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Original Research

Anti-Fatigue Effect of *Viola odorata* L. in Forced Swimming Test in Rat

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Abstract

Fatigue is a complex phenomenon that is explained as difficulty starting or keeping voluntary physical or mental activity leading to negative impacts on life and work performance. This study aimed to investigate the anti-fatigue effects of *Viola odorata* L. in an animal model. Aqueous and ethanol extracts of *V. odorata* were prepared and total phenolics content was determined. Then, the anti-fatigue activity of the extracts was evaluated via a weight-loaded forced swimming test in the rat. To this end, 48 male Wistar rats were randomly classified into 6 groups. The control group using distilled water and other groups with ethanol (EVO; 50, 100, 200 mg/kg) and aqueous extracts of *V. odorata* (WVO; 50, 100mg/kg) were gavaged once daily for four weeks. Then, the forced swimming was conducted and swimming time, as a fatigue factor was measured. In addition, to validate the effect of *V. odorata* on the endurance capacity of the rats, biochemical factors including glucose, lactate dehydrogenase (LDH), and tumor necrosis factor (TNF-*α*) were examined in the serum. Hepatotoxicity was also assessed using hematoxylin-eosin staining. Our data indicated that the forced swimming time of the EVO-100, EVO-200, and WVO-100 groups was significantly increased. The serum glucose in the group which received EVO-200 was increased significantly; while serum LDH levels in all treated groups were significantly decreased. Also, the serum level of TNF-α in the groups which received EVO-100 or 200 was increased significantly. However, there was no considerable difference in serum TNF-*α* level and no hepatotoxicity within aqueous extract groups. Pathology results showed fewer effects of the aqueous extract rather than ethanol extract on the liver. The results provide evidence for the development and use of *V. odorata* aqueous extract as an anti-fatigue supplement.

Keywords: Fatigue; Forced swimming test; *Viola odorata*

Introduction

Fatigue is a complex phenomenon that is explained as mental or physical weariness leading to negative influences on exercise severity, family life, and work performance. The incidence of physical fatigue can be described through at least two mechanisms of energy exhaustion and oxidative stress. Exhaustion theory indicates that fatigue results from energy source depletion, extra metabolite accumulation, and reactive free radicals leading to tissue damage [1]. Fatigue categorize as secondary, chronic, or physiologic. Secondary fatigue is caused by disturbed sleep, excess exertion, depression, and side effects of medication. Chronic fatigue syndrome includes determined unexplainable

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fatigue taking for over 6 months; however, there is no clear etiology [2]. Physiological fatigue is the consequence of extreme physical loading, mental pressure, and insufficient rest that further categorize as peripheral and central fatigue [3]. Up to now, pharmacological drugs or treatments for fatigue cannot yet satisfy the requirement of people; therefore, along with the speed of modern life and increasing fatigue in society, the requirement to find out anti-fatigue health, natural foods, and medications with no side effects is crucial. The useful effects of nutraceuticals and herbal medicine on physical fatigue have been broadly explored in recent years [4]. So far, several plant species have been used to relieve fatigue and many of them have been retrieved from traditional or folklore medicine. Ethnobotanical investigations performed by Traditional Medicine and Materia Medica Research Center (TMRC) to assess the utilization of medicinal plants by local people [5] have proved the usage of *Viola odorata* as an anti-fatigue tea in the Golestan province of Iran.

The medicinal plant, *V. odorata* L. (Violaceae), is commonly identified as "Banafshah" and sweet violet in north & west Asia and north Europe. According to numerous studies, *V. odorata* was reported to demonstrate extensive bioactivities, such as antihypertensive, antidyslipidemic, antimutagenic, diuretic, laxative, expectorant, antibacterial, antimalarial, and antioxidant effects [6,7]. However, inadequate evidence exists regarding the effects of *V. odorata* on exercise performance and physical fatigue. The evidence demonstrates the advantageous effects of antioxidants on fatigue. Different defensive paths were expressed by antioxidants versus the free radical-induced oxidative stress, some of them can play role in reducing physical fatigue [8]. Several pharmacological models exist for evaluating anti-fatigue properties. The forced swimming test is a rodent behavioral test for examining the anti-fatigue effect of agents [9]. In this study, the anti-fatigue effect of *V. odorata* has been investigated through the forced swimming test in rats.

Materials and Methods

Ethics

The processes in this study were consistent with the Animal Ethics Committee of the Shahid Beheshti University of Medical Sciences (code: IR. SBMU. RETECH. REC. 1398. 030).

Plant material and extraction

Viola odorata was collected from Mazandaran, Lavij to Lariz, Baharsare region in spring 2018 and identified in the herbarium of TMRC with voucher number 1467.

Preparation of aqueous extract

The total part of *V. odorata* was crushed into small parts and infused for 10 min at 90°C with water (1:10). The extract (WVO) was centrifuged at 2000 rpm for 5 min and the supernatants were filtered via filter paper and dried by freeze-drying method.

Preparation of ethanol extract

Dried powder of *V. odorata* was extracted by ethanol 96% (1:10), three times. The filtered ethanol extract (EVO) was evaporated to dryness using a rotary evaporator at 40°C.

Total phenolic contents investigation

The total phenolic content of aqueous and alcoholic extracts was determined using the Folin-Ciocalteu reagent [10,11].

Animals and experiment design

Total of 48 male Wistar rats (180-200 g, 12 weeks old) were bought from the animal care laboratory of Shahid Beheshti University of Medical Sciences. The rats were kept at %40-60 relative humidity and ambient temperature (23±2°C), with a 12:12 hour light-dark cycle. Water and food were made available.

One week before the experiments, the animals were adapted to the diet and environment. First, the rats were classified into six groups (8 rats in each group): the control group receiving 0.5 mL D.W, EVO-50, EVO-100, and EVO-200 groups which received ethanol extract including 50,100,200 mg/kg/day respectively, WVO-50 and WVO-100 groups receiving the aqueous extract of *V. odorata* with 50 or 100 mg/kg/day. All groups were administered 0.5 mL samples by oral gavage once daily for four weeks.

Forced swimming test

The forced swimming test was performed for one hour followed by the last administration [1]. The rats were located individually in a container (a radius of 20 cm and length of 65 cm) filled with 40 cm fresh water depth kept at 25 ± 1°C. A constant weight equal to %5 of body weight was attached to the rat tail's root. Fatigue was defined by detecting loss of harmonized actions and failure in returning to the surface within 10 seconds while recording the swimming time directly.

Biochemical parameters

After the swimming test, the blood was sampled from the cardiac puncture and collected in a micro-tube without the anti-coagulant. The blood samples were centrifuged at 2000 rpm for 10 min for separating the serum. Serum glucose and lactate dehydrogenase (LDH) levels were determined using an auto-analyzer and the serum level of tumor necrosis factor α (TNF-α) was evaluated by ELISA reader.

Histopathology in liver

In the end, the animals were killed to collect their liver tissues and fix them in 10% buffered formalin. Then they were embedded in paraffin and then cut into slices with 4-mm thickness. Tissue slices were marked with hematoxylin and eosin for histological examination.

Statistical analysis

All data were processed utilizing prism v6.07 and expressed as means ± SEM. Statistical analysis was done through one-way analysis of variance (ANOVA). A p-value less than 0.05 was considered statistically significant.

Results

Total phenolics contents

The results showed that the phenolics content in aqueous and alcoholic extracts using pyrogallol for standard calibration curve was 5.075% and 14.15%, respectively.

Effects of V. odorata on swimming time

As shown in figure 1, the administration of 100 mg/kg ($p<0.001$) and 200 mg/kg ($p<0.05$) of ethanol extract of *V. odorata* significantly increased the duration of forced swimming in rats compared to the control group. Also, 100 mg/kg of the aqueous extract group compared to the control group led to a significant increase in the duration of forced swimming in rats ($p<0.05$), while 50 mg/kg aqueous and ethanolic extracts could not significantly increase the duration of forced swimming.

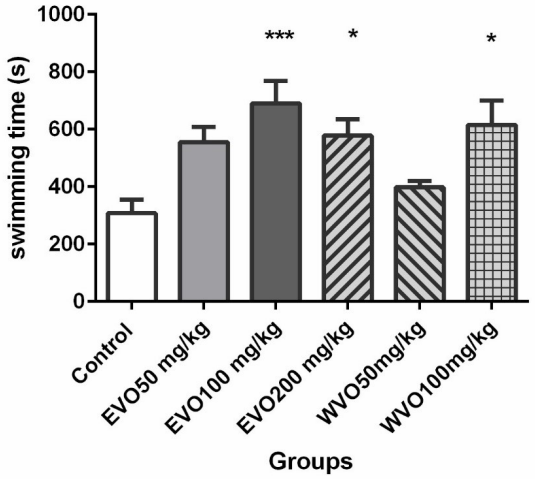

Figure 1. Effect of *Viola odorata* on swimming time in the rat. Rats were pretreated with distilled water, ethanol extracts (EVO-50, EVO-100, and EVO-200), and aqueous extracts (WVO-50, WVO-100) for 30 days and then 1 h later underwent an exhaustive swimming test with a 5% body weight load attached to the rat tail. Data are mean ± SEM (n =8). *p < 0.05, ***p <0.001

*The effects of V. odorata on serum biochemical pa*rameters

According to figure 2, the EVO-200 group showed a significant increase in serum glucose ($p<0.05$). Although, the glucose level in other groups was higher than in the control group but there was no considerable difference.

As illustrated in figure 3, the serum level of LDH in the group that received the ethanol extract at the concentrations of 50, 100, and 200 mg/kg was sig-

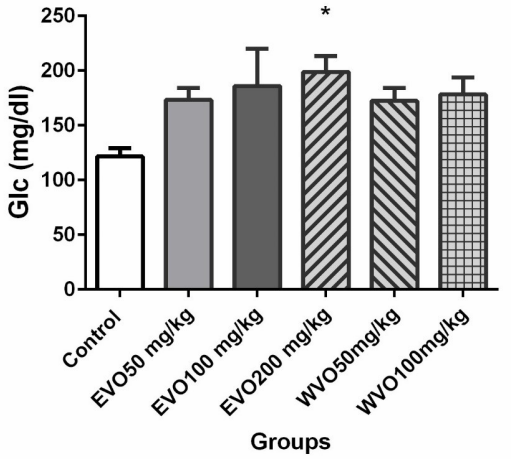

Figure 2. Effects of *V. odorata* on serum Glc level in the rat. Rats were pretreated with distilled water, ethanol extracts (EVO- 50, EVO-100, and EVO-200), and aqueous extracts (WVO-50 and WVO-100) for 30 days and then experienced the forced swimming test. Data are mean ± SEM (n =8). *p < 0.05

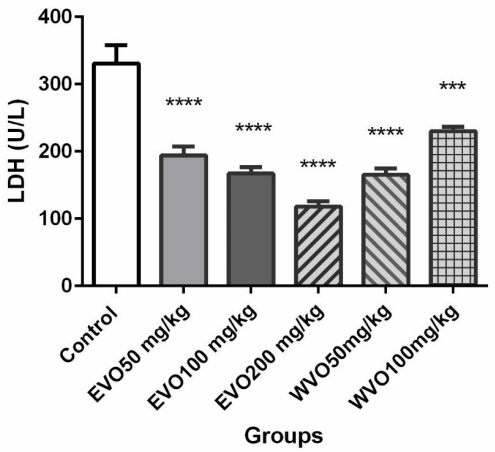

Figure 3. Effects of *V. odorata* on serum LDH level in the rat. Rats were pretreated with distilled water, ethanol extracts (EVO- 50, EVO-100, and EVO-200), and aqueous extracts (WVO-50 and WVO-100 mg/kg) for 30 days and after the forced swimming test. Data are mean ± SEM (n =8). ***P < 0.001, ****p<0.0001

nificantly reduced compared to the control group ($p<0.0001$). Serum LDH in the group that received 50 mg/kg ($p<0.0001$) and 100 mg/kg ($p<0.001$) of aqueous extract decreased significantly compared to the control group.

Diagram 4 shows the comparison of serum levels of TNF-α in the control and treatment groups. Administering the 50 mg/kg of ethanol extract did not cause any changes in the serum level of TNF-α compared to the control group ($p>0.05$). While the serum level of TNF-α in the group that received the 100 mg/kg ($p<0.01$) and 200 mg/kg ($p<0.0001$) ethanol extract increased significantly compared to the control group. The administration of the 50,100 mg/kg aqueous extracts did not cause any changes in the serum level of TNF-α in rats compared to the control group ($p>0.05$).

Effect of V. odorata on histopathology

To investigate the hepatotoxicity of *V. odorata*, 100 mg/kg of ethanol and aqueous extracts were examined by histopathology through hematoxylin-eosin staining.

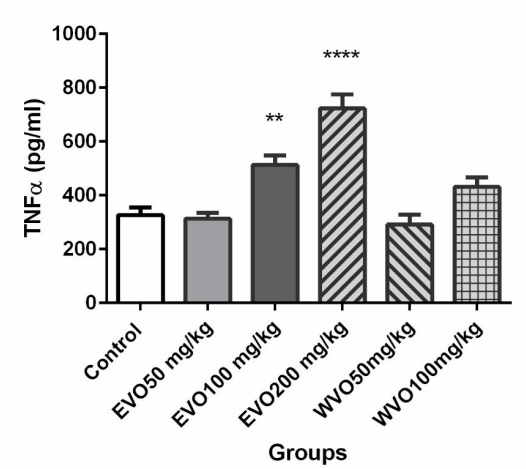

Figure 4. Effects of *V. odorata* on serum TNF-α level in the rat. Rats were pretreated with distilled water, ethanol extracts (EVO- 50, EVO-100, and EVO-200), and aqueous extracts (WVO-50 and WVO-100 mg/kg) for 30 days and after the swimming test. Data are mean ± SEM (n =8). **p < 0.01, ****p<0.0001.

As shown in figure 5, liver parenchyma with mild portal lymphocytic inflammation was seen in the control group (A). No significant liver fibrosis was observed. Also, no evidence of bile duct injury was realized. In group B which received EVO-100, liver tissue with moderate eosinophil-rich, lymphoplasma cell inflammation in the parenchyma and portal tract with interface hepatitis was observed. Mild hydropic change, few piecemeal necroses, and portal and periportal fibrosis were seen. No steatosis and cholestasis were found.

Liver parenchyma in group C showed mild hydropic changes, with mild eosinophilic inflammation and no confluent necrosis. The Portal tract indicated mild lymphocytic inflammation and minimal fibrosis.

Discussion

The energy metabolism over muscular activity level determines physiological fatigue. The fatigue behaviors are decreased in the muscle strength and the highest output power of the motion energy system. Exercise durability is a key variable in assessing delayed fatigue treatment [1]. Medicinal plants are rich in natural anti-fatigue compounds that many researchers have been interested in them to find their anti-fatigue effects [12].

So far, many plants have been researched to relieve fatigue. Many mechanisms are involved in the anti-fatigue properties of plants including the level of blood lactate and serum urea nitrogen reduction, the malondialdehyde content decrease, mitochondria protection, antioxidant superoxide dismutase enhancement, blood sugar, and triglyceride levels improvement [13]. For example, *Pinus koraiennsis* extract has demonstrated significant anti-fatigue effects by increasing the swimming time of mice and restoring muscle and liver glycogen loss [14].

In another research, Wang et al. observed that polysaccharides of *Radix Astragali* can prevent fatigue and prolong forced swimming time of mice, delaying the increases of blood lactic acid, and increasing the tissue glycogen [13].

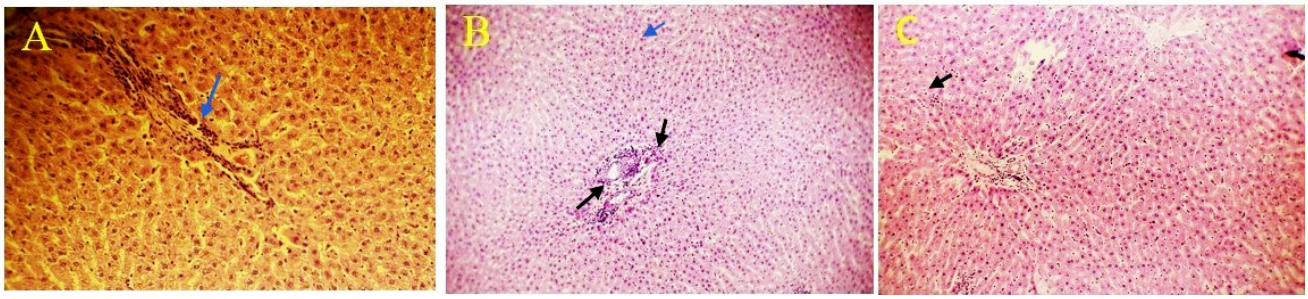

Figure 5. Effect of *V. odorata* treatment on the morphology of liver tissues. Rats were pretreated with (A) control; (B) ethanol extract (EVO-100); (C) aqueous extract (WVO-100) for 30 days. Samples were photographed with a light microscope (hematoxylin and eosin staining)

Among plants, *V. odorata* is used in folklore medicine of the Golestan province of Iran as an anti-fatigue agent. In this study, we examined the anti-fatigue effects of this medicinal plant in the forced swimming test in rats. Dawson and Horvath indicated the advantages of swimming over other kinds of exercise, such as the treadmill [15].

Our result suggested that *V. odorata* (EVO-100, 200; WVO-100) could encompass the forced swimming time of rats which suggests that *V. odorata* might have anti-fatigue activity. Some biochemical variables, including glucose, LDH, and TNF-*α* are imperative indicators of post-exercise muscle fatigue. During exercise, plasma glucose increase through the combined effect of norepinephrine, epinephrine, glucagon, and cortisol. Although glucose is activated by insulin to enter cells, it decreases during prolonged exercise. Blood glucose is a key fuel for increasing ATP production upon skeletal muscle contraction during exercise, and skeletal muscle glucose uptake is enhanced by muscle contraction during exercise using a mechanism independent of the insulin signaling pathway [16,17]. Our results indicated that plasma glucose at 200 mg/kg of *V. odorata* was considerably greater than the control group followed by immediate fatigue. This result may be because of secondary metabolites of *V. odorata* such as flavonoids [6].

The excessive free radicals generated by intense exercise lead to lipid peroxidation and loss of DNA integrity [6]. Moreover, skeletal muscle contractile dysfunction is promoted by high reactive oxygen species levels leading to muscle fatigue [18]. Accumulating the reactive free radicals will cause oxidative stress and hurt organs [19]. Under oxidative stress-induced cellular damage, the cell membrane integrity is damaged while penetrating the cytosolic enzymes into the serum. Those enzymes, such as LDH, creatine kinase (CK), alanine aminotransferase, and aspartate aminotransferase are the parameters revealing the tissue damage under high-intensity exercise [20]. Moreover, LDH catalyzes the interconversion of lactate and pyruvate. Thus, the LDH level increments immediately after exercise [21]. Remarkably, in this research, the serum LDH level of all groups decreased significantly in comparison to the control group after the forced swimming test.

In normal conditions, the muscle cell construction is healthy, and LDH hardly permeates the cell membrane. High-intensity exercise can cause tissue injury, which is connected to exercise-induced fatigue; thus, LDH is found plentifully in plasma.

The results of our study demonstrated that the activity of LDH in different groups of *V. odorata* was significantly lower than those in the control group. Consequently, one possible explanation of the anti-fatigue activity of *V. odorata* is that it could preserve and keep the integrity of the cell membrane after forced swimming. Consistent with our data, Zhou et al. observed that turnip extract could powerfully improve muscle damage by reducing the LDH and CK activities in plasma [22].

Recent studies indicated that TNF-*α* is synthesized mechanically by receiving the stress via the muscle cells that probably have a key role to initiate fatigue. Moreover, the intracellular glycogen stock is reduced by pro-inflammatory cytokines, interleukin-6 and TNF-*α*, leading to fatigue [23].

In this study, although the 200 mg/kg of *V. odorata* ethanol extract could increase the serum glucose and decrease serum LDH level, it increases the level of serum TNF-*α* which suggested this response may be caused by an acute period of physical activity that is similar in numerous aspects to those triggered by sepsis, infection, or trauma [15]. Therefore, ethanol extract of *V. odorata* is not a suitable candidate as an anti-fatigue treatment.

The aqueous extract of *V. odorata* had an anti-fatigue effect in rats, as demonstrated by the increased forced swimming. Also, in addition to serum LDH reduction, no significant difference in the serum level of TNF-*α* was observed in the WVO-100 group. Pathology results showed that the aqueous extract has fewer side effects on the liver during 4 weeks. Considering all the aspects, it can be concluded that the aqueous extract is a suitable candidate as an anti-fatigue agent, although clinical trials and toxicity studies are necessary.

Conclusion

Our findings showed that *V. odorata* aqueous extract improved the swimming time of the rat test, by decreasing the serum level of LDH and no change in TNF-*α* level. Therefore, the aqueous extract of *V. odorata* is suggested as an anti-fatigue supplement.

Conflict of Interests

No conflict of interest was declared by the authors.

Acknowledgments

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References

[1] Liu Y, Liu C. Antifatigue and increasing exercise performance of Actinidia arguta crude alkaloids in mice. J Food Drug Anal 2016;24:738-745.

[2] Huang C-C, Hsu M-C, Huang W-C, Yang H-R, Hou C-C. Tri-

terpenoid-rich extract from Antrodia camphorata improves physical fatigue and exercise performance in mice. Evid Based Complement Alternat Med 2012;2012:364741.

[3] Hsu Y-J, Huang W-C, Chiu C-C, Liu Y-L, Chiu W-C, et al. Capsaicin supplementation reduces physical fatigue and improves exercise performance in mice. Nutrients 2016;8:648.

[4] Yuan T, Wu D, Sun K, Tan X, Wang J, et al. Anti-fatigue activity of aqueous extracts of Sonchus arvensis L. in exercise trained mice. Molecules 2019;24:1168.

[5] Naghibi F, Esmaeili S, Malekmohammadi M, Hassanpour A, Mosaddegh M. Ethnobotanical survey of medicinal plants used traditionally in two villages of Hamedan, Iran. Res J Pharmacogn 2014;1:7-14.

[6] Asheesh K, Suresh C, Meenakshi P. A brief knowledge of banafsha (*Viola odorata* linn.) & other Viola species. Int J Ayurveda Pharma Res 2017;5:73-78.

[7] Ghiaee A, Naghibi F, Esmaeili S, Mosaddegh M. Herbal remedies connected to malaria like fever in Iranian ancient medicinal books-Brief review article. Iran J Parasitol 2014;9:553-559.

[8] Lee J-C, Kao J-Y, Kuo D-H, Liao C-F, Huang C-H, et al. Antifatigue and antioxidant activity of alcoholic extract from Saussurea involucrata. J Trad Complement Med 2011;1:64-68.

[9] Kim N-H, Moon P-D, Pak SC, Kim H-M, Jeong H-J. Anti-fatigue effect of Zizania caudiflora (Turczaninow) Nakai. Am J Chin Med 2012;40(01):111-120.

[10] Zakerin S, Hajimehdipoor H, Mortazavi SA, Sabetkasaei M, Choopani R, et al. A herbal syrup: formulation and antidepressant effect in male rat. Journal of Reports in Pharmaceutical Sciences. 2021;10:101-109.

[11] Fahimi S, Mortazavi SA, Abdollahi M, Hajimehdipoor H. Formulation of a traditionally used polyherbal product for burn healing and HPTLC fingerprinting of its phenolic contents. Iran J Pharm Res 2016;15:95-105.

[12] Rahimifard N, Haji MH, Hedayati M, Bagheri O, Pishehvar H, et al. Cytotoxic effects of essential oils and extracts of some Mentha species on Vero, Hela and Hep2 cell lines. J Med Plants 2010;9:88-92.

[13] Zhou SS, Jiang JG. Anti-fatigue effects of active ingredients from traditional Chinese medicine: a review. Curr Med Chem 2019;26:1833-1848.

[14] Lee SM, Kim YH, Kim YR, Lee BR, Shin S, et al. Anti-fatigue potential of Pinus koraiensis leaf extract in an acute exercise-treated mouse model. Biomed Pharmacother 2022;153:113501.

[15] Kpatcha T, Dosseh K, Idoh K, Agbonon A, Gbéassor M. Effect of adansonia digitata l. on physical performance and haematological parameters in rats. Afr J Sci Res 2016;5:1-6.

[16] Huang C-C, Chen Y-M, Kan N-W, Chao H-L, Ho C-S, et al. Cornu cervi pantotrichum supplementation improves exercise performance and protects against physical fatigue in mice. Molecules 2014;19:4669-4680.

[17] Egan B, Zierath JR. Exercise metabolism and the molecular regulation of skeletal muscle adaptation. Cell metab 2013;17:162-184.

[18] Powers SK, Jackson MJ. Exercise-induced oxidative stress: cellular mechanisms and impact on muscle force production. Physiol Rev 2008;88:1243-1276.

[19] Jin H-M, Wei P. Anti-fatigue properties of tartary buckwheat extracts in mice. Int J Mol Sci 2011;12:4770-4780.

[20] Wu R-E, Huang W-C, Liao C-C, Chang Y-K, Kan N-W, et al. Resveratrol protects against physical fatigue and improves exercise performance in mice. Molecules 2013;18:4689-4702.

[21] Lee B-R, Lee J-H, An H-J. Effects of Taraxacum officinale on fatigue and immunological parameters in mice. Molecules 2012;17:13253-13265.

[22] Zhou Y, Li Z. Anti-fatigue activities and phytochemical compositions of turnip (brassica rapa l.) extracts. Pharmacogn Mag 2021;17:857-864.

[23] Chen Y-M, Tsai Y-H, Tsai T-Y, Chiu Y-S, Wei L, et al. Fucoidan supplementation improves exercise performance and exhibits anti-fatigue action in mice. Nutrients 2014;7:239-252.