Anti-fatigue Effects of Polysaccharides from *Morchella esculenta*

Fuwei Yin[1][a] and Lantao Liu[2,*][b]
[1]*School of Physical Education, Hunan Normal University, Changsha 410012, China*
[2]*Department of Physical Education, Central South University, Changsha 410083, China*

Keywords: Polysaccharides, *M. esculenta,* Anti-Fatigue, Mechanism, Forced Swimming Test.

Abstract: Fatigue has caused indirect damage to the body or a direct cause of disease, which has led to the research and development of anti-fatigue natural medicines and supplements that have become a hot spot at home and abroad. The aim of present work was to evaluate the anti-fatigue effects of polysaccharides from *M. esculenta* (PMe) by using a swimming exercise animal model. The mice were assigned into a normal control group and three PMe treatment groups. The treatment groups received different doses of PMe (100, 200, and 400 mg/kg) through gastric gavage once per day for 4 weeks, and normal control group received distilled water. On the last day of the experiment, the forced swimming test was performed, and fatigue-related biochemical parameters were analyzed. The results showed that PMe significantly prolonged ($p<0.05$) the swimming time to exhaustion, reduced ($p<0.05$) the levels of lactate, urea nitrogen and malondialdehyde in serum, increased ($p<0.05$) the levels of non-esterfied fatty acid in serum, as well as the glycogen levels in liver and muscle. In conclusion, PMe has the anti-fatigue effects and its mechanisms might be related to the fact that PMe could reduce the production of metabolites or delay the accumulation of metabolites, attenuate protein and amino acid metabolism, and enhance fat metabolism, reduce oxidative stress and protect oxidative damage induced by exercise, and improve the energy substance storage or reduce energy substance consumption.

1 INTRODUCTION

Morchella esculenta (L.) Pers. (*M. esculenta*) belongs to the family of *Ascomycota* of the order *Discomycetes*, which grows in temperate regions of Asia, Europe and Americas. It is a class of rare edible and medicinal mushrooms with rich nutrition and delicious taste (Nitha, Fijesh, Janardhanan, 2013). As early as 2000 years ago, *M. esculenta* was used in Traditional Chinese medicine (TCM) for the treatment of spleen and stomach weakness, indigestion, phlegm and shortness of breath, dizziness, insomnia and other diseases (Cui, Chen, Wang, Kai, Fang, 2011). Various bioactive ingredients from *M. esculenta* have been isolated and reported, such as polysaccharides, tocopherol, carotenoids, organic acids and polyphenols (Yang, Yin, Zhang, 2015). Modern pharmacological studies have demonstrated that polysaccharides is one of the most important bioactive ingredients of *M. esculenta* and have a variety of pharmacological actions, including immunomodulatory, antioxidation, antibacterial, anti-viral, antimicrobial, antitumor, anti-proliferation, anti-inflammatory, hepatoprotective, and many other effects (Liu, Pan, 2016). However, little is known about anti-fatigue activity of polysaccharides from *M. esculenta* (PMe). The aim of present work was to evaluate the effects of PMe on physical fatigue by using a swimming exercise animal model. Further, its possible mechanism of anti-fatigue effects also will be investigated, which will provide a scientific basis for the use of this active ingredient

[a] https://orcid.org/0000-0001-6749-4912
[b] https://orcid.org/0000-0003-1498-3864

2 MATERIALS AND METHODS

2.1 Materials and Chemicals

Dry *M. esculenta* mycelium was obtained from ZhongZhiKang Mushroom Science & Technology Development Co., Ltd. (Hangzhou, China). Assay kits for determination of lactate (LA) and glycogen

Yin, F. and Liu, L.
Anti-fatigue Effects of Polysaccharides from Morchella esculenta.
DOI: 10.5220/0011191100003443
In *Proceedings of the 4th International Conference on Biomedical Engineering and Bioinformatics (ICBEB 2022)*, pages 132-138
ISBN: 978-989-758-595-1
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were provided by the LeiGen Biotechnology Co. (Beijing, China). Assay kits for determination of urea nitrogen (UN) and non-esterfied fatty acid (NEFA) were provided by the HuiLi Biochemical Reagents Co. (Changchun, China). Assay kits for determination of malondialdehyde (MDA) were provided by the JianCheng Biotechnology Co. (Nanjing, China). All the other chemicals and reagents used in this study were of analytical grade and were provided by the commercial channels. All solutions were prepared with deionized water to eliminate metal ion contamination.

2.2 Polysaccharides from *M. Esculenta* Preparation

PMe was extracted according to the pre-described method by published literature and subjected to small modification (Cui, Chen, Wang, Kai, Fang, 2011). The dry *M. esculenta* mycelium were further dried to constant weight at 45°C, and ground into fine powder (100 mesh) using a shredder. Then, the powder was extracted by refluxing in 80% ethanol for 7 h to remove the ethanol-soluble materials, including colored substances, small molecule substances, monosaccharides, and oligosaccharides. The ethanol was volatilized and the pretreaed dry powder was obtained. Pretreated powder was extracted with 10 volumes of distilled water at 90°C for 3 h. After centrifugation (2432 × g, 15 min), the residue was extracted for another 3 h at 90ºC. All supernatants were combined and concentrated in a rotary evaporator under low pressure. The concentrated supernatants were mixed with 3 volumes of 95% ethanol at 4°C for 24 h. After centrifugation (2432 × g, 15 min), the precipitate was washed twice with 100% ethanol and acetone, subsequently re-dissolved in distilled water, and again precipitated with 95% ethanol. The resulting precipitate was collected by centrifugation (2432 × g, 15 min) and the proteins were removed by Sevag method. Finally, the supernatant was collected and lyophilized to obtain PMe.

2.3 Experimental Animals and Care Conditions

Healthy male Kunming mice weighting between 18 and 22g were housed in standard conditions with maintained relative humidity (50 ± 10%), controlled temperature (22 ± 1°C), and an artificial 12-h light/dark cycle (lights on 07:30-19:30 h). The mice were allowed free access to standard food particles (unless otherwise stated) and tap water *ad libitum* throughout the experimental period. All animals are subjected to humanitarian care in compliance with the "Measures for the Administration of Laboratory Animals in Hunan Province", which strictly comply with the orders issued by the Ministry of Science and Technology of China in 1988. The experimental protocol was approved by the Ethics Committee of Central South University

2.4 Experimental Design

After 7 days of adaptation to the feeding environment, the mice were randomly assigned into the following four groups (10 mice per group). Group 1: normal control (NC) group, the mice were treated with distilled water. Group 2: low dose PMe treatment (LPT) group, the mice were treated with 100 mg/kg of PMe. Group 3: middle dose PMe treatment (MPT) group, the mice were treated with 200 mg/kg of PMe. Group 4: high dose PMe treatment (HPT) group, the mice were treated with 400 mg/kg of PMe. PMe was dissolved in distilled water. The treatment groups received different doses of PMe through gastric gavage once per day for 4 weeks, and NC group received the same volume of distilled water.

On the last day of the experiment, 30 min after the last treatment, the forced swimming test was performed as previously reported. The apparatus used was a plastic water tank (length: 65 cm, width: 50 cm, depth: 50 cm) filled with water of 30 cm deep at room temperature (25 ± 1°C). Each mouse tail tied a wire bundle (equivalent to 10% body weight) to swim in order to shorten the test time. Mice are considered exhausted when the animal sink into the water and can not float on the water surface within 10 s (Zhang, 2015), and their swimming time to exhaustion was immediately recorded.

2.5 Biochemical Analysis

After the forced swimming test, the mice were anesthetized by intraperitoneal injection of 10% (w/v) chloral hydrate (350 mg/kg body weight) and sacrificed via decapitation. Blood samples were collected and serum were prepared by centrifugation (1800 × g, 15 min) at 4°C for the estimations of levels of LA, UN, NFFA, and MDA. Then the liver and quadriceps femoris muscle were quickly resected, washed with physiological saline, and stored in liquid nitrogen at -80°C for the estimations of glycogen contents. All biochemical parameters were measured using the corresponding commercial

assay kits according to the manufacturer's recommended instructions.

2.6 Statistical Analysis

Values are presented as Mean ± standard deviation (SD). Statistical analysis was performed using SPSS data analysis software (version 18.0, Chicago, USA). Statistical significance was done using one way analysis of variance (ANOVA) test and then by Dunnett's test. $p<0.05$ was considered significant.

3 RESULTS

3.1 Effect of PMe on the Swimming Time to Exhaustion of Mice

As shown in Fig. 1, the swimming time to exhaustion of mice in the LPT, MPT, and HPT groups (7.94 ± 1.04, 8.21 ± 0.86, and 8.98 ± 1.15 min, respectively) were significantly longer ($p<0.05$) than that in the NC group (6.87 ± 0.97 min).

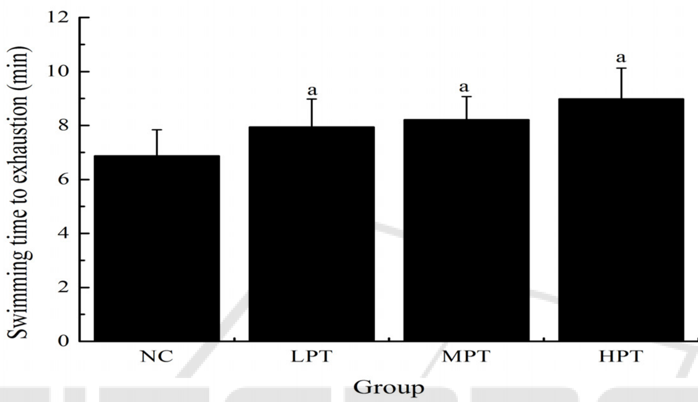

Figure 1: Effect of PMe on the swimming time to exhaustion of mice. Values are presented as Mean ±SD. ap<0.05 compared to the NC group.

3.2 Effect of PMe on the LA and UN in Serum of Mice

As shown in Fig. 2, the serum LA levels of mice in the LPT, MPT and HPT groups (13.49 ± 1.84, 12.16 ± 2.23, and 10.83 ± 1.78 mmol/L, respectively) were significantly lower ($p<0.05$) than that in the NC group (15.21 ± 2.16 mmol/L). Meanwhile, the serum UN levels of mice in the MPT and HPT groups (8.74 ± 0.75 and 8.21 ± 0.97 mmol/L) were significantly lower ($p<0.05$) than that in the NC group (9.85 ± 1.16 mmol/L).

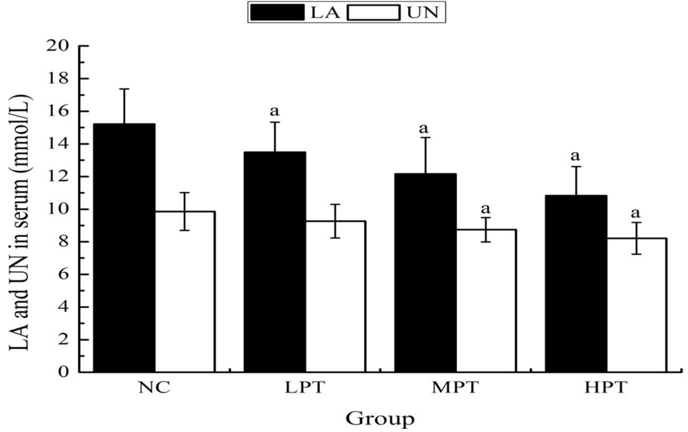

Figure 2: Effect of PMe on the LA and UN in serum of mice. Values are presented as Mean ±SD. ap<0.05 compared to the NC group.

3.3 Effect of PMe on the Serum NEFA of Mice

As shown in Fig. 3, the serum NEFA levels of mice in the MPT and HPT groups (39.47 ± 2.06 and 46.23 ± 6.12 umol/L) were significantly higher ($p<0.05$) than that in the NC group (34.56 ± 5.13 umol/L).

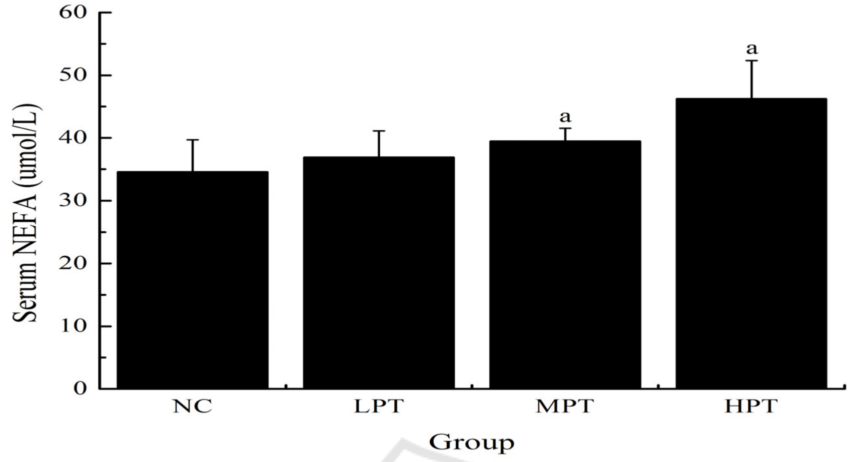

Figure 3: Effect of PMe on the serum NEFA of mice. Values are presented as Mean ±SD. ap<0.05 compared to the NC group.

3.4 Effect of PMe on the Serum MDA of Mice

As shown in Fig. 4, the serum MDA levels of mice in the MPT and HPT groups (10.34 ± 1.40 and 8.15 ± 1.03 nmol/mL) were significantly lower ($p<0.05$) than that in the NC group (12.51 ± 1.47 nmol/mL).

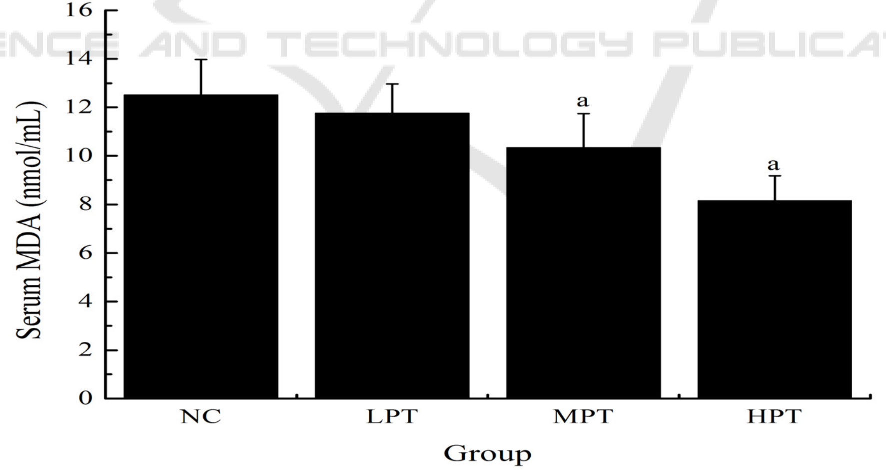

Figure 4: Effect of PMe on the serum MDA of mice. Values are presented as Mean ±SD. ap<0.05 compared to the NC group.

3.5 Effect of PMe on the Glycogen in Liver and Muscle of Mice

As shown in fig. 6, the liver glycogen levels of mice in the LPT, MPT and HPT groups (8.86 ± 0.94, 9.47 ± 1.16, and 11.32 ± 1.03 mg/g, respectively) were significantly higher ($p<0.05$) than that in the NC group (7.69 ± 0.89 mg/g). Similarly, the muscle glycogen levels of mice in the MPT and HPT groups (1.75 ± 0.27 and 1.94 ± 0.22 mg/g) were significantly higher ($p<0.05$) than that in the NC group (1.43 ± 0.21 mg/g).

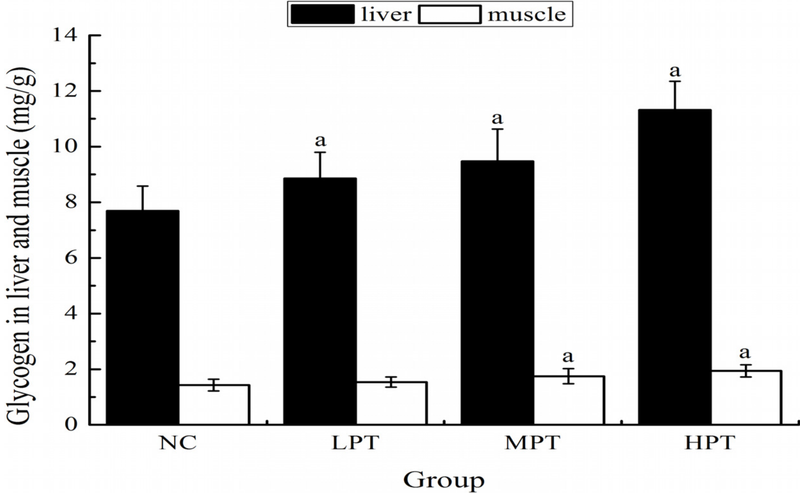

Figure 5: Effect of PMe on the glycogen in liver and muscle of mice. Values are presented as Mean ±SD. ap<0.05 compared to the NC group.

4 DISCUSSIONS

Exercise tolerance is the most direct and important indicators to reflect the physical fatigue. Enhanced exercise tolerance in an exercise test means a lessening of fatigue (Xu, Zhang, 2013). The animal model for evaluating exercise tolerance mainly includes forced wheel running test, forced treadmill running test, forced climbing test and forced swimming test and so on. Forced swimming test rather than other forced exercise tests in this study was chosen as an animal model because it can cause minimal damage to animals, and has a high reproducibility (Jin, Wei, 2011). The lengths of swimming time to exhaustion can reflect the degree of exercise tolerance. The data obtained from this study show that different dose of PMe significantly prolonged the swimming time to exhaustion of mice, which indicated that PMe could improve exercise tolerance and had the anti-fatigue effects.

Energy supply from the glycolysis is the main energy source of strenuous exercise (Yan, Hao, 2016). The increase in muscle oxygen consumption would lead to hypoxia of body, resulting in accelerated glycolysis reaction, and produce a lot of LA during strenuous exercise. The accumulation of serum LA could cause the cell pH value to decrease, leading to a series of biochemical changes, which finally lead to fatigue. Therefore, the accumulation of serum LA could show the speed and extent of fatigue development. UN is a product of protein and amino acid catabolic metabolism. During strenuous exercise, protein and amino acid catabolic metabolism would be strengthened when for a long time body cannot get enough energy by means of sugar and fat catabolic metabolism (Lin, Liu, 2014). Meanwhile, nucleotides metabolism would also be quickened. These two metabolic pathways eventually form UN. Less produced serum UN indicated stronger exercise tolerance and bearing capability of body (Liu, Ji, Li, 2013). So, serum UN is another sensitive indicator of fatigue. In this study, middle and high dose PMe significantly decreased the LA and UN levels in serum of mice, which indicated that PMe could reduce the production of serum LA or delay the accumulation of serum LA, decrease serum UN levels by attenuating protein and amino acid metabolism, thereby delaying fatigue.

Enhancing the proportion of energy supply from fat catabolic metabolism during strenuous exercise could save the glycogen consumption of body, keeping the blood glucose in a physiological range in order to meet the needs of the brain central nervous system (Xu, 2012). This could improve exercise tolerance and delay the occurrence of fatigue. It is reported that the increased availability of NEFA results in greater fat metabolism in the muscle. In this study, middle and high dose PMe significantly increased the serum NEFA levels of mice, which indicated that PMe could improve exercise tolerance might due to enhanced fat metabolism by increasing availability of NEFA.

Strenuous exercise increases the production of free radicals and ROS, which thus attacks the membrane lipid and causes the lipid peroxidation product to form. In turn, the formation and

accumulation of lipid peroxidation will damage the cells, especially membrane structure and genetic material changes, to further cause the body's oxidative damage, accelerating the development of fatigue (Yan, Hao, 2016). MDA, one of the degradation products from lipid peroxidation, is known to be the most sensitive parameter reflecting oxidative damage (Chen, Li, Wang, Zhang, 2013). In this study, middle and high dose PMe significantly decreased the serum MDA levels of mice, which indicated that the anti-fatigue effects of PMe might be due to protecting oxidative damage induced by strenuous exercise through reducing lipid peroxidation.

Exercise energy is originally derived from the decomposition of glycogen, which can supplement blood glucose consumption, and maintain blood glucose levels stable in the physiological range (Yu, Huang, 2012). The increase in muscle glycogen consumption in strenuous exercise will promote the liver glycogen decomposition of glucose to speed up to maintain blood glucose levels stable. Glycogen storage directly affects exercise endurance. Thus, the glycogen is another important indicator related to fatigue. In this study, middle and high dose PMe significantly increased the glycogen levels in liver and muscle of mice, which indicated that anti-fatigue effects of PMe might be due, at least in part, to improving glycogen storage, or reducing glycogen consumption during strenuous exercise.

In recent years, a series of mechanisms on physical fatigue have been explored, such as free radical theory, exhaustion theory, metabolic matter accumulation theory, internal environmental imbalance theory, mutation theory, protective inhibition theory and so on (Wang, Xing, 2014). In this study, we reveal the anti-fatigue mechanisms of PMe from three aspects of energy metabolism and storages, metabolite accumulation, and free radical induced oxidative stress.

5 CONCLUSION

Based on the above tests and analysis, it can be concluded that PMe has the anti-fatigue effects as evidenced by prolonging the swimming time to exhaustion of mice, reducing the levels of LA, UN and MDA in serum, and increasing the levels of NEFA in serum, as well as the glycogen levels in liver and muscle. The anti-fatigue mechanisms of PMe might be through the following pathways.

(1) PMe could reduce the production of metabolites or delay the accumulation of metabolites.

(2) PMe could attenuate protein and amino acid metabolism, and enhance fat metabolism.

(3) PMe could reduce oxidative stress, and protect oxidative damage induced by exercise.

(4) PMe could improve the energy substance storage or reduce energy substance consumption.

Further research is needed to clarify the detailed mechanism of PMe's anti-fatigue effects.

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