



## Anti-fatigue effects of *Elaeagnus multiflora* fruit extracts in mice

Myung-A Jung<sup>1</sup> · Ara Jo<sup>1</sup> · Jawon Shin<sup>1</sup> · Huwon Kang<sup>1</sup> · Yujin Kim<sup>1</sup> ·  
Dool-Ri Oh<sup>1</sup> · Chul-yung Choi<sup>1</sup>

Received: 4 February 2020 / Accepted: 24 February 2020 / Published Online: 31 March 2020  
© The Korean Society for Applied Biological Chemistry 2020

**Abstract** The fruit, leaves, and roots of *Elaeagnus multiflora* Thunb. have been used in traditional Chinese medicine to treat cough, diarrhea, and itching. However, the anti-fatigue effects of *E. multiflora* fruit (EMF) extract have not been studied in detail. The aim of this study was to examine the effect of EMF on fatigue and exercise performance in BALB/c mice. EMF was orally administered to mice at four doses (10, 50, 100, and 200 mg/kg/day) for 2 weeks. The anti-fatigue activity was evaluated by determining the exhaustive swimming time. Blood lactate and glucose levels and serum lactate levels after a 10 min swimming time, as well as ammonia, creatine kinase (CK), blood urea nitrogen (BUN), lactate dehydrogenase (LDH), and glycogen contents after exhaustive swimming time were measured. The exhaustive swimming time of the EMF 200 group was significantly increased ( $p < 0.01$ ). The EMF groups showed significantly low levels of CK, BUN, LDH, and lactate compared with the control group ( $p < 0.05$ ). Increased liver glycogen was observed in the EMF 200 group ( $p < 0.05$ ). These results suggest that EMF can be utilized as an efficacious natural resource for its anti-fatigue effects.

**Keywords** Anti-fatigue · Blood urea nitrogen · Creatine kinase · *Elaeagnus multiflora* · Glycogen; lactate · Swimming time

### Introduction

Fatigue is best characterized as trouble initiating and maintaining voluntary mental and physical activities and is accompanied by a feeling of extreme physical or mental exhaustion resulting from hard physical labor, mental work, and social relationships [1]. It is generally characterized by physiological, psychological, and pathological fatigue [2]. Fatigue is accompanied by a feeling of extreme physical or mental exhaustion, resulting from hard physical labor, mental work, and social relationships [3]. Thus, fatigue can cause various disorders related to bio-regulatory, autonomic nervous, endocrine, and immune systems [4]. These disorders can result in a reduction in exercise intensity or even interruption of activity [5]. According to the exhaustion theory, many energy sources such as glucose and liver glycogen are exhausted during exercise, leading to physical fatigue [6]. Other biomarkers, such as lactate, ammonia (NH<sub>3</sub>), creatine kinase (CK), lactic dehydrogenase (LDH), blood urea nitrogen (BUN), and glucose, are widely used to assess fatigue [7-9]. Serum BUN is the by-product of protein and amino acid metabolism. CK and LDH are known to be accurate indicators of muscle damage. Lactate has been proposed as an active metabolite that plays vital roles in producing muscle glycogen and in causing muscle fatigue [11,12]. There are many factors other than renal disease that can alter BUN levels, such as protein breakdown, stress, and fatigue. In particular, BUN levels were found to increase significantly after exercise [13].

Previous studies have shown that herbal medicines and foods are important resources that improve exercise performance and reduce fatigue [14].

*Elaeagnus multiflora* Thunb., commonly called oriental cherry silverberry, belongs to the family Elaeagnaceae. Several parts of *Elaeagnus multiflora* Thunb., commonly called oriental cherry silverberry, have long been used in traditional Chinese medicine for the treatment of cough, diarrhea, and itching [15-17]. *E. multiflora* extracts have been shown to have pharmacological properties including anticancer and antioxidant activities [18,19].

Chul-yung Choi (✉)  
E-mail: blockstar@hanmail.net

<sup>1</sup>Jeonnam Bioindustry Foundation, Center of Natural Resources Research (JCNR), Jeollanamdo 59338, Republic of Korea

This is an Open Access article distributed under the terms of the Creative Commons Attribution Non-Commercial License (<http://creativecommons.org/licenses/by-nc/3.0/>) which permits unrestricted non-commercial use, distribution, and reproduction in any medium, provided the original work is properly cited.

In addition, compounds isolated from EMF have been shown to have physiological effects [20–22]. However, the anti-fatigue effects of EMF extract have not been investigated in detail.

In this study, we aimed to investigate whether EMF improves exercise performance and physical fatigue by using an exhaustive swimming test, biomarker analysis, and tissue glycogen measurement in mice.

## Materials and Methods

### Preparation of EMF

The EMF used in this study were collected in Yeosu, Jeollanamdo, Republic of Korea and authenticated by Dr. Choi at the Jeonnam Bioindustry Foundation, Center of Nature Resources Research (Jangheung, Jeolloanamdo, Republic of Korea). EMF were washed two times with distilled water. Briefly, the plant material (1 kg) was extracted with 10 L distilled water at 100 °C for 4 h. The residue was removed by filtration through a 150 mm mesh. The filtrate was evaporated under vacuum and freeze-dried to obtain EMF powder (124 g). The dried EMF extract was stored at 4 °C until it was used in the assays.

### Animals

Male, 5-week-old BALB/c mice weighing approximately 20–23 g were purchased from Samtako Bio Korea (Osan, South Korea). The mice were housed in plastic cages at a controlled temperature (22±2 °C) and humidity (50±5%), with 100 % fresh HEPA-filtered air and a 12:12 h light:dark cycle (lights on at 08:00 AM). All experimental procedures were conducted in accordance with the guidelines for the care of experimental animals and approved by the Institutional Animal Care and Use Committee (IACUC) of the Jeollanamdo Institute of Natural Resources Research (approval number JINR-1803).

### Experimental groups and drug administration

The mice were acclimated for a one-week period and were then divided into six groups based on body weight (20–23 g, n=6 per group): no administration of EMF (control), 100 mg/kg of taurine (Taurine), 10 mg/kg of EMF (EMF 10), 50 mg/kg of EMF (EMF 50), 100 mg/kg of EMF (EMF 100), and 200 mg/kg of EMF (EMF 200). Taurine, a known anti-fatigue agent, was used as the positive control. Control group mice were fed only distilled water. The EMF and Taurine doses were administered for 2 weeks. The mice were trained to swim twice per week during the 2-week administration period to accustom themselves to swimming. Body weight was measured at the end of each week. After the end of the treatment period, the mice were fasted for 12 h.

### Adjustable-current swimming pool

One hour after administration of the last treatment, swimming capacity was measured as described previously [23]. An acrylic

plastic pool (90×45×45 cm) was used to determine the swimming capacity. The pool was filled with water to a depth of 35 cm (temperature: 30±1 °C). The pool current was generated by circulating water with a pump, which included spout and suction parts. The water current was adjusted by controlling the voltage supplied to the pump and monitored with a water flowmeter. Swimming time was measured at a flow rate of 8 L/min. Exhaustion, defined by the stop of movement and failure to return to the surface within 7 s, was evaluated.

### Blood lactate and glucose analysis

At the end of the 12 h fasting period, after the last administration of EMF, blood was collected from the tail vein (before swimming and after 10 min swimming). Lactate and glucose levels were measured using an autoanalyzer.

### Biochemical analysis

At the end of the 12 h fasting period and completion of the swimming exercise, blood was collected from the abdominal artery. Serum was obtained by centrifuging the blood at 9,000× g for 20 min. BUN was measured in the serum using the appropriate kit (DRI-CHEM 4000i; Fujifilm, Tokyo, Japan). LDH and CK contents were measured in the serum using the respective kits (Biovision, CA, USA) according to the manufacturer's instructions.

### Tissue glycogen determination

The mice were killed immediately after blood collection, and the liver and muscle tissues were excised and weighed for glycogen content analysis. Glycogen was measured using a glycogen colorimetric assay kit (Biovision, CA, USA), according to the manufacturer's instructions.

### Statistical analysis

The results are presented as the mean standard error of the mean (SE). The data were statistically evaluated using one-way analysis of variance (ANOVA), followed by Dunnett's multiple comparison test using the GraphPad Prism (GraphPad Inc., San Diego, CA, USA) software program. P-values less than 0.05 were considered statistically significant.

## Results and Discussion

### Body and organ weights

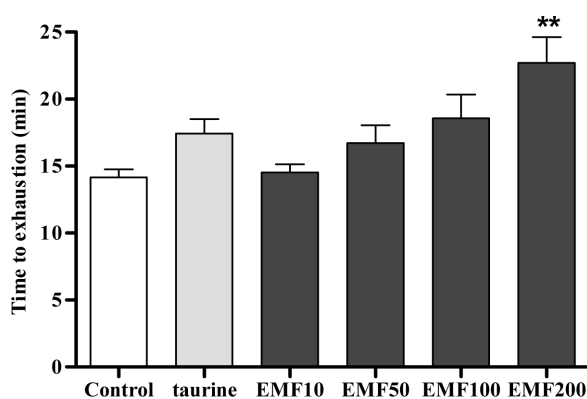
The body, gastrocnemius muscle, and liver weights of mice treated with EMF for 2 weeks are shown in Table 1. In the control group, the body weights did not change during the first week of treatment but gradually increased from week 2 until the end of the experiment. These results indicated no significant changes in body, gastrocnemius muscle, and liver weights among the groups. EMF effects on exhaustive swimming test

Swimming time is considered an appropriate scale for measuring

**Table 1** Effect of *Elaeagnus multiflora* fruit extract on relative body and organ weights of mice

Group	Initial BW (g)	Final BW (g)	Muscle (g)	Liver (g)
Control	21.47±0.33	22.31±0.44	0.23±0.01	1.03±0.05
Taurine	21.51±0.32	22.58±0.58	0.24±0.01	1.06±0.01
EMF 10	21.47±0.29	22.58±0.58	0.25±0.01	1.08±0.05
EMF 50	21.46±0.29	21.95±0.43	0.23±0.01	1.07±0.02
EMF 100	21.47±0.29	22.95±0.28	0.25±0.01	1.04±0.02
EMF 200	21.44±0.29	22.12±0.33	0.24±0.01	0.98±0.01

Control, no administration of EMF; Taurine, 100 mg/kg taurine; EMF 10, 10 mg/kg EMF; EMF 50, 50 mg/kg EMF; EMF 100, 100 mg/kg EMF; EMF 200, 200 mg/kg EMF per day; BW, body weight. The values are the mean ± standard error (SE)



**Fig. 1** Effect of *Elaeagnus multiflora* fruit (EMF) extract on swimming exercise performance in BALB/c mice. The mice were assessed after 14 days. An exhaustive swimming exercise was performed 1 h later. Control, no administration of EMF; Taurine, 100 mg/kg taurine; EMF 10, 10 mg/kg EMF; EMF 50, 50 mg/kg EMF; EMF 100, 100 mg/kg EMF; and EMF 200, 200 mg/kg EMF per day. The values are the mean standard error (SE). Asterisks above the bars indicate significant differences, as determined by the Dunnett's multiple comparison test ( $p < 0.01$ )

fatigue level objectively and quantitatively [24]. The anti-fatigue activity of EMF was determined as the swimming endurance capacity of mice by using an adjustable current swimming pool. The results showed that the swimming time to exhaustion of each EMF-treated group increased when compared with that of the control group (Fig. 1). The exhaustive swimming time of EMF 200 group was significantly longer than that of the control group ( $p < 0.01$ ). These results suggest that EMF enhanced swimming capacity by delaying the onset of physical fatigue in mice.

#### Effect of EMF on blood lactate and glucose

Lactate is produced by glycolysis under anaerobic conditions during high-intensity exercise. Therefore, blood lactate is an important parameter for determining the degree of fatigue after exercise [25,26]. Increased lactate level results in decreased pH value, which can have a detrimental effect on glycolysis, enzyme activities, and muscular contractions due to calcium ion release [27]. In the present study, the blood lactate levels before the swimming test did not significantly differ among the treatment

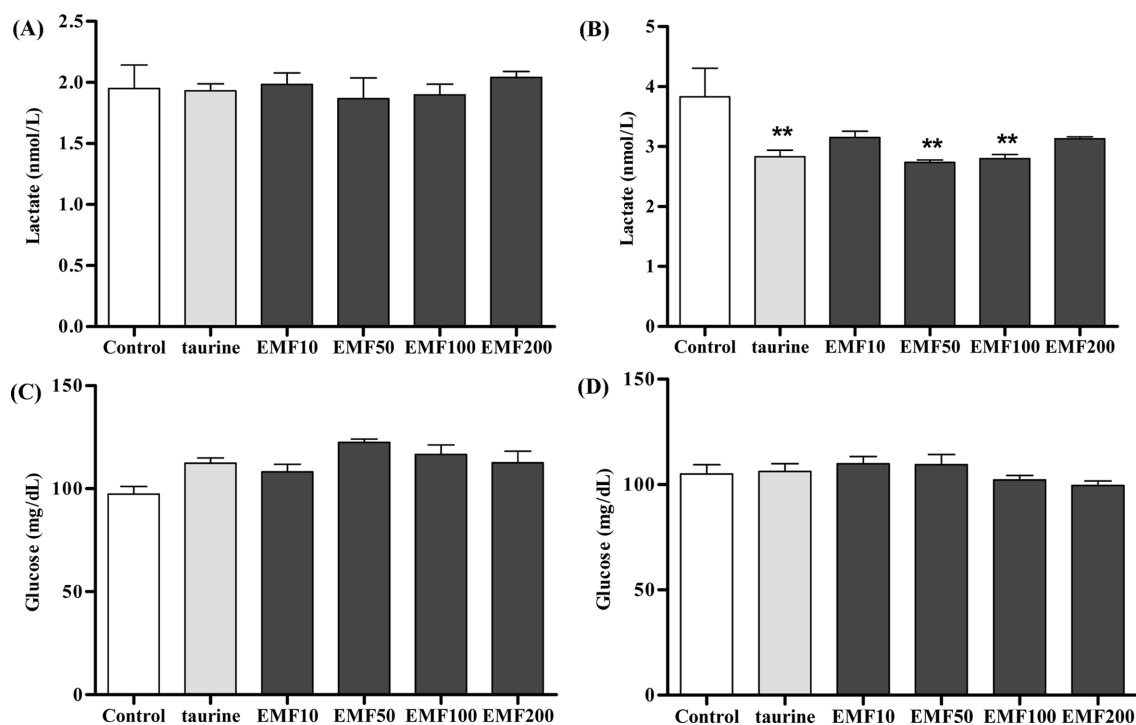
and control groups (Fig. 2A). However, after the 10 min swimming test, the blood lactate levels were significantly lower in the EMF groups than in the control group (Fig. 2B,  $p < 0.01$ ). Likewise, glucose levels did not differ significantly among the treatment and control groups either before or after the 10 min swimming test (Figs. 2C, D).

#### Effect of EMF on clinical biochemistry tests after acute exercise challenge

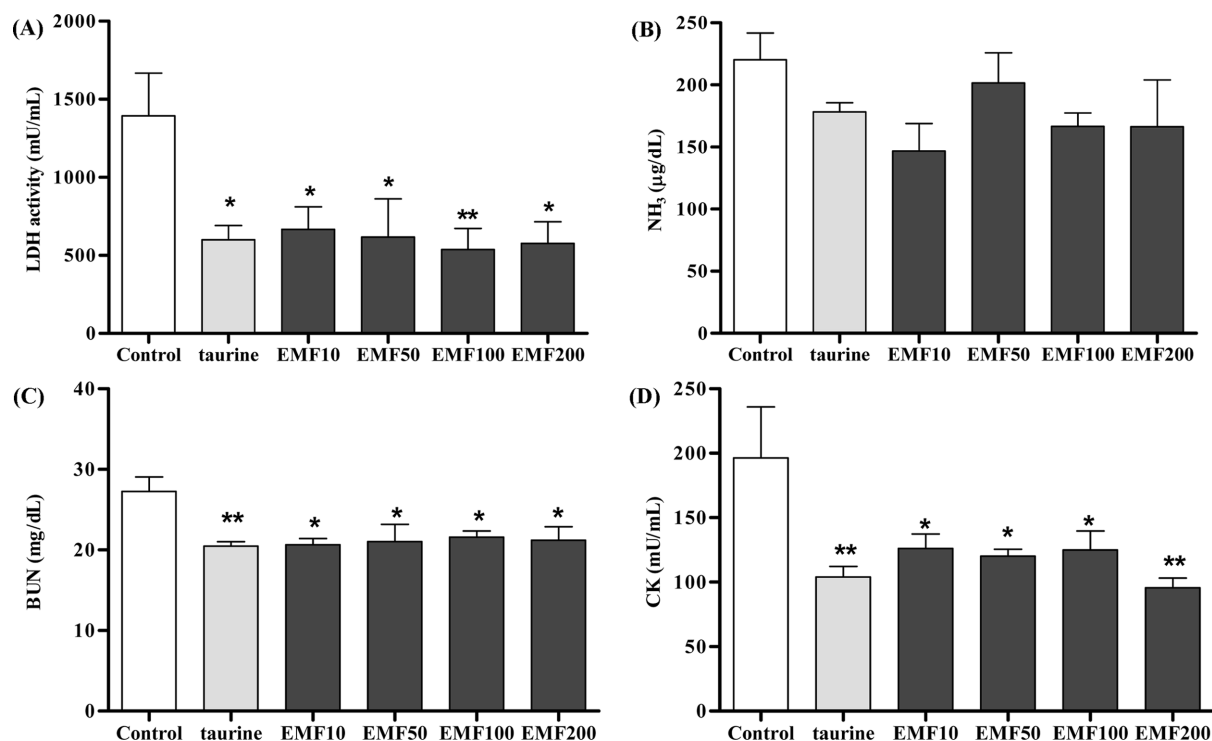
LDH activity was significantly lower in the EMF groups than in the control group (Fig. 3A). EMF decreased blood lactate levels (Fig. 2B) by decreasing LDH activity, thereby reducing the fatigue of muscles by preventing energy depletion during exercise (Fig. 3A) and reducing the excessive accumulation of fatigue indicators. The increase in  $\text{NH}_3$  levels is associated with peripheral as well as central fatigue during exercise [28]. The EMF groups tended to show low  $\text{NH}_3$  levels. However, the difference in  $\text{NH}_3$  levels between the control and EMF groups was not significant (Fig. 3B). Several factors including protein breakdown, stress, and fatigue can cause BUN alteration [25,29]. The BUN levels are shown in Fig. 3C. After swimming, the BUN levels were significantly lower in the EMF groups than in the control group ( $p < 0.05$ ). CK serum activity has been shown to delay increase in structural muscle damage [30]. In the present study, compared with the control, the EMF groups showed a significant decrease in CK activity (Fig. 3D). The rapid recovery of serum CK with EMF could have contributed to exercise performance by ameliorating fatigue.

#### Effect of EMF on liver and muscle glycogen after acute exercise challenge

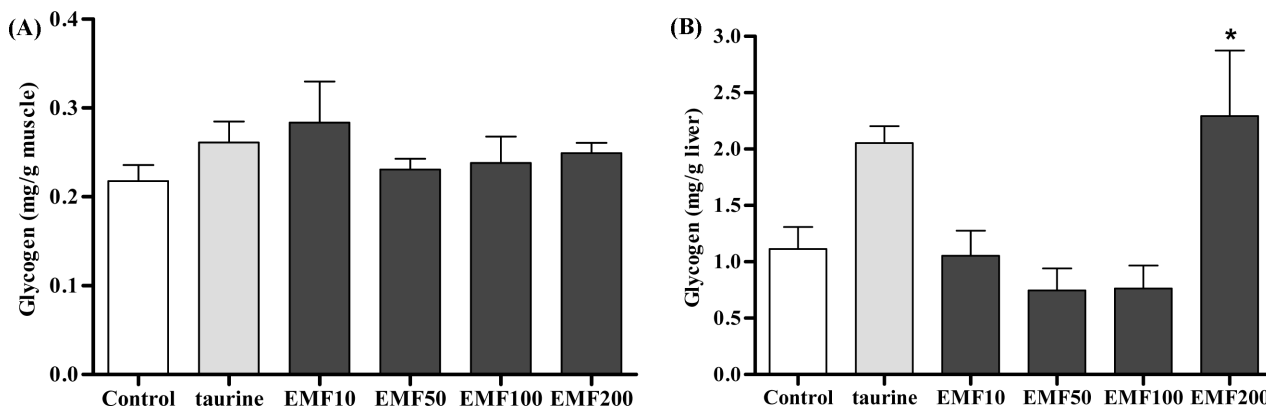
Glucose is stored as glycogen, which is mainly found in the liver and muscle tissues [31]. The endurance capacity of the body is markedly decreased if energy is exhausted. Since glycogen is the most important source of glucose during fatigue, an increase in liver glycogen level contributes to augmented endurance during exercise [26]. Previous studies have shown the importance of muscle glycogen levels in endurance exercise [32] and suggested that low muscle glycogen was a factor causing fatigue and exhaustion [33]. As shown in Fig. 4A, the concentration of liver



**Fig. 2** Effect of *Elaeagnus multiflora* fruit (EMF) extract on serum (A, B), lactate (C, D) and glucose levels before (A, C) and after (B, D) 15 min swimming test. BALB/c mice were administered 10, 50, 100, and 200 mg/kg EMF for 14 days. Control, no administration of EMF; Taurine, 100 mg/kg taurine; EMF 10, 10 mg/kg EMF; EMF 50, 50 mg/kg EMF; EMF 100, 100 mg/kg EMF; and EMF 200, 200 mg/kg EMF per day. The values are the mean SE. Asterisks above the bars indicate significant differences, as determined by the Dunnett's multiple comparison test ( $p < 0.01$ )



**Fig. 3** Effect of *Elaeagnus multiflora* fruit (EMF) extract on serum (A) lactate dehydrogenase (LDH); (B) ammonia; (C) blood urea nitrogen (BUN); and (D) creatine kinase (CK) levels after acute exercise. BALB/c mice were administered 10, 50, 100, and 200 mg/kg EMF for 14 days. Control, no administration of EMF; Taurine, 100 mg/kg taurine; EMF 10, 10 mg/kg EMF; EMF 50, 50 mg/kg EMF; EMF 100, 100 mg/kg EMF; and EMF 200, 200 mg/kg EMF per day. The values are the mean standard error (SE). Asterisks above the bars indicate significant differences, as determined by the Dunnett's multiple comparison test ( $*p < 0.05$  and  $**p < 0.01$ )



**Fig. 4** Effect of *Elaeagnus multiflora* fruit (EMF) extract on muscle (A) and hepatic (B) glycogen levels at the end of the experiments. Control, no administration of EMF; Taurine, 100 mg/kg taurine; EMF 10, 10 mg/kg EMF; EMF 50, 50 mg/kg EMF; EMF 100, 100 mg/kg EMF; and EMF 200, 200 mg/kg EMF per day. The values are the mean standard error (SE). Asterisk above the bars indicate significant differences, as determined by the Dunnett's multiple comparison test ( $p < 0.05$ )

glycogen in EMF 200 group was higher than that in the control group ( $p < 0.05$ ). The glycogen contents of muscle did not differ significantly among the groups (Fig. 4B). The mechanism underlying the effects of EMF in exercise performance should be elucidated in future studies.

**Acknowledgments** This study was supported by the Support Program for Creative Industry Institutes (Commercial Biotechnology Sophistication Platform Construction Program, R0003950), funded by the Ministry of Trade, Industry & Energy (MOTIE, Korea).

## References

- You L, Zhao M, Regenstein JM, Ren J (2011) In vitro antioxidant activity and *in vivo* anti-fatigue effect of loach (*Misgurnus anguillicaudatus*) peptides prepared by papain digestion. *Food Chem* 124: 188–194
- Ament W, Verkerke GK (2009) Exercise and fatigue. *Sports Med* 39: 389–422
- Huang LZ, Huang BK, Ye Q, Qin LP (2011) Bioactivity guided fractionation for anti-fatigue property of *Acanthopanax senticosus*. *J Ethnopharmacol* 133: 213–219
- Maes M, Song C, Lin A, De JR, Van GA, Keins G, Bosmans E, De Meester I, Benoy I, Neels H, Demedts P, Janca A, Scharpe S, Smith RS (1998) The effects of psychological stress on humans: increased production of pro-inflammatory cytokines and a Th1-like response in stress-induced anxiety. *Cytokine* 10: 313–318
- Davis JM, Bailey SP (1997) Possible mechanisms of central nervous system fatigue during exercise. *Med Sci Sports Exerc* 29: 45–47
- Wang L, Zhang HL, Lu R (2008) The decapeptide CMS001 enhances swimming endurance in mice. *Peptides* 29: 1176–1182
- Huang CC, Tsai SC, Lin WT (2008) Potential ergogenic effects of L-arginine against oxidative and inflammatory stress induced by acute exercise in aging rats. *Exp Gerontol* 43: 571–577
- Ni W, Gao T, Wang H, Du Y, Li J, Li C, Wei L, Bi H (2013) Anti-fatigue activity of polysaccharides from the fruits of four Tibetan plateau indigenous medicinal plants. *J Ethnopharmacol* 150: 529–535
- Huang WC, Lin CI, Chiu CC, Lin YT, Huang WK, Huang HY, Huang CC (2014) Chicken essence improves exercise performance and ameliorates physical fatigue. *Nutrients* 6: 2681–2696
- Cori TG, Cori CF (1993) Changes in hexosephosphate, glycogen, and lactic acid during contraction and recovery of mammalian muscle. *J Biol Chem* 99: 493–505
- Fabiato A, Fabiato F (1978) Effects of pH on the myofilaments and the sarcoplasmic reticulum of skinned cells from cardiac and skeletal muscles. *J Physiol* 276: 233–255
- Kristensen M, Albertsen J, Rentsch M, Juel C (1993) Lactate and force production in skeletal muscle. *J Physiol* 562: 521–526
- Wu IT (1999) The effects of serum biochemical value with different beverage to replenish and intermittent exercise in high intensity. *Tahan Jr Coll Eng Bus J* 13: 387–400
- Yeh TS, Chuang HL, Huang WC, Chen YM, Huang CC, Hsu MC (2015) Astragalus membranaceus improves exercise performance and ameliorates exercise-induced fatigue in trained mice. *Molecules* 19: 2793–2807
- Sakamura F, Suga T (1987) Changes in chemical components of ripening oleaster fruits. *Phytochemistry* 26: 2481–2484
- Kim CG (1984) Illustrated natural drugs encyclopedia. Namsandang, Seoul
- Yoon KY, Hong JY, Nam HS, Moon YS, Shin SR (2007) Antioxidant activities and xanthine oxidase inhibitory effects of hot-water extracts from fruits of *Elaeagnus multiflora* Thunb. in maturity. *J Korean Soc Food Sci Nutr* 36: 14–19
- Hong JY, Nam HS, Lee YS, Yoon KY, Kim NW, Shin SR (2006) Study on the antioxidant activity of extracts from the fruit of *Elaeagnus multiflora* Thunb. *Korea J Food Preserv* 13: 413–419
- Kim SA, Oh SI, Lee MS (2007) Antioxidative and cytotoxic effects of solvent fractions from *Elaeagnus multiflora*. *Korean J Food Nutr* 20: 134–142
- Kim NW, Joo EY, Kim SL (2003) Analysis on the components of the fruit of *Elaeagnus multiflora* Thunb. *Korean J Food Preserv* 10: 534–539
- Hong JY, Nam HS, Lee YS, Kim NW, Shin SR (2006) Anti-oxidant activity of ethanol extracts from fruits of *Elaeagnus multiflora* Thunb. during maturation. *Korean J Food Preserv* 13: 643–648
- Shin SR, Hong JY, Yoon KY (2008) Antioxidant properties and total phenolic contents of cherry elaeagnus (*Elaeagnus multiflora* Thunb.) leaf extract. *Food Sci Biotechnol* 17: 608–612
- Matsumoto K, Ishihara K, Tanaka K, Inoue K, Fushiki T (1996) An adjustable-current swimming pool for the evaluation of endurance capacity of mice. *J Appl Physiol* 81: 1843–1849
- Tanaka M, Nakamura F, Mizokawa S, Matsumura A, Nozaki S, Watanabe Y (2003) Establishment and assessment of a rat model of fatigue. *Neurosci Lett* 352: 159–162
- Wang JJ, Shieh MJ, Kuo SL, Lee CL, Pan TM (2006) Effect of red mold rice on antifatigue and exercise. *Appl Microbiol Biotechnol* 70: 247–253

26. Yu B, Lu ZX, Bie MR, Lian XZ (2008) Scavenging and anti-fatigue activity of fermented defatted soybean peptides. *Eur Food Res Technol* 226: 415–421
27. Cairns SP (2006) Lactic acid and exercise performance: Culprit or friend? *Sports Med* 36: 279–291
28. Wang SY, Huang WC, Liu CC, Wang MF, Ho CS, Huang WP, Hou CC, Chuang HL, Huang CC (2012) Pumpkin (*Cucurbita moschata*) fruit extract improve physical fatigue and exercise performance in mice. *Molecules* 17: 11864–11876
29. Shang H, Cao S, Wang JH, Zheng H, Putheti R (2009) Glabridin from Chinese herb licorice inhibits fatigue in mice. *Afr J Tradit Complement Altern Med* 7: 17–23
30. Appell HJ, Soares JMC, Duarte JAR (1992) Exercise, muscle damage and fatigue. *Sports Med* 13: 108–115
31. Hsu YJ, Huang WC, Chiu CC, Liu YL, Chiu WC, Chiu CH, Chiu YS, Huang CC (2016) Capsaicin supplementation reduces physical fatigue and improves exercise performance in mice. *Nutrients* 8: 648
32. Dohm GL, Tapscott EB, Barakat HA, Kasperek GL (1983) Influence of fasting on glycogen depletion in rats during exercise. *J Appl Physiol Respir Environ Exerc Physiol* 55: 830–833
33. Jung K, Kim I, Han D (2004) Effect of medicinal plant extracts on forced swimming capacity in mice. *J Ethnopharmacol* 93: 75–81