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Hot Water Extract of Leather Carp (*Cyprinus carpio nudus*) Improves Exercise Performance in Mice.

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ABSTRACT: The hot water extract of leather carp (*Cyprinus carpio nudus*) has been used as a nourishing tonic soup and as an aid for recovery from physical fatigue. In this study, we investigated the effect of leather carp extract on exercise performance in mice. Swimming endurance and forelimb grip strength were assessed following oral administration of the extract (once per day for 7 days) at a dose of 0.5 mg/10 μL/g body weight. After 7 days, mice given the leather carp extract had significantly greater swimming endurance [105±18 s ($P<0.05$); 52% longer than day 0] and forelimb grip strength [1.18±0.05 Newton ($P<0.01$); 17% greater than day 0]. The extract increased muscle mass, but had little effect on body weight. Following the swimming exercise, blood glucose, glutathione peroxidase, and superoxide dismutase levels in extract-fed mice were significantly higher (145%, 131%, and 106%, respectively) than in the saline control group. Blood levels of high-density lipoprotein cholesterol were also significantly increased (128%) in mice given the extract compared to the controls. These results suggest that leather carp extract can improve physical exercise performance and prevent oxidative stress caused by exhaustive workouts.

Keywords: anti-fatigue, exercise performance, leather carp (*Cyprinus carpio nudus*)

INTRODUCTION

Leather carp [*Cyprinus carpio nudus* (Linnaeus)], known as Israeli carp, German carp, or fragrant fish in Korea, is a part of the Cyprinidae family. Leather carps have large and thick scales distributed along the lower dorsal fin, but lack scales on the rest of the body. They inhabit warm, deep, slow-flowing, and still waters such as lowland rivers and vegetated lakes. They grow fast and have a strong appetite, feeding on a variety of benthic organisms and plant material. The flesh has a firm, chewy texture, with no fine bones or earthy musty odor (1). Thus, leather carp has become popular as a raw flesh or as a hot chowder food. Additionally, the hot water extract of leather carp has been used as a nourishing tonic soup and as an aid for recovery from physical fatigue (2). Leather carp is commercially important in the inland aquaculture industry as one of the major food fish species. In 2013, the production of leather carp by inland water fisheries in Korea was 1,068 metric tons (wet weight), worth 7.4 million US dollars (3).

The effectiveness of various natural food products in improving exercise performance is of interest to sport and healthcare businesses. During physical exercise, the contracting muscles generate force/power, metabolites, and heat, which ultimately induce fatigue and exhaustion (4). 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 oxygen species (ROS), increasing the level of oxidative stress in the body (5). Extracts from a variety of natural food sources have been studied as potential exercise supplements to help improve performance and recover from physical fatigue (6,7). Most active compounds in exercise supplements, such as peptides (8), polysaccharides (9-11), flavonoids (12), and terpenoids (13), originate from herbal sources. Recently, compounds from animal sources, such as antioxidant peptides from grass carp *Ctenopharyngodon idellus* (14) and pig spleen (15), have been identified as enhancers of swimming endurance. To date, few studies have examined compounds from aquatic organisms as potential exercise performance enhancers. To investigate the health and an-

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ti-fatigue claims related to leather carp extract, we evaluated its effect on swimming endurance, forelimb grip strength, and blood biochemical factors in mice.

MATERIALS AND METHODS

Extract preparation and reagents

Fresh leather carp [*Cyprinus carpio nudus* (Linnaeus)] fillets were obtained from the Kumgu Aquaculture Farm (Gimje, Korea), and a voucher specimen is kept in the author's laboratory (M.R. Kim). Fillets (2 kg) were extracted with boiling water (20 L) for 25 h. The residue and oil layer were removed by centrifugation at 3,000 *g* for 10 min, and the aqueous portion was concentrated using a rotary evaporator to obtain the extract (327 g). The extract was adjusted to 50 mg/mL (or 30 mg/mL as protein) with distilled water. Bovine serum albumin was used as the standard for determining protein content (16). Assay kits for determining glucose (AM201-K), urea (AM165-K), glutamic oxaloacetic transaminase (GOT; AM101-K), glutamic pyruvic transaminase (GPT; AM101-K), triglyceride (AM157S-K), high-density lipoprotein (HDL) cholesterol (AM203-K), and total cholesterol (AM202-K) were purchased from Asan Pharmaceutical (Seoul, Korea). The lactate (K627-100), glutathione peroxidase (GPx; K762- 100), and superoxide dismutase (SOD; K335-100) kits were purchased from BioVision (Milpitas, CA, USA). The other reagents used were of analytical grade and purchased from Sigma-Aldrich Co. (St. Louis, MO, USA).

Swimming endurance test

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 5 animals per cage. Feeding consisted of standard animal pellets (Formula^{TM} M07; FeedLab, Guri, Korea) and water *ad libitum*. 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 per day for 7 days at a dose of 10 μL/g body weight (17). One hour after each administration, mice underwent body weight and swimming time measurements. Pure octacosanol (Ferngrove Pharmaceuticals Pty, Ltd., Sydney, Australia) was used as a positive control using an oral dose of 6.7 ng/10 μL/g body weight as per the supplier's protocol. The control group was orally administered saline. For the swimming endurance test, mice were placed individually in a 1-L beaker (25-cm height, 10-cm diameter) filled with water (10 cm deep) at 24±1°C. The total duration of immobility, after a delay of 2 min, was measured for a period of 4 min (6). Each mouse was considered to be immobile when it ceased struggling and remained floating motionless in the water, making only movements necessary to keep its head above water. During the 4-min swim test, the duration of swimming time was calculated by subtracting the total immobilized time. Swimming endurance (s) was measured three times: before treatment (day 0); 1 h after administrating treatment on the third day (day 3); and 1 h after administrating treatment on the seventh day (day 7).

Forelimb grip strength

Mice (n=12 per group per test) were orally administered hot water extract, octacosanol (positive control), or saline (negative control) as described above. One hour after each administration, mice underwent forelimb grip strength measurements. A low-force testing system (Model-RX-10, Aikoh Engineering Co., Osaka, Japan) was used to measure grip strength. The amount of tensile force generated by each mouse was measured using a force transducer with a rectangular 4×5-cm^2 metal net (18). 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. This caused a counter-pull, and the grasping force was recorded by the grip strength in Newton (N). Prior to the experiment, mice were trained to perform this procedure for 3 days. Grip strength (N) was measured 3 times: before treatment (day 0); 1 h after treatment on the third day (day 3); and 1 h after the seventh treatment (day 7). Each mouse was subjected to 3 grip trials with a 1-min rest between trials. The maximum force exerted by the mouse counter-pull was recorded as the forelimb grip strength.

Muscle mass

Mice (n=12 per group per test) were orally administered leather carp extract, octacosanol (positive control), or saline (negative control) as described above. One hour after the last administration, mice were humanely euthanized by cervical dislocation. After peeling the skin off, forelimb and hindlimb were disarticulated from scapula to carpus and from ilium to medial malleolus, respectively, in a consistent manner. They were quickly weighed and the volume was measured in a 10 mL syringe. The volume was given with muscle and bone. After boiling for 1 min to enable muscles to be cleaned easily, only the bone was weighed. Muscle mass of forelimbs and hindlimbs were calculated using lean mass excluding bone mass.

Biochemical assays

Thirty min after the final swimming trial, blood samples from each mouse were collected. Mice were anesthetized with Zoletil 50 (Virbac, Carros, France; 10 mg/kg, i.m.), and blood was drawn using the facial vein technique (19). Blood was allowed to clot for 10 min, followed by centrifugation at 3,000 *g* for 15 min to obtain serum. Using a UV/Vis spectrophotometer (Optizen 2120UV, Mecasys Co., Daejeon, Korea), serum levels of glucose, lactate, urea, GPx, SOD, GOT, GPT, triglyceride, HDL cholesterol, and total cholesterol were determined by glucose oxidase, lactate dehydrogenase, urease-indophenol, GPx colorimetric assay, SOD colorimetric assay, Reitman-Frankel method, lipoprotein lipase, HDL cholestase, and cholesterol esterase-oxidase methods, respectively.

Statistical analysis

Statistical analysis was performed using the Student's *t*-test. All of the animal experiments were done with a minimum of 12 mice per group. Data are reported as the mean±standard error.

RESULTS AND DISCUSSION

In a comparison test evaluating the ability of various fish extracts to enhance exercise capacity, leather carp had the most potent enhancing effect on swimming time among seven common freshwater fish species (bagrid catfish, channel catfish, freshwater eel, leather carp, marsh snail, softshell turtle, and trout; data not shown). Given this finding, the effect of leather carp extract on exercise performance was further investigated. Following oral administration of leather carp extract in mice, swimming time averaged 82±13 s and 105±18 s ($P<0.05$) on days 3 and 7, respectively (Fig. 1); this represents increases of 19% and 52%, respectively, compared with the swimming time on day 0 (69±13 s). Swimming capacity increased significantly with the number of days the mice were fed. On day 7, swimming time was 3-fold longer in the leather carp group than in the saline control group. Octacosanol treatment, acting as a positive control, marginally increased swimming endurance by 8% and 15% on days 3 and 7, respectively. Similarly, increases of 8% (day 3) and 17% (day 7) were observed in the saline group. Our data demonstrate that leather carp extract has a much stronger enhancing effect on swimming endurance than does octacosanol or saline. The forelimb grip strength of mice given the leather carp extract averaged 1.08±0.05 N ($P<0.05$) and 1.18±0.05 N ($P<0.01$) on days 3 and 7, respectively (Fig. 2); this represents increases of 7% and 17%, respectively, compared with the grip strength on day 0 (1.01±0.03 N). Grip strength increased significantly with the number of days the mice were fed. The increase in grip strength observed on day 7 was 19-fold stronger in the leather carp group than in the saline control group. Octacosanol treatment increased grip strength by 8% and 11% on days 3 and 7, respectively. Grip strength in the saline group changed by −1% (day 3) and −2% (day 7). Our data show that leather carp extract and octacosanol have similar effects on grip strength in mice. Body weights of mice given the leather carp extract averaged 24.8±1.0 g on days 3 and 7 (Fig. 3), which represents a 2% increase compared with day 0 (24.3±1.1 g). In mice given octacosanol, body weight increased by 8% ($P<0.05$) and 12% ($P<0.01$) on days 3 and 7, respectively, compared with day 0. Thus, octacosanol treatment had a greater effect on body weight than the leather carp extract. No change in body weight was observed in mice given saline. Overall, the leather carp extract significantly increased swimming capacity and grip strength, but had little effect on body weight. Forelimb muscle mass of mice given the leather carp extract averaged 0.76±0.02 g

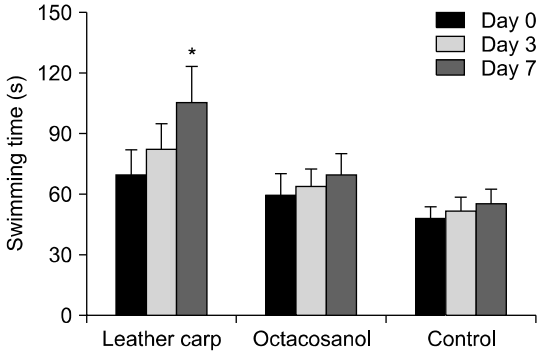

Fig. 1. Effect of leather carp extract on swimming endurance. Mice were orally administered the extract once per day for 7 days at a dose of 0.5 mg/10 μL/g body weight, and underwent the swimming test 1 h after administration. Octacosanol, the positive control, was given at a dose of 6.7 ng/10 μL/g body weight. Data are presented as the mean±SE (n=12). $^{*}P<0.05$ compared with day 0.

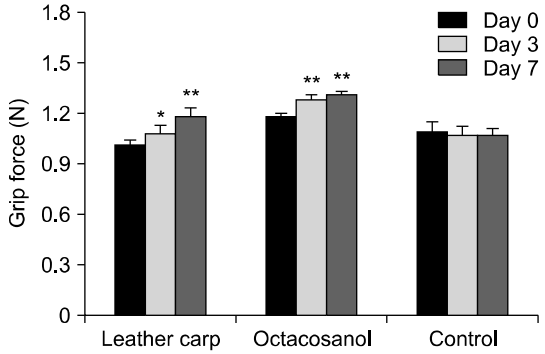

Fig. 2. Effect of leather carp extract on forelimb grip strength. Mice were orally administered the extract once per day for 7 days at a dose of 0.5 mg/10 μL/g body weight, and underwent the grip test 1 h after administration. Octacosanol, the positive control, was given at a dose of 6.7 ng/10 μL/g body weight. Data are presented as the mean±SE (n=12). $^{*}P<0.05$ and $^{**}P<0.01$ compared with day 0.

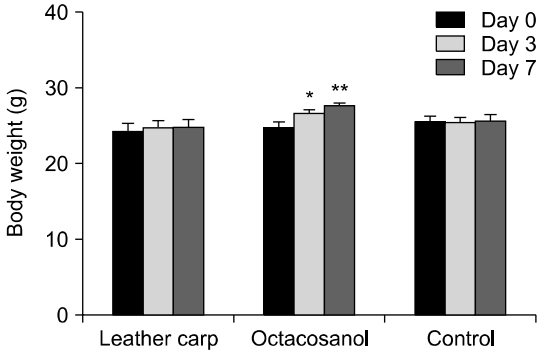

Fig. 3. Effect of leather carp extract on body weight. Mice were orally administered the extract once per day for 7 days at a dose of 0.5 mg/10 μL/g body weight, and body weight was measured 1 h after administration. Octacosanol, the positive control, was given at a dose of 6.7 ng/10 μL/g body weight. Data are presented as the mean±SE (n=12). $^{*}P<0.05$ and $^{**}P<0.01$ compared with day 0.

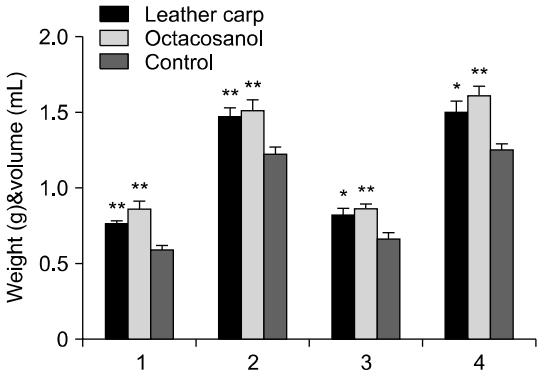

Fig. 4. Effect of leather carp extract on muscle mass of forelimb and hindlimb. Mice were orally administered the extract once per day for 7 days at a dose of 0.5 mg/10 μL/g body weight or the octacosanol at a dose of 6.7 ng/10 μL/g body weight. Weight (1&2) and volume (3&4) of forelimbs (1&3) and hindlimbs (2&4), respectively, were measured 1 h after the final administration. Data are presented as the mean±SE (n=12). $^{*}P<0.05$ and $^{**}P<0.01$ compared to control.

($P<0.01$) on day 7 (Fig. 4), which represents a 30% increase compared with saline (0.59±0.03 g). In mice given octacosanol, forelimb muscle increased by 46% compared with saline. Hindlimb muscle mass of mice given the leather carp extract averaged 1.47±0.06 g ($P<0.01$) on day 7, which represents a 20% increase compared with saline (1.22±0.05 g). In mice given octacosanol, hindlimb muscle increased by 24% compared with saline. Thus, the leather carp extract and octacosanol had great effects on muscle mass of forelimbs and hindlimbs. Significant increases in volumes of forelimb and hindlimb were also observed. Overall, the leather carp extract significantly increased muscle weight and volume, but had little effect on total body weight.

To investigate the anti-fatigue or fatigue recovery properties of the leather carp extract, biochemical analyses were performed on blood samples collected 30 min after the swimming test

Table 1. Effect of leather carp extract on serum glucose, lactate, urea, GPx activity, SOD activity, GOT activity, GPT activity, triglyceride, HDL cholesterol, and total cholesterol after the swimming test

	Glucose (mmol/L)	Lactate (μmol/L)	Urea (mmol/L)	GPx (mU/mL)	SOD (mU/mL)	GOT (IU/L)	GPT (IU/L)	Triglyceride (mmol/L)	HDL cholesterol (mmol/L)	Total cholesterol (mmol/L)
Saline	7.7±0.6	545.5±13.7	1.2±0.1	949.8±54.4	856.4±13.9	29.9±3.9	19.1±1.5	2.3±0.1	3.6±0.3	7.8±0.4
Leather carp	11.2±2.0**	515.1±11.4	1.1±0.0	1,240.4±92.8*	907.3±9.9**	23.7±2.3	14.6±2.5	2.2±0.1	4.6±0.4*	8.2±0.2
Relative activity (%)[1]	145	94	92	131	106	79	76	96	128	105
Octacosanol	12.2±0.8**	822.9±25.5**	0.9±0.0**	1,104.00±67.9	1,344.30±61.5**	28.6±5.8	24.3±3.2	1.7±0.1**	3.2±0.1	5.7±0.2**
Relative activity (%)	158	151	75	116	157	96	127	74	89	73

GPx, glutathione peroxidase; SOD, superoxide dismutase; GOT, glutamic oxaloacetic transaminase; GPT, glutamic pyruvic transaminase; HDL, high-density lipoprotein.
Values are the means±SE (n=12). $^{*}P<0.05$ and $^{**}P<0.01$ compared to control.
[1]Relative activities are expressed as percentages of the values against the saline control group.

the swimming test on day 7. In mice given the leather carp extract, serum glucose levels averaged 11.2±2.0 mM/L, a 145% increase over saline-treated mice ($P<0.01$) (Table 1). The levels of lactate and urea were 515.1 ±11.4 μM/L and 1.1±0.0 mM/L, respectively, which were lower than the levels in the control mice. Thus, the extract induced higher levels of blood glucose, an energy source for exercise performance and fatigue recovery. The activities of GPx and SOD, antioxidant enzymes, in the extract-fed mice were 1,240.4±92.8 mU/mL ($P<0.05$) and 907.3±9.9 mU/mL ($P<0.01$), respectively, which were significantly higher than those in the control group. Therefore, the leather carp extract decreased oxidative stress caused by an exhaustive workout. Activities of GOT and GPT, measures of liver toxicity, were all within the normal range of 0∼40 IU/L. Serum HDL cholesterol was 4.6±0.4 mM/L, which was significantly higher than in the control group (128%, $P<0.05$). The triglyceride and total cholesterol levels stayed relatively constant. Even after 7 days of leather carp extract exposure, mice did not accumulate triglyceride or total cholesterol, both of which are markers for obesity at high concentrations.

Leather carps grow fast and are used as a tonic food. Aquaculture of leather carp has become popular in freshwater farms and ponds. Ingestion of raw carp bile is known to induce acute hepatotoxicity and nephrotoxicity in humans (20). The flesh fillet or whole body, after removing the gall bladder, is generally used for cooking. Leather carp flesh has a relatively high amount of lysine (3.5 g/100 g wet tissue) (21), which is a limiting essential amino acid in major plant-food protein sources (22). Leather carps have high nutritional value, especially for plant-food consumers. Additionally, lysine is considered to have better antioxidant properties (23), although all 20 of the amino acids found in proteins have the potential to interact with free radicals (24). Antioxidant mechanisms of many proteins are dependent on amino acid composition. *In vitro* radical scavenging activities of leather carp extract against 2,2'-azinobis[3-ethylbenzothiazoline]-6-sulfonic acid (ABTS), superoxide, and hydroxyl radicals have ranged from 50 to 60% (25), which is similar to the values obtained with silver carp protein hydrolysate (26) and loach protein hydrolysate (27). The antioxidant properties of leather carp extract allow neutralization of free radicals, which may help in delaying fatigue and limiting oxidative damage of tissues.

Among blood biochemical parameters, the homeostasis of serum glucose level is very important for improving exercise performance. Physical exercise necessitates a higher rate of metabolism to handle the increased energy demands. When hypoglycemia is prolonged, it can overwhelm the glycogen storage and reduce blood glucose (28). 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 an accumulation of lactic acid (29). When mice were fed the leather carp extract, blood glucose levels increased to near hyperglycemic levels. It is known that hyperglycemia may change the organization of membrane lipids, which is correlated with increased formation of lipid hydroperoxide (LOOH), an intermediate product of oxidative lipid damage (30). Leather carp extract also increased GPx and SOD antioxidant activities in the blood, which may help protect against oxidative lipid damage. Additionally, we found that mice given the leather carp extract did not show any problematic symptoms of hyperglycemia, such as body weight loss or physical tiredness. Thus, the extract appeared to facilitate the release of glucose from glycogen, allowing the animals to regain energy after a workout. Similar results were obtained when fungus (13) or fenugreek (31) extracts were used to improve exercise performance and decrease physical fatigue. Exhaustive heavy exercise generates ROS (5). The activities of GPx and SOD, major antioxidant enzymes, were used as a measure of the anti-fatigue effect of leather carp extract. GPx and SOD activities in the extract-fed mice were much higher than those in the saline control group. This suggests that the extract decreases oxidative stress by counteracting the oxidative effects of free radicals produced during exhaustive workouts, similar to phenolic compounds (32) and some dietary antioxidants (33). The anti-fatigue capacity of the extract may have also been facilitated by lower levels of ROS, such as LOOH, via increased blood HDL. HDL in the blood acts as an antioxidant by reducing levels of LOOH (34). HDL has been found to increase peripheral glucose uptake through the activation of 5'-adenosine monophosphate-activated protein kinase in muscle cells (35) and insulin secretion by pancreatic β cells (36). This suggests a potential metabolic role for HDL in the modulation of plasma glucose homeostasis, in addition to its well-established role in reverse cholesterol transport and modulation of inflammation (37). Though human data are still unavailable, it is plausible that the plasma concentration of HDL may be one of the factors that modulate the duration and intensity of transient hyperglycemia. For prevention of cardiovascular diseases and diabetes, many studies have shown that a diet supplemented with fruit and vegetables has beneficial effects for reduction of cholesterol and glucose (38,39). Although the mechanisms are unclear regarding the leather carp extract-induced increases in blood HDL, glucose, GPx, and SOD, the observed enhancements in exercise performance are likely to be complex, possibly involving a combination of bioactive components in the extract. Further research is necessary to investigate the isolated bioactive components. Nevertheless, our study indicates that leather carp extract

is beneficial for preventing the oxidative stress caused by exhaustive workouts. Comparing to the octacosanol as a positive control, the leather carp extract reduced blood lactate significantly. The triglyceride/HDL ratio is considered a better predictor of coronary arterial disease than lipid alone, and a stronger predictor of myocardial infarction than classical atherogenic indices (low density lipoprotein : HDL cholesterol ratio) (40). The triglyceride/HDL ratio in mice given the leather carp extract was 0.48, compared with 0.63 in the saline control group. The ratio in the octacosanol group was 0.53 even though the levels of triglycerides and cholesterol were lower than in the leather carp extract and saline groups. Thus, it is proposed that leather carp extract alleviates, rather than induces, cardiovascular disease and atherosclerotic progression. In summary, our findings indicate that leather carp (*Cyprinus carpio nudus*) extract may be useful in health food products for enhancing physical performance and managing the onset of fatigue.

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AUTHOR DISCLOSURE STATEMENT

The authors declare no conflict of interest.

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