Ethanol Extract of Eriobotrya japonica Leaves Enhanced Swimming Capacity in Mice

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Abstract Eriobotrya japonica is known as ‘Loquat’ and cultivated in Korea, China, Japan, and India. The present study aims to investigate the effect of Eriobotrya japonica leaves ethanol extract (EJLE) on swimming capacity in mice. Mice were administered distilled water (EX-CON) or 1 g kg⁻¹ per day of EJLE (EX-EJLE) for 14 days. Exhaustive swimming time was significantly prolonged (by 1.4-fold) in EX-EJLE group compared to that in EX-CON group (P < 0.05). EX-EJLE group showed high nonesterified fatty acid (NEFA) and glycogen levels during swimming (P < 0.05). When compared to EX-CON, the EX-EJLE exhibited significant increases in antioxidant enzymes activities including catalase (CAT), superoxide dismutase (SOD), and glutathione peroxidase (GPx) and glutathione (GSH) level as well as and decrease in malondialdehyde (MDA) level (P < 0.05). EJLE elevated the mRNA expression of carnitine palmitoyltransferase-1, β-hydroxyacyl coenzymes dehydrogenase, peroxisomal proliferator activated receptors-δ, and uncoupling protein-3 in muscle (P < 0.05). These results suggest that EJLE supplementation could improve swimming capacity by ameliorate the physical exhaustion through enhancing utilization of fatty acid, facilitating lipid catabolism, and elevating antioxidant capability.

Keywords: eriobotrya japonica, leaves, ethanol extract, swimming capacity


1. Introduction

Exercise capacity is important both for athletic endurance performance and maintaining a healthy lifestyle. The energy metabolism in skeletal muscle plays a key role in exercise capacity [1,2]. Previous studies have reported that selection of exogenous supplements may improve exercise capacity [2,3,4] by sparing glycogen utilization and increasing fatty acid utilization [4,5], and facilitating fatty acid oxidation [3,4,6].

Strenuous and exhaustive exercise can lead to the production of excess reactive oxygen species (ROS), which results in an imbalance between ROS generation and the antioxidant defense activities of cellular antioxidants and enzymes such as glutathione (GSH), catalase (CAT), glutathione peroxidase (GPx), and superoxide dismutase (SOD). This imbalance eventually damages biological molecules and key cellular components and interferes with processes such as lipid peroxidation. Because oxidative stress may be responsible for muscle soreness, fatigue, and muscle damage, it has been suggested that antioxidant supplementation may relieve these problems [7,8]. In addition, deteriorating function of skeletal muscle is closely related to lower exercise capacity [9,10].

Exogenous antioxidants may prevent exercise-induced oxidative damage, since they can detoxify certain free radicals and peroxides while enhancing endogenous antioxidants [11,12]. In addition, antioxidants from plant leaves may improve physiological conditions by promoting and interacting with endogenous antioxidants [3,4,13]. Thus, antioxidants may be key to combating and reducing exhaustion on exercise.

Eriobotrya japonica (EJ) is a large evergreen shrub or small tree, with a rounded crown, short trunk, and woolly new twigs. The leaves are more frequently used for medicinal purposes than the other plant parts. In fact, its leaves have been used as traditional medicine for the treatment of diabetes mellitus, chronic bronchitis, coughs, and skin diseases. Various triterpenes, sesquiterpenes, flavonoids, tannins, and megastigmane glycosides have been detected in the leaves, and some of these compounds have been reported to have anti-inflammatory, antioxidant, anti-mutagenic, neuroprotective, and anti-tumor activities [14,15]. Recent studies have provided evidence that they may possess antioxidative and hypoglycemic effects [16,17]. However, the effects of EJ are unknown on exercise capacity and energy metabolism with antioxidant
activities. Therefore, the aim of this study was to investigate the effects of an ethanol extract of EJ leaves on swimming capacity in mice.

Also, the possible mechanisms underlying its effects on energy metabolism and endogenous antioxidant activities will be in the scope of this investigation.

2. Materials and Methods

2.1. Preparation of EJLE

_Eribotrya japonica_ (EJ) leaves were collected in Jangheung (Jeollanamdo, Korea). The leaves were cleaned by washing thoroughly with water and were powdered with a grinder (Hanil Electric, Seoul, Korea). Powered EJ leaves (100 g) were refluxed with 1.5 L of 5% ethanol at 100°C for 3 h using soxhlet apparatus and heating mantle, filtered through Whatman paper No. 6 (NJ, USA) and concentrated in a rotary evaporator (EYELA, Tokyo, Japan) under reduced pressure. The concentrate was lyophilized and kept at -20°C until use. The lyophilized powder generated from the 5% ethanol extract of EJ leaves was named EJLE. The yield of EJLE based on the dried weight was 8.4%.

2.2. Animals and Experimental Design

Four-week-old male ICR mice (29.4 ± 1.2 g per each) were obtained from Orient Bio (Seongnam, Korea) and housed in cages under climate-controlled conditions (temperature, 22 ± 2°C; humidity, ~ 60%; and a 12-h light/dark cycle). The mice were allowed access to AIN-76 diet (G-Bio, Seongnam, Korea) and water _ad libitum_. The protocol for the animal study was approved by the Chonnam National University Institutional Animal Care and Use Committee (CNU-IACUC-YB-R-2015-44), and animals were cared for in accordance with the “Guidelines for Animal Experiments” established by the university.

An acrylic plastic pool (90 × 45 × 45 cm) was used to determine swimming capacity [10,18]. The pool was filled with water to a depth of 38 cm, and the temperature was maintained at 34 ± 1°C. The current strength in the swimming pool was adjusted to 7.5 L/min by controlling the voltage in the pool pump, and was monitored using a water flow meter.

Mice were acclimatized for one week prior to use in the experiments. The mice were forced to swim twice at 3-day intervals to measure exhaustive swimming time. The mice were divided into two sets (administered distilled water and EJLE) [EX-C: administered distilled water, EX-E: administered EJLE; n = 10 per group] with similar mean swimming capacities. Each set was evaluated exhaustive swimming capacities. The first set were sacrificed to collect gastrocnemius muscle. Antioxidant enzyme activities (CAT, SOD, and GSH) were determined in accordance with previous studies [10,18].

2.3. Measurement of Exhaustive Swimming Capacity

On day 7 and day 14, two groups in the first set were administered distilled water and EJLE, and then 60 min later, were exhaustively exercised without any load. The experiments were conducted from 13:00 to 15:00, a time period in which minimal variation in endurance capacity in rodents has been confirmed. The mice were determined to be exhausted when they failed to rise to the surface to breathe within a 5–7 s period [18].

2.4. Analysis of Biochemical Parameters

On day 14, two groups in the second set were administered distilled water and EJLE 60 min before swimming and collected blood from the tail. At 10 min after swimming, blood was collected from the tail. And then, mice were sacrificed to collect gastrocnemius muscle. The plasma used for NEFA was collected by centrifugation at 1,000 × g for 10 min at 4°C. The plasma used for NEFA assay by the enzymatic method with a commercial kit (Wako Pure Chemical, Osaka, Japan). Muscle glycogen was estimated in accordance with that mentioned in our previous study [18].

2.5. Tissue Antioxidant Activities

Immediately after the exhaustive swimming, mice in the first set were sacrificed to collect gastrocnemius muscle. Antioxidant enzyme activities (CAT, SOD, and GSH) were determined in accordance with previous studies [10,18]. The amount of protein was measured using the Bradford assay [19].

2.6. Real-time Polymerase Chain Reaction

The gastrocnemius muscle was homogenized with a pestle under liquid nitrogen. Total RNA was isolated with the easy-BLUE™III kit (INtRON Biotechnology, Seongnam, Korea) according to the manufacturer’s instructions. Real-time polymerase chain reaction (PCR) was performed using specific primer sets with Quantifast SYBR Green PCR Master Mix (Qiagen, Valencia, CA, USA) according to the manufacturer’s instructions. The primer sequences were as follows: carnitine palmitoyltransferase-1 (CPT-1, NM_013495): 5'- GTG ACT GGT GGG AGG AAT AC-3' and 5'- GAG CAT CTC CAT GGC GTA G-3', β-hydroxyacyl coenzymes A dehydrogenase (β-HAD, NM_145558): 5'-GAC AGG GTC ATG CTA TGA TTG TG-3' and 5'- TCCT GTC GCC TCC TTC TAG AG-3', peroxisomal proliferator activated receptors delta (PPAR-δ, NM_011145): 5'-CGC AAG CCC TTC ATG GAC AT-3' and 5'-CGC ATT GAA CTT GAC AGG AAA AA-3', uncoupling protein-3 (UCP-3, NM_009464): 5'-CCA GAG CAT GGT GCC TTC GCT GCT-3' and 5'- GTG TGA GCA GCA GTG-3', β-actin (NM_007393): 5'- ACG GCC AGG TCA CTA TTG-3' and 3'- AAG AAG GAA GGC TGG AAA AGA-5'. mRNA expression was quantified using the ΔΔ CT method.
2.7. Statistical Analysis

Data are presented as mean ± standard errors (SE). The data were statistically evaluated using one-way analysis of variance (ANOVA) and Duncan’s test to determine the significance differences between the groups at $P < 0.05$.

3. Results

3.1. Effect of EJLE on Exhaustive Swimming Time

EJLE was prepared from 5% ethanol extract of EJ leaves. Exhaustive swimming time of EX-CON and EX-EJLE groups was evaluated on 7 d and 14 d after orally EJLE-administration. The exhaustive swimming time of the EX-EJLE group was significantly longer than that of the EX-CON group on 14 day ($P < 0.05$, Figure 1). The increased exercise time of the EX-EJLE group was not associated with a significant change in body or tissue weight which suggests that the prolonged time to exhaustion was mainly due to the effect of EJLE (data not shown).

Figure 1. Effect of EJLE on exhaustive swimming time in mice. Data are expressed as the mean ± SE (n=10). * $P < 0.05$ compare to EX-CON

3.2. Effect of EJLE on Biochemical Parameters during Swimming

The blood NEFA level in the EX-CON and EX-EJLE groups were similar before swimming. Compared with the EX-CON, NEFA levels in the EX-EJLE mice were higher than in the EX-CON mice ($P < 0.05$, Figure 2A). Muscle glycogen level in EX-EJLE was significantly increased as compared with the EX-CON ($P < 0.05$, Figure 2B).

Figure 2. Effect of EJLE on the levels of NEFA (A) and glycogen (B). Data are expressed as the mean ± SE (n=10). Different letters above the bar indicate significant difference at $P < 0.05$ by one-way ANOVA. Pre-exercise, before swimming; post-exercise, 10 min after swimming

3.3. Effect of EJLE on Endogenous Antioxidants Activities in Muscle

The activities of CAT, SOD, and GPx in the EX-EJLE group were significantly increased compared with the EX-CON group ($P < 0.05$, Table 1). GSH level in the EX-EJLE group was elevated compared to the EX-CON ($P < 0.05$, Table 2). MDA level in the EX-EJLE was decreased as compared to that in the EX-CON group ($P < 0.05$, Table 2).

Table 1. Effects of EJLE on antioxidant activities.

<table>
<thead>
<tr>
<th></th>
<th>CAT (U/mg protein)</th>
<th>SOD (U/mg protein)</th>
<th>GPx (U/mg protein)</th>
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<tbody>
<tr>
<td>EX-CON</td>
<td>1.35±0.12</td>
<td>8.04±0.43</td>
<td>0.18±0.01</td>
</tr>
<tr>
<td>EX-EJLE</td>
<td>1.68±0.12 *</td>
<td>9.25±0.41 *</td>
<td>0.21±0.02 *</td>
</tr>
</tbody>
</table>

Data are expressed as the mean ± SE (n=10). * $P < 0.05$ compare to EX-CON.

Table 2. Effects of EJLE on GSH and MDA levels.

<table>
<thead>
<tr>
<th></th>
<th>GSH (nmol/mg protein)</th>
<th>MDA (µmol/mg protein)</th>
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<tbody>
<tr>
<td>EX-CON</td>
<td>2.77±0.14</td>
<td>0.82±0.07</td>
</tr>
<tr>
<td>EX-EJLE</td>
<td>3.23±0.12 *</td>
<td>0.66±0.03 *</td>
</tr>
</tbody>
</table>

Data are expressed as the mean ± SE (n=10). * $P < 0.05$ compare to EX-CON.

3.4. Effects of EJLE on the mRNA Expression of Energy Metabolism-related Enzymes and Transcription Factors in Muscle

The levels of CPT-1 and β-HAD in the muscle of mice in the EX-EJLE group were significantly higher than their corresponding expression levels in the EX-CON group. The expression levels of PPAR-δ in the muscle of mice in the EX-EJLE group were significantly higher than the expression in the EX-CON group. The levels of UCP-3 in the muscle of the EX-EJLE group were significantly higher than in the EX-CON, group ($P < 0.05$, Figure 3).
The energy substrates available during exercise are plasma glucose, intramuscular glycogen, and free fatty acids. Fatty acids are utilized by energy-generating metabolic pathways, including fatty acid β-oxidation. CPT-1 and β-HAD are the rate-limiting enzyme in fatty acid transport across the mitochondrion and central enzyme in fatty acid β-oxidation [27]. PPAR-δ controls fatty acid oxidation by regulating the genes involved in fatty acid transport and beta-oxidation in skeletal muscle [28]. UCP-3 may contribute to skeletal muscle energy metabolism and protect mitochondria against fatty acid and ROS accumulation (lipotoxicity) [29]. In this study, the mRNA expression of CPT-1, β-HAD, PPAR-δ, and UCP-3 was significantly upregulated in EX-EJLE mice compared to their expression in EX-CON mice, respectively. This indicated that EJLE may enhance the utilization of energy substrates and activate energy-generating metabolic pathways. Therefore, we speculated that EJLE improves metabolic efficiency, and related molecules may improve swimming capacity.

Strenuous and exhaustive exercise may lead to an imbalance between ROS generation and antioxidant defense, resulting in oxidative stress [30]. During prolonged exercise, energy-generating systems, including fatty acid oxidation and energy production by the mitochondrial electron transport chain (ETC), function as powerful sources of ROS [31]. During exercise, ROS generation occurs via fatty acid oxidation as a result of fat utilization [32,33]. The antioxidant defense system in the body plays an important protective role against oxidative stress. These systems are important for scavenging free radicals and their metabolic products and in maintaining normal cellular physiology [34,35]. Previous studies reported decreased antioxidant enzyme activities after similar exhaustive exercise [10,35]. In this study, the EJLE-treated mice showed increased antioxidant enzyme activities including CAT, SOD, and Gpx and GSH level. Exercise-induced ROS can rapidly attack lipids in the cell and induce the production of peroxidation byproducts such as MDA [18]. In the present study, administration of EJLE decreased MDA levels after exhaustive swimming. These results indicate that administration of EJLE for 14 d effectively enhanced the antioxidant defense systems in cellular organelles. We speculated that the increase in ROS levels following exhaustive swimming might be prevented by EJLE administration.

4. Discussion

EJ, which is also known as “Loquat”, belongs to the Rosaceae family. Its leaves have been widely used as a traditional medicine in Korea, China, and Japan, because of their beneficial effect on fever, back pain, gastroenteric diseases, and diabetes mellitus. EJ leaves have been reported to have various health-promoting properties, such as anti-obesity and anti-inflammatory effects [20,21]. Recently, muscle function in dexamethasone-induced muscle atrophy and sarcopenia rats was improved following [22,23]. However, little is known about the effect of a 5% ethanol extract of EJ leaves on physiological function such as swimming capacity. In this study, the stimulatory effects of a 5% ethanol extract from EJ leaves (EJLE) on exercise capacity was investigated using forced swimming. To clarify the effects of EJLE administration during and after exercise, NEFA and glycogen were measured in the blood and muscle.

Extracts from natural sources have been reported to increase exercise capacity in similar models [18,24]. Exhaustive swimming time was significantly enhanced in the EX-EJLE group compared to that in the EX-CON group. Hence, EJLE was shown to possess exercise-enhancing activity.

Enhancement of exercise capacity could be accounted for by a greater potential for fatty acid metabolism and by a reduced rate of muscle glycogen breakdown. Swimming capacity could be improved by increasing the availability of fatty acids and that this effect is mediated by a slowing of glycogen depletion [25,26]. Several plant extracts were reported as meaningful sources because the metabolic effects of those on increasing endurance performance appear to be caused by the increase in fatty acid utilization as an energy source, with sparing of glycogen [18,19,25,26]. During swimming, the NEFA levels and glycogen levels in the EX-EJLE group were significant higher than those in the EX-CON, respectively. EJLE supplementation can effectively enhance the exercise capacity of mice by supplying energy sources by facilitating of fatty acid utilization with sparing of muscle glycogen.

5. Conclusion

In this study, supplementation with EJLE (1 g kg⁻¹ bw per day) improved the exhaustive swimming time by fatty acid utilization and sparing glycogen during swimming. In addition, EJLE supplementation was exhibited increasing fatty acid beta-oxidation related mRNA expression including CPT-1, β-HAD, PPAR-δ, and UCP-3, and enhancing antioxidant capability including CAT, SOD, Gpx, GSH, and MDA in mice. These findings suggest that EJLE could be a potential extract for functional supplementation with improving swimming capacity by up-regulating energy-supporting metabolism and antioxidant capability.
Acknowledgments

This research was financially supported by the Ministry of Trade, Industry and Energy (MOTIE) and Korea Institute for Advancement of Technology (KIAT) through the Promoting Regional Specialized Industry.

Statement of Competing Interests

The authors have no competing interests.

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