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FULL PAPER

Physiology

Ludwigia octovalvis (Jacq.) raven extract supplementation enhances muscle glycogen content and endurance exercise performance in mice

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ABSTRACT. *Ludwigia octovalvis* extract (LOE) is a widely used traditional Chinese herbal medicine. To date, few studies have demonstrated the effect of LOE supplementation on exercise performance, physical fatigue and biochemical profile. The purpose of this study is to evaluate the potential beneficial effects of LOE extract on fatigue and ergogenic functions following physiological challenge. Male ICR mice from 3 groups (n=8 per group) were orally administered LOE for 4 weeks at 0 (vehicle), 61.5 (LOE-1X) or 307.5 (LOE-5X) mg/kg/day. LOE supplementation was able to dose-dependently increase endurance swimming time (P<0.0001) and decrease levels of serum lactate (P=0.0022), ammonia (P<0.0001), creatine kinase (P<0.0001), blood urea nitrogen (P<0.0001) and glucose utilization (P<0.0001) after acute exercise challenge. The glycogen in gastrocnemius muscle also increased with LOE treatment in a dose-dependent manner (P<0.0001). Biochemically, AST, ALT, LDH, CK, BUN, creatinine and UA levels were decreased with LOE treatment. Our study shows that 4-week supplementation with LOE increases muscle glycogen content storage to enhance exercise performance and anti-fatigue effects.

KEY WORDS: anti-fatigue, exercise performance, glycogen

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Ludwigia octovalvis (Jacq.) P. H. Raven (Family: Onagraceae) is an aquatic plant commonly grown in the wet areas of Taiwan (Fig. 1). *L. octovalvis* extract (LOE) is widely consumed as a healthful drink in a number of countries [15] and has been extensively used in traditional Chinese medicine to provide immunoregulatory, hepatoprotective, cardiovascular-protective properties and anti-aging effects [20]. Numerous antioxidative flavonoids, such as quercetin, luteolin, apigenin, and gallic acid, have been isolated from *L. octovalvis* [15]. In previous studies, LOE has been shown to contain high levels of polyphenols, flavonoids and β-sitosterol [18, 20, 28]. Quercetin is one of the active components of flavonoids which exist in natural plants, particularly in LOE [29]. Intake of quercetin has been inversely associated with coronary heart disease [2]. In addition to antibacterial activity, quercetin also contains antioxidant activity, preventing oxidation of low density lipoproteins *in vitro*. Besides quercetin, LOE's active ingredient, β-sitosterol, has been shown to significantly extend the lifespan of fruit flies [21] and activate the AMP-activated protein kinase (AMPK) pathway [21, 27]. AMPK is a major cellular energy sensor and metabolic regulator, and is considered to be a therapeutic target for treatment of diabetes mellitus via glucose handling [7]. AMPK also plays a major role in the physiological effects of energy metabolism and metabolic adaptation to fasting and exercise in skeletal muscle [3]. Based on these, there is potential for LOE to be used as a sport nutrition supplementation to enhance exercise performance or to counteract fatigue. Given there are very few studies that directly

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address LOE's anti-fatigue properties and ability to improve exercise performance, this study seeks to evaluate the potential ergogenic, anti-fatigue and beneficial health effects of LOE supplementation in mice after 28 days by using our previously established *in vivo* platform [12].

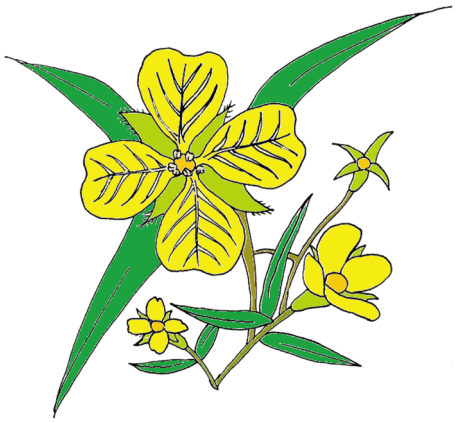

Fig. 1. Schematic diagram of wild *Ludwigia octovalvis* (Jacq.) P. H. Raven.

MATERIALS AND METHODS

Preparation of L. octovalvis extract (LOE)

Air-dried whole plants of *L. octovalvis* were obtained from the Taiwan Herbal Biopharma Co., Ltd. (Tainan, Taiwan). Briefly, 100 g of dry *L. octovalvis* powder was soaked in 400 m*l* of 95% ethanol overnight for 4 hr, extracted and then concentrated in a rotary evaporator at 55°C for 30 min. The extract was decanted and filtered through Whatman No. 2 filter paper to remove debris and lyophilized for 40 hr. The lyophilized powder was dried in oven at 55°C. The yield of LOE was 2.5% (2.5/100 g × 100%).

Materials, animals, and experiment design

Male ICR mice (8-weeks old) grown under specific pathogen-free conditions were purchased from BioLASCO (Yi-Lan, Taiwan). All mice were provided a standard laboratory diet (No. 5001; PMI Nutrition International, Brentwood, MO, U.S.A.), distilled water *ad libitum* and housed at 12-hr light/12-hr dark cycle at room temperature (22 ± 1°C) and 50–60% humidity. The Institutional Animal Care and Use Committee (IACUC) of National Taiwan Sport University (NTSU) inspected all animal experiments, and this study conformed to the guidelines of protocol IACUC-10502 approved by the IACUC ethics committee. The 1X dose of LOE extract used for humans is typically 300 mg per day. The 1X mouse dose (61.5 mg/kg) we used was converted from a human-equivalent dose (HED) based on body surface area according to the US Food and Drug Administration formula: Assuming a human weight of 60 kg, the HED for 300 (mg)/60 (kg)=5 × 12.3=61.5 mg/kg; the conversion coefficient 12.3 is used to account for differences in body surface area between mice and human as previously described [5]. In total, 24 mice were randomly assigned to 3 groups (8 mice/group) for daily oral LOE treatment for 4 weeks: vehicle; 61.5 mg/kg (LOE-1X); and 307.5 mg/kg (LOE-5X). The vehicle group received the same volume of solution equivalent to individual body weight (BW). Mice were randomly housed in groups of 4 per cage.

Forelimb grip strength test

A low-force testing system (Model-RX-5, Aikoh Engineering, Nagoya, Japan) was used to measure the forelimb grip strength of treated mice as previously described [6].

Swimming exercise performance test

The swim-to-exhaustion test involved loads corresponding to 5% of the mouse BW attached to the tails to evaluate endurance times as previously described [5]. The swimming endurance time of each mouse was recorded from beginning to exhaustion, which is determined by observing loss of coordinated movements and failure to return to the surface within 7 sec.

Determination of fatigue-associated biochemical variables

The effect of LOE supplementation on fatigue-associated biochemical indices was evaluated after exercise as previously described [22]. One hour after LOE supplementation, all mice underwent a 15-min swim exercise without weight loading. After the 15-min swim exercise, blood samples were immediately collected and centrifuged at 1,500 × *g* at 4°C for 10 min to obtain the serum. Serum lactate, ammonia, glucose, creatine kinase (CK) and blood urea nitrogen (BUN) levels were determined by using an autoanalyzer (Hitachi 7060, Hitachi, Tokyo, Japan).

Clinical biochemical profiles

At the end of the experimental period, mice were euthanized by 95% CO_2 asphyxiation and blood collected immediately. Serum was separated by centrifugation and levels of the clinical biochemical variables, including aspartate aminotransferase (AST), alanine aminotransferase (ALT), lactic dehydrogenase (LDH), creatine kinase (CK), total protein (TP), albumin, blood urea nitrogen (BUN), creatinine, uric acid (UA), total cholesterol (TC), triacylglycerols (TG) and glucose, were measured by using an autoanalyzer (Hitachi 7060).

Tissue glycogen determination and visceral organ weight

The stored form of glucose is glycogen, which exists mostly in liver and muscle tissue. Liver and muscle tissues were excised after the mice were euthanized and weighed for glycogen content analysis as described previously [6, 22]. The weights of the liver, kidney, heart, lung, muscle and epididymal fat pad (EFP) related visceral organs were recorded.

Histology of tissues

All tissues were carefully removed, minced and fixed in 10% formalin. Samples were embedded in paraffin and cut into 4-μm

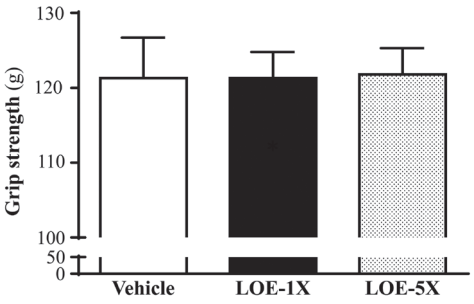

Fig. 2. Effect of LOE (*L. octovalvis* extract) supplementation for 4 weeks on forelimb grip strength. Mice were treated with vehicle, LOE-1X, or LOE-5X for 4 weeks before the forelimb grip strength test was administered. Data is shown as mean ± SEM, *n*=8 mice/group. Analysis is using one-way ANOVA, with different letters (a, b) indicating a significant difference at $P<0.05$.

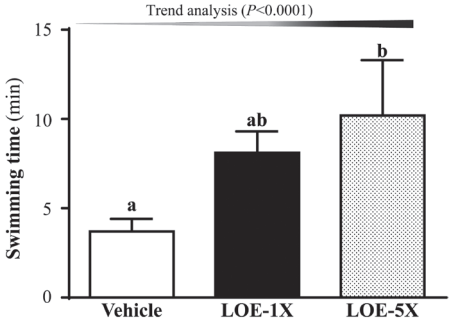

Fig. 3. Effect of LOE supplementation on swimming exercise performance. Mice were treated with vehicle, LOE-1X or LOE-5X for 4 weeks. One hr after the last treatment, the exhaustive swimming test was performed. Loads used for the tests were attached to the tails of the mice and equivalent to 5% of the body weight. Data is shown as mean ± SEM, *n*=8 mice/group. Analysis is using one-way ANOVA with different letters (a, b) indicating a significant difference at $P<0.05$.

thick slices for morphological and pathological evaluations. Tissue was stained with hematoxylin and eosin (H&E) and examined by a veterinary pathologist using an optical microscope equipped with a CCD camera (BX-51, Olympus, Tokyo, Japan).

Statistical analysis

All data are expressed as mean ± SEM, n=8 mice/group. Statistical differences among groups were analyzed by a one-way analysis of variance (ANOVA) and the Cochran-Armitage test for dose-effect trend analysis with SAS 9.0 (SAS Inst., Cary, NC, U.S.A.). Differences between groups were analyzed by one-way analysis of variance (ANOVA) and using Duncan's *post-hoc* test. All *P*-values <0.05 were considered significant.

RESULTS

Effects of LOE on exercise performance in forelimb grip strength and weight-loaded swimming test

After supplementation of LOE for 4 weeks, we examined the effect of LOE on forelimb grip strength in the mice. The forelimb grip strengths for the vehicle, LOE-1X and LOE-5X groups were found to be 121 ± 5, 121 ± 4 and 121 ± 8 g, respectively (Fig. 2). The difference in forelimb grip strength was shown to be statistically insignificant with the LOE supplementation. We further evaluated the anti-fatigue effects of LOE supplementation using exercise endurance as an index. Endurance swimming times were 3.7 ± 0.7, 8.1 ± 1.2 and 10.2 ± 3.1 min with the vehicle, LOE-1X and LOE-5X treatment groups, respectively (Fig. 3). The exhaustive swimming time was higher by 2.72-fold in the LOE-5X group than vehicle group ($P=0.0295$). In the trend analysis, absolute exhaustive swimming time dose-dependently increased with LOE supplementation ($P<0.0001$).

Effect of LOE supplementation on serum lactate, ammonia, glucose, CK and BUN levels after acute exercise challenge

Lactate levels in vehicle, LOE-1X, and LOE-5X groups were 7.5 ± 0.3, 5.5 ± 0.4 and 4.7 ± 0.2 mmol/*l*. Compared with the vehicle group, lactate level was decrease by 27.24% ($P=0.0005$) and 38.21% ($P<0.0001$) in the LOE-1X and LOE-5X groups, respectively (Fig. 4A). In the trend analysis, serum lactate levels decreased in a dose dependent manner with increased LOE dose ($P<0.0001$). These results indicate that LOE supplementation may have the potential for clearance or utilization of blood lactate during exercise.

Serum ammonia levels of the vehicle, LOE-1X and LOE-5X groups were 129 ± 6, 61 ± 7 and 56 ± 5 μmol/*l*, respectively (Fig. 4B). The ammonia levels of LOE-1X and LOE-5X groups were significantly lower by 52.52% ($P<0.0001$) and 56.41% ($P<0.0001$), compared with vehicle group. Trend analysis revealed that serum ammonia level dose-dependently decreased with increasing LOE dose ($P<0.0001$), suggesting that continuous supplementation with LOE for 4 weeks could decrease ammonia accumulation during exercise.

Serum CK level is an important clinical biomarker of muscle damage with lower CK levels representing greater recovery capacity from exercise-induced muscle damage. The serum CK levels of vehicle, LOE-1X and LOE-5X groups were shown to be 1097 ± 191, 518 ± 51 and 302 ± 30 (mg/d*l*), respectively (Fig. 4D). Compared with vehicle treatment, the CK level was decreased by 52.79% ($P=0.0046$) and 72.45% ($P=0.0003$) for the LOE-1X and LOE-5X groups, respectively. Our findings suggest that LOE supplementation could ameliorate skeletal muscle injury induced by acute exercise challenge. Trend analysis showed that LOE treatment had a significant dose-dependent effect on CK level ($P<0.0001$).

Blood glucose level is an important index for performance maintenance during exercise. As exercise continues, there is an increase in glucose uptake and a decrease in intramuscular glucose concentration as the hexokinase inhibition is relieved by a lower glucose 6-phosphate (G-6-P) concentration [17]. Serum glucose levels in the vehicle, LOE-1X and LOE-5X groups were 123 ±

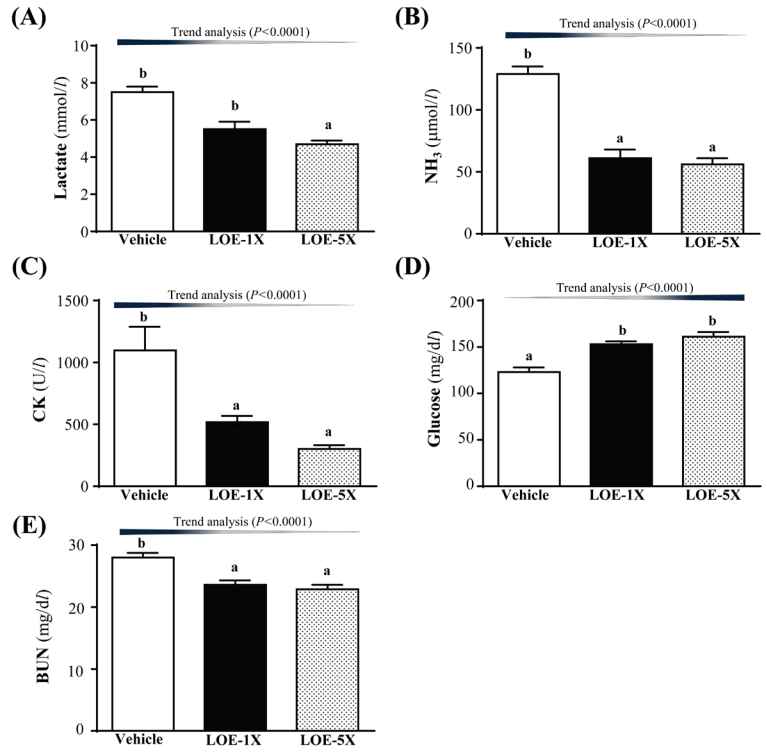

Fig. 4. Effect of LOE supplementation on serum levels of (A) lactate, (B) ammonia, (C) creatine kinase (CK), (D) glucose and (E) blood urea nitrogen (BUN) after acute exercise challenge. Data is expressed as mean ± SEM, n=8 mice/group. Analysis is using one-way ANOVA. Different letters (a, b) indicate a significant difference at $P<0.05$.

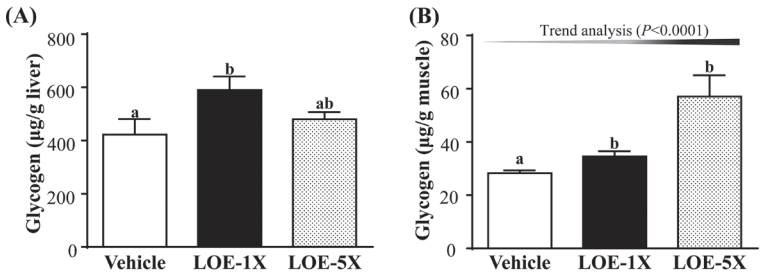

Fig. 5. Effect of LOE supplementation on glycogen levels in (A) liver and (B) muscle. Data is expressed as mean ± SEM, n=8 mice/group, by one-way ANOVA. Different letters (a, b) indicate a significant difference at $P<0.05$.

5, 153 ± 3 and 161 ± 5 mg/d*l*, respectively (Fig. 4C). The glucose levels of the LOE-1X and LOE-5X group were higher by 1.24- ($P=0.0004$) and 1.31-fold ($P<0.0001$) compared with the vehicle group. Trend analysis showed dose-dependent serum glucose level increase with increased LOE supplementation ($P<0.0001$). Taken together, our data indicates that continuous supplementation with LOE for 4 weeks could increase serum glucose levels and improve glucose uptake capacity toward beneficial anti-fatigue activity.

BUN is an important biochemical parameter related to fatigue and BUN level measures the amount of nitrogen in blood from the waste product of urea. Urea serves an important role in the metabolism of nitrogen-containing compounds. Serum BUN levels of the vehicle, LOE-1X and LOE-5X were 28.0 ± 0.8, 23.6 ± 0.7 and 22.9 ± 0.7 (mg/d*l*), respectively (Fig. 4E). Compared with vehicle group, the BUN level of the LOE-1X and LOE-5X was significantly decreased by 15.63% ($P=0.0009$) and 18.81% ($P=0.0002$), respectively. The trend analysis revealed that LOE treatment had a significant dose-dependent effect on BUN level ($P<0.0001$).

Effect of LOE supplementation for 4 weeks on hepatic and muscle glycogen level

We measured glycogen content of liver and muscle tissues (Fig. 5A and 5B). The liver glycogen levels in the vehicle, LOE-1X and LOE-5X groups were 4.22 ± 0.59, 5.90 ± 0.51 and 4.80 ± 0.27 mg/g liver, respectively. The LOE-1X group showed a significantly higher (1.40-fold, $P=0.0214$) liver glycogen level compared to the vehicle control group. The muscle glycogen in vehicle, LOE-1X and LOE-5X groups were 0.28 ± 0.01, 0.35 ± 0.02 and 0.57 ± 0.08 mg/g muscle, respectively. The muscle glycogen content for the LOE-5X group was significantly higher (2.02-fold, $P=0.0004$) than the vehicle group, suggesting that LOE supplementation could help increase muscle glycogen storage in the mice leading to enhanced energy utilization. In the trend analysis, muscle glycogen increased with the LOE dosage ($P<0.0001$).

General characteristics of mice with LOE supplementation for 4 weeks

Initial BW and final BW of the mice did not differ among the vehicle, LOE-1X and LOE-5X groups (Table 1). Similarly, daily

Table 1. General characteristics of mice with *L. octovalvis* extract (LOE) supplementation

Characteristic	Vehicle	LOE-1X	LOE-5X	Trend analysis
Initial BW (g)	34.59 ± 0.32	34.48 ± 0.48	34.53 ± 0.32	0.9721
Final BW (g)	37.61 ± 0.49	37.88 ± 0.38	38.00 ± 0.31	0.5334
Food intake (g/day)	7.59 ± 0.12	7.69 ± 0.13	7.71 ± 0.20	0.5726
Water intake (ml//day)	7.97 ± 0.13	7.90 ± 0.26	8.02 ± 0.13	0.5999
Liver (g)	1.82 ± 0.08	1.87 ± 0.02	1.81 ± 0.02	0.2179
Kidney (g)	0.60 ± 0.00	0.60 ± 0.02	0.62 ± 0.01	0.1561
Heart (g)	0.22 ± 0.01	0.21 ± 0.01	0.22 ± 0.01	0.9788
Lung (g)	0.21 ± 0.00	0.22 ± 0.00	0.22 ± 0.00	0.4959
Muscle (g)	0.38 ± 0.01	0.39 ± 0.00	0.38 ± 0.01	0.6695
EFP (g)	0.36 ± 0.02	0.36 ± 0.02	0.37 ± 0.02	0.8280
BAT (g)	0.075 ± 0.009[a)]	0.094 ± 0.003[b)]	0.090 ± 0.004[a,b)]	0.1720
Relative liver weight (%)	4.83 ± 0.17	4.93 ± 0.08	4.78 ± 0.07	0.4016
Relative Kidney weight (%)	1.60 ± 0.02	1.58 ± 0.04	1.63 ± 0.03	0.3901
Relative Heart weight (%)	0.58 ± 0.03	0.56 ± 0.02	0.57 ± 0.02	0.9419
Relative Lung weight (%)	0.57 ± 0.02	0.57 ± 0.01	0.58 ± 0.02	0.7591
Relative Muscle weight (%)	1.00 ± 0.02	1.04 ± 0.02	1.00 ± 0.02	0.9404
Relative EFP weight (%)	0.95 ± 0.05	0.95 ± 0.05	0.97 ± 0.06	0.9576
Relative BAT weight (%)	0.20 ± 0.02[a)]	0.25 ± 0.01[b)]	0.24 ± 0.01[a,b)]	0.1760

Data are mean ± SEM, n=8 mice/group. Different letters (a, b) in the same row indicate a significant difference at $P<0.05$. Food efficiency ratio: body weight (BW) gain (g/day)/food intake (g/day). Muscle mass includes both gastrocnemius and soleus muscles in the back part of the lower legs. BAT: brown adipose tissue; EFP: epididymal fat pad. Mice were treated with vehicle, LOE-1X, or LOE-5X for 4 weeks.

intake of diet and water were no different between the vehicle and treatment groups. We observed that LOE treatment did not affect water intake, diet or BW. The BW of the mice in each group steadily increased throughout the experimental period (Table 1). Analyses of the mice body compositions after 4-week LOE supplementation showed no significant difference in weights of liver, kidney, heart, lung, muscle and epididymal fat pad (EFP). Only the brown adipose fat (BAT) saw slight increase (1.25-fold, $P=0.0396$) in the LOE-1X group compared with vehicle treatment. We also examined the relative tissue weights at the end of 4 weeks LOE supplementation, which is calculated by the different tissue weights adjusted for by individual BW %. The relative BAT weight with the LOE-1X group was higher by 1.24-fold ($P=0.0396$) than vehicle group, suggesting a slight beneficial effect of LOE supplementation on BAT weight. Besides that, all relative weight of all other tissues did not differ significantly among the vehicle, LOE-1X and LOE-5X groups.

Effect of LOE supplementation on biochemical variables

We observed that 4-weeks LOE supplementation increased exhaustive exercise challenge time and optimized anti-fatigue indicators including lactate, ammonia, glucose, CK and BUN levels. Both liver and muscle glycogen storage capacity could also be increased by LOE supplementation. We further investigated whether the 4-week LOE treatment could affect other biochemical markers in the healthy mice by examining tissue- and health status-related biochemical variables and major organs including the skeletal muscle, heart, kidney and lung (Table 2).

Total protein and albumin levels did not differ among the groups ($P>0.05$, Table 2). Serum AST levels were lower by 24.28% ($P=0.0003$) and 27.43% ($P<0.0001$) with the LOE-1X and LOE-5X group respectively, compared with the vehicle group. Similarly, ALT levels were reduced by 28.31% ($P=0.0214$) and 24.62% ($P=0.0422$) with LOE-1X and LOE-5X, respectively. LDH levels were also significantly lower in the LOE-1X (30.44%, $P=0.0046$) and LOE-5X (28.09%, $P=0.0081$) than vehicle group. The serum CK of the mice in the LOE-1X and LOE-5X groups were lower by 46.78% ($P<0.0001$) and 54.78% ($P<0.0001$), respectively. BUN levels in LOE-1X and LOE-5X were significantly lowered by 19.18% ($P=0.0030$) and 20.74% ($P=0.0016$), respectively, compared with vehicle treatment. Serum creatinine levels in LOE-5X group was 17.49% ($P=0.0074$) lower than in vehicle group. The serum UA of the mice in the LOE-1X and LOE-5X groups were reduced by 20.38% ($P=0.0014$) and 38.86% ($P<0.0001$), respectively, while the serum TC and TG of the mice in the LOE-5X group were lowered by 14.73% ($P=0.0063$) and 27.11% ($P<0.0005$), respectively, compared to the vehicle group.

Trend analysis revealed that LOE treatment had significant dose-dependent reduction in levels of AST ($P<0.0001$), ALT ($P=0.0450$), LDH ($P=0.0016$), CK ($P<0.0001$), BUN ($P<0.0001$), creatinine ($P=0.0021$), UA ($P<0.0001$), TC ($P=0.0034$) and TG ($P<0.0001$). In addition, serum glucose was shown to be 1.11-fold higher ($P=0.0243$) in the LOE-5X compared to the vehicle group. Trend analysis also revealed significant ($P=0.0108$) dose-dependent increase on serum glucose levels. Taken together, our findings indicate that long-term daily supplementation with LOE may potentially protect muscle damage by decreasing AST, ALT, LDH, CK, BUN, creatinine and UA levels. Additionally, our results also suggest that LOE supplementation may have the potential to prevent lipid accumulation through reduced TC and TG.

Table 2. Biochemical analysis with LOE supplementation at the end of the experiment

Parameter	Vehicle	LOE-1X	LOE-5X	Trend analysis
AST (U/*l*)	135 ± 5[b)]	102 ± 5[a)]	98 ± 4[a)]	<0.0001 (↓)
ALT (U/*l*)	75 ± 5[b)]	54 ± 5[a)]	56 ± 6[a)]	0.0450 (↓)
LDH (U/*l*)	766 ± 71[b)]	533 ± 30[a)]	551 ± 24[a)]	0.0016 (↓)
CK (U/*l*)	835 ± 22[b)]	444 ± 24[a)]	377 ± 61[a)]	<0.0001 (↓)
TP (g/d*l*)	5.1 ± 0.0[a)]	5.1 ± 0.0[a)]	5.1 ± 0.1[a)]	0.9224
Albumin (g/d*l*)	3.1 ± 0.0[a)]	3.2 ± 0.0[a)]	3.1 ± 0.0[a)]	0.7858
BUN (mg/d*l*)	25.7 ± 1.4[b)]	20.8 ± 0.6[a)]	20.4 ± 0.5[a)]	<0.0001 (↓)
Creatinine (mg/d*l*)	0.28 ± 0.01[b)]	0.25 ± 0.01[b)]	0.23 ± 0.01[a)]	0.0021 (↓)
UA (mg/d*l*)	2.6 ± 0.1[b)]	2.1 ± 0.1[a)]	1.6 ± 0.1[a)]	<0.0001 (↓)
TC (mg/d*l*)	141 ± 4[b)]	143 ± 4[b)]	120 ± 4[a)]	0.0034 (↓)
TG (mg/d*l*)	145 ± 6[c)]	131 ± 6[b,c)]	106 ± 7[a)]	<0.0001 (↓)
Glucose (mg/d*l*)	147 ± 3[a)]	161 ± 4[a,b)]	164 ± 6[b)]	0.0108 (↑)

Data are mean ± SEM, n=8 mice/group. Different letters (a, b) in the same row indicate a significant difference at $P<0.05$ by one-way ANOVA. AST, aspartate aminotransferase; ALT, alanine aminotransferase; LDH, lactic dehydrogenase; CK, creatine kinase; TP, total protein; BUN, blood urea nitrogen; UA, uric acid; TC, total cholesterol; TG, triglycerides.

Effect of LOE supplementation on histological examinations at the end of the experiment

LOE supplementation for 4 weeks in the mice had no adverse effects on major organs such as the liver, skeletal muscle, heart, kidney, lung and EFP based on tissue morphology (Fig. 6). This suggests that the dose for LOE supplementation used in this study was safe.

DISCUSSION

According to previous studies, LOE has been shown to be an AMP activated protein kinase (AMPK) activator. AMPK is a major cellular energy sensor and metabolic regulator, and is considered to be a therapeutic target for treatment of diabetes mellitus [21]. Additionally, AMPK is a sensor of cellular energy status and plays a central role in skeletal muscle metabolism [14]. Either nutritional supplementation or exercise is able to activate AMPK in the heart [8]. Activation AMPK could increase skeletal muscle strength and cardiac function, especially during endurance exercise [19]. In our study, although the mice did not undergo a training intervention, LOE supplementation was able to extend endurance exercise times to improve exercise performance, possibly through LOE's activation of the AMPK pathway [20, 23, 29].

Exercise-induced muscle fatigue can be evaluated by various biochemical indicators, of which the most well-known biomarkers include lactate, ammonia, glucose, CK and BUN levels [13, 24]. Lactate threshold is a useful measure for deciding exercise intensity for training and racing in endurance exercise, with lactate accumulation being caused by changes in extracellular pH. A decrease in pH is required for lactate to efflux from the muscle [1]. Lactate clearance is expected to reduce peripheral neuromuscular fatigue and has positive effects on muscle function [26].

During exercise, the consumption of ATP exceeds the ATP supply, causing ATP/ADP (adenosine diphosphate) ratio decreases. The production of ammonia during exercise is the result of adenylate kinase transferring one energy-rich phosphate group from one ADP molecule to another, resulting in the formation of one ATP and one AMP molecule. AMP is deaminated to inosine monophosphate (IMP) and ammonia via the enzyme AMP deaminase [10]. Ammonia accumulation in blood has been observed in endurance athletes and exercise-related increase in blood ammonia is almost linear, regardless of the exercise intensity [16]. In previous studies, lower ammonia accumulation has been observed in endurance athletes [9]. Both serum lactate and ammonia concentration are useful biomarkers for exercise-related metabolic responses in competitive explosive force, as well as in endurance-trained athletes [16]. In this respect, LOE may have potential as an ergogenic supplement by improving the removal of these metabolic wastes during exercise.

Oleanolic acid and related derivatives such as triterpene acids and ursolic acid have been isolated from LOE [4]. Synthesis and biological evaluation of oleanolic acid and related derivatives have shown them to be novel inhibitors of glycogen phosphorylase [25]. Inhibition of glycogen phosphorylase leads to inhibition of glycogenolysis. ATP and glucose are also known glycogen phosphorylase inhibitors, but high concentrations of AMP can activate previously inactive glycogen phosphorylase to accelerate glycogenolysis [11]. Our findings suggest that LOE could increasing blood glucose and muscle glycogen breakdown redirecting blood flow toward skeletal muscle for enhanced performance, especially in enhance glucose storage in muscle, concomitantly enhancing endurance exercise performance.

In this study, we found that a 4-week supplementation with LOE extract could significantly improve performance of endurance exercise, with the mice showing extended swimming times to exhaustion. LOE's anti-fatigue activity is reflected by the decrease in plasma lactate, ammonia, CK, BUN and increased glucose levels, after acute exercise in mice. LOE also had positive effects on the liver, kidney, muscle protection and prevention of lipid accumulation by decreasing AST, ALT, LDH, CK, BUN, creatinine, UA levels, as well as TC and TG levels. In terms of glycogen storage capacity, our data clearly indicates that muscle glycogen increased with 4-week LOE-supplementation in a dose-dependent manner, suggesting that enhanced muscle glycogen storage was the main reason for extended swimming time to exhaustion.

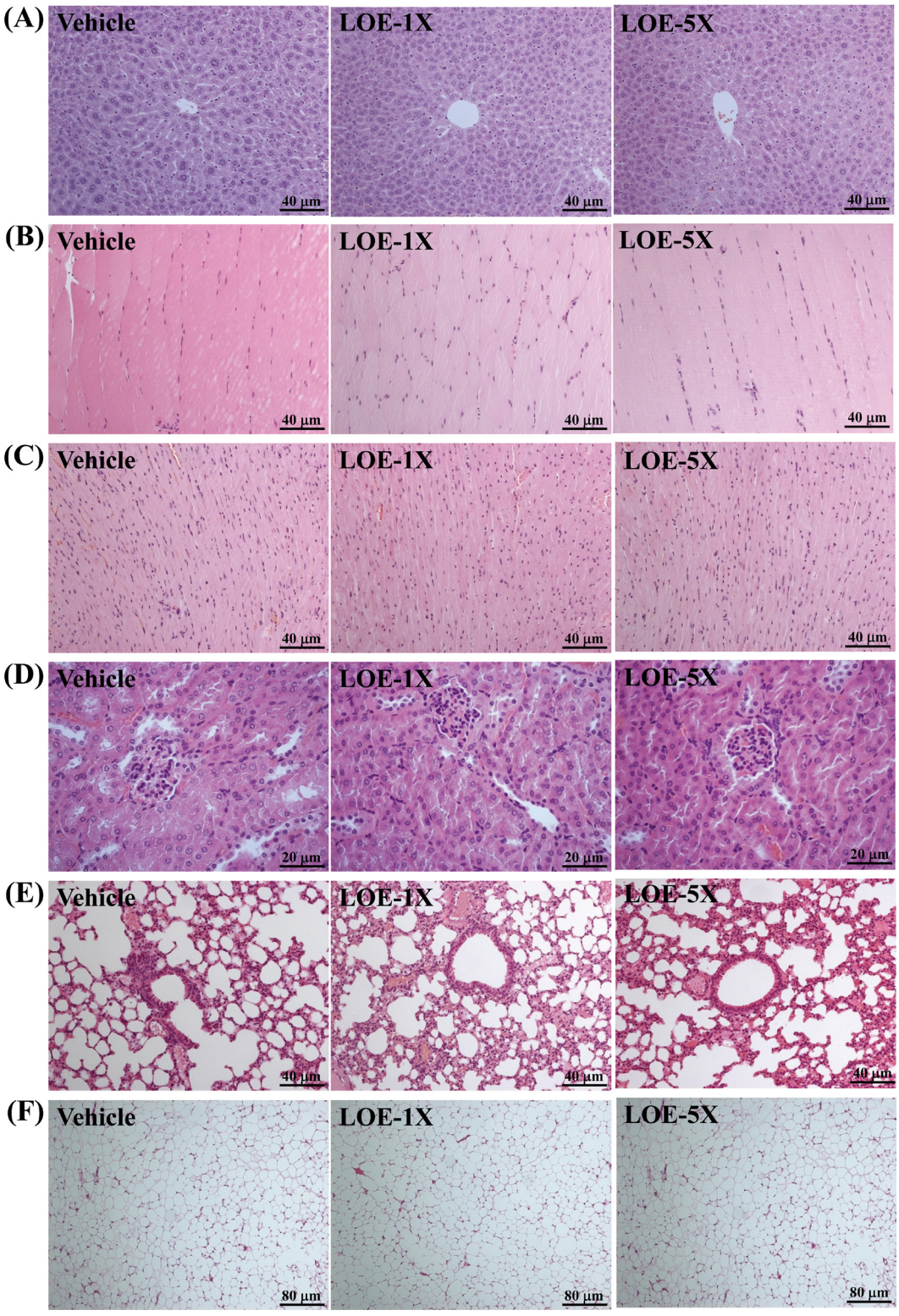

Fig. 6. Effect of LOE supplementation on morphology of (A) liver, (B) skeletal muscle, (C) heart, (D) kidney, (E) lungs and (F) epididymal fat pad (EFP). Specimens were photographed by light microscopy and stained with H&E. Magnification: × 200 (A–E) and × 100 (F).

In conclusion, our study has found that LOE supplementation for 4 weeks is able to considerably enhance endurance exercise performance, improve fatigue as assessed by the biochemical markers, as well as increase muscle glycogen. This suggests that LOE could potentially serve well as a nutrient supplement or healthy ergogenic aid to enhance muscle glycogen storage and maximize energy utilization during exercise.

CONFLICTS OF INTEREST. The authors declare no conflict of interest.

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