000 *g* for 10 min, the aqueous portion was concentrated using a rotary evaporator to obtain the extract (220 g; yield 11%). The extract was adjusted to 88 mg/mL (or 50 mg/mL as protein) with distilled water. Bovine serum albumin was used as the standard when determining the protein content (8). Assay kits for determining glucose (AM201-K),

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Correspondence to Yong-Ki Hong, Tel: +82-51-629-5862, E-mail: ykhong@pknu.ac.kr

urea (AM165-K), lactate dehydrogenase (LDH; LDH-LQ), triglyceride (AM157S-K), and cholesterol (AM202-K) were purchased from Asan Pharmaceutical (Seoul, Korea). The glutathione peroxidase (GPx) kit (K762-100) was purchased from BioVision (Milpitas, CA, USA). The other reagents used in this study were of analytical grade.

Forelimb grip strength

More than 48 ICR mice (6~8 weeks old) weighing 23~27 g were purchased from Hyochang Science (Daegu, Korea). Mice were kept in a controlled environment at 24±1°C under a 12 h light/12 h dark cycle at 65% humidity, with a maximum of five animals per cage. The animals were fed standard animal pellets (Formula™ M07; FeedLab, Guri, Korea) and water *ad libitum*. The mice were treated in compliance with current laws and guiding principles for the care and use of laboratory animals approved by the Animal Ethics Committee of Pukyong National University (Busan, Korea). The ethics committee approved this study under protocol AEC-201405. The mice (n=12 per group per test) were orally administered the extract once a day for 7 days with each 1 μL/g body weight, and then underwent body weight and grip strength measurements 1 h after each administration. The vehicle group was orally administered saline as a control. A low-force testing system (Model-RX-10, Aikoh Engineering Co., Osaka, Japan) was used to measure the forelimb grip strength of the mice. The amount of tensile force generated by each mouse was measured using a force transducer with a rectangular 4×5 cm^2 metal net (9). The mouse was grasped at the base of the tail and pulled slightly backward by the tail while the two forelimbs gripped the metal net, which caused a counter-pull. The grip strength meter recorded the grasping force in Newtons (N). Before the experiment, the mice were trained to perform this procedure for 3 days. Grip strength (N) and body weight (g) were measured three times: before treatment (day 0); 1 h after treatment on the third day (day 3); and 1 h after the seventh day (day 7). Each mouse was subjected to three grip trials with at least a 1 min rest between trials. The maximum force exerted by the mouse counter-pull was recorded as the forelimb grip strength.

Biochemical assays

Thirty min after the last grip strength trial, blood samples were collected from mice. The mice were anesthetized with Zoletil 50 (Virbac, Carros, France; 10 mg/kg, intramuscular), and then blood was drawn using the facial vein technique (10). After clotting the blood for 10 min, it was centrifuged at 3,000 *g* for 15 min to obtain serum. The serum levels of glucose, urea, LDH, GPx, triglyceride, and total cholesterol were determined by glucose oxidase, urease-indophenol, lactate substrate, GPx colorimetric assay, lipoprotein lipase, and cholesterol esterase-oxidase methods, respectively, using an UV/Vis spectrophotometer (Optizen 2120UV, Mecasys Co., Daejeon, Korea).

Statistical analysis

Statistical analysis was performed using the Student's *t*-test. All of the animal experiments were done with at least 12 mice per group. Data are reported as the means ±standard deviation (SD).

RESULTS AND DISCUSSION

The forelimb grip strength of the mice fed the softshell turtle extract averaged 1.19±0.06 N ($P<0.01$) and 1.25±0.07 N ($P<0.01$) on days 3 and 7, respectively (Fig. 1A), which represented an increase of 6.9% and 16.8%, compared to the force on day 0 (1.04±0.06 N). Control treatment increased the strength by −0.2% and 0.3% on days 3 and 7, respectively. Grip strength significantly increased with the time that the mice were fed the extract. The body weights of mice fed the extract

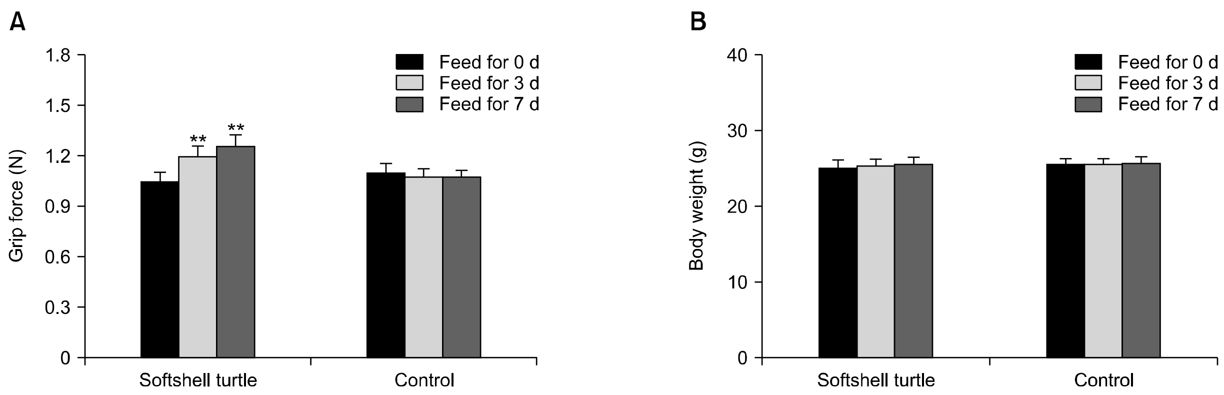

Fig. 1. Effects of the softshell turtle extract on forelimb grip strength (A) and body weight (B) in mice. Values are the means±SD (n=12). $^{**}P<0.01$ compared to day 0.

averaged 25.3±1.1 g and 25.6±1.0 g after 3 and 7 days, respectively (Fig. 1B), which were marginally increased by 1.0% and 2.3% compared to day 0 (25.0±1.1 g). With the control treatment, body weight increased by −0.2% and 0.3% after 3 and 7 days, respectively compared to day 0. Therefore, the *T. sinensis* extract significantly increased grip strength, and resulted in a marginal increase in body weight.

To investigate the anti-fatigue or fatigue recovery effects of the *T. sinensis* extract, blood samples for biochemical analysis were collected at 30 min after trials on day 7.

In the extract-fed mice, serum glucose levels averaged 9.9±1.0 mmol/L, which was 202% higher ($P<0.01$) than that in the control mice. Thus, presence of the extract led to the provision of more blood glucose as energy source for the forelimb exercise and fatigue recovery. In addition, urea level averaged 3.5±0.8 mmol/L, which was 73% lower ($P<0.01$) than that in control mice (Table 1). The *T. sinensis* extract may prevent the release of blood urea nitrogen via protein catabolism. The activity of LDH in the mice fed the extract averaged 2,275.4±697.3 U/L ($P<0.01$), which was 314% higher than that of the control group. As an antioxidant enzyme, the activity of GPx in the extract-fed mice was 514.7±153.8 mU/mL ($P<0.01$), which was 165% higher than that of the control group. Therefore, the *T. sinensis* extract decreased the oxidative stress caused by an exhaustive workout. Serum triglyceride and total cholesterol levels were 0.8±0.3 mmol/L ($P<0.05$) and 1.9±0.4 mmol/L ($P<0.01$), respectively. Even after being fed the softshell turtle extract for 7 days, the mice did not accumulate triglyceride and cholesterol. Rather, the triglyceride and cholesterol levels decreased by 62% and 49%, respectively, upon feeding of the extract. This may be caused by the increased breakdown of triglycerides to free fatty acids which are subsequently oxidized.

Among biochemical parameters, the homeostasis of blood serum glucose is very important for improving exercise performance. Training frequently leads to hypoglycemia, which necessitates a higher rate of metabolism to handle the increasing energy demands. When hypoglycemia is prolonged, it can overwhelm the glycogen storage and reduce blood glucose (11,12). Therefore, blood glucose levels reflect the grade and speed of fatigue development. A modest workout begins with an increase in aerobic muscle activity; however, rigorous exercise triggers anaerobic metabolism, leading to a decrease in blood glucose and the accumulation of lactic acid (13). When mice were fed the *T. sinensis* extract, blood glucose levels were maintained at twice the level of the control group. Thus, the extract appeared to facilitate the release of glucose from glycogen and increase LDH activity, allowing the animals to regain energy after a workout. Similar results were obtained when extracts of a fungus (4) or fenugreek (14) were used to improve exercise performance and decrease physical fatigue. Aging (15) and overloading muscle for extended periods (16) decrease LDH enzyme activity. Even though the mechanism is not clear, the *T. sinensis* extract might also prevent the decreased muscle functional capacity that accompanies aging or a workout by enhancing the enzyme activity.

Blood urea nitrogen is another blood biochemical index associated with physical fatigue. It is formed in the liver as the end product of protein and amino acid metabolism. The urea level reflects kidney function, although many other factors affect its level, including protein breakdown, dehydration, stress, and fatigue (17). After prolonged physical activity, blood urea levels normally increase. Upon giving mice the *T. sinensis* extract, blood urea levels remained as low as 73% compared to the control group. This suggests that the turtle extract prevents protein catabolism, even during intense exercise, which reflects enhanced endurance. Exhaustive heavy exercise generates free oxygen radicals (2).

The activity of GPx, a major antioxidant enzyme, was examined to determine the anti-fatigue effect of the *T. sinensis* extract. The higher enzyme activity in the extract-fed mice explains that the extract decreases oxidative stress by counteracting possible harmful aspects of radicals associated with oxidative stress and exhaustive workouts, similar to phenolic compounds (18) and some dietary antioxidants (19). The *T. sinensis* extract enhanced the exercise capacity of mice, perhaps in part, by increasing fat utilization via decreasing triglyceride lev-

Table 1. Effects of the softshell turtle (*T. sinensis*) extract on serum glucose and urea levels, LDH and GPx activities, triglyceride, and total cholesterol after the forelimb grip exercise

	Glucose (mmol/L)	Urea (mmol/L)	LDH (U/L)	GPx (mU/mL)	Triglyceride (mmol/L)	Cholesterol (mmol/L)
Control	4.9±1.5	4.8±0.9	725.1±326.3	312.7±44.4	1.3±0.4	3.9±1.3
Softshell turtle	9.9±1.0**	3.5±0.8**	2,275.4±697.3**	514.7±153.8**	0.8±0.3*	1.9±0.4**
Relative activity (%)[1)]	202	73	314	165	62	49

LDH, lactate dehydrogenase; GPx, glutathione peroxidase.
Values are the means±SD (n=12). *$P<0.05$ and **$P<0.01$ compared to control.
[1)]Relative activities are expressed as percentages of the values against the control group.

els (to 62%) in blood. Although the mechanisms by which the extract decreased triglyceride and cholesterol levels are unclear, the effect might be beneficial during extended exercise, because better utilization of triglyceride might spare glycogen and glucose (20,21). As a result, the extract prevented the onset of fatigue.

In combination with tonic properties of the *T. sinensis* extract, it also reduced the levels of triglyceride and total cholesterol in blood by almost half. Therefore, further studies are needed to elucidate the precise mechanisms of the *T. sinensis* extract on exercise, fatigue prevention, and reducing triglyceride and cholesterol, with the goal of developing commercial food products to manage these activities.

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AUTHOR DISCLOSURE STATEMENT

The authors declare no conflict of interest.

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