Saudi Journal of Biological Sciences 26 (2019) 2052–2056

Contents lists available at ScienceDirect

Saudi Journal of Biological Sciences

journal homepage: www.sciencedirect.com

The anti-fatigue and anti-anoxia effects of *Tremella* extract

Dongsheng Yang [a,c], Junhui Lian [a,c], Lanying Wang [a,c], Xiaomei Liu [a,c], Yanzhen Wang [a,c], Xi Zhao [a,c], Xiaoming Zhang [b], Wenzhong Hu [a,c,*]

[a] School of Pharmacy and Food Science, Zhuhai College of Jilin University, Jinwan District, Zhuhai 519041, China
[b] School of Chemical Engineering and New Energy Materials, Zhuhai College of Jilin University, Zhuhai 519041, China
[c] Sub-center of Medicine and Food Homology and Health Research, National Engineering Research Center for Modernazation of Traditional Chinese Medicine, Zhuhai 519041, China

ARTICLE INFO

Article history:
Received 25 July 2019
Revised 9 August 2019
Accepted 14 August 2019
Available online 14 August 2019

Keywords:
Tremella
Anti-fatigue
Anti-hypoxia
Antioxidant

ABSTRACT

Objective: The present study intended to explore the anti-fatigue and anti-hypoxia efficacy of Tremella extract in mouse model.
Methods: Kunming mice were randomized into 5 groups randomly (n = 12/group; half male and female), and given 0.9%Nacl (10 mL/Kg Serving as blank control group)orally, 0.6 g/kg rhodiola capsule (Serving as positive group) and Tremella extract at doses of high (3.0 g/kg), middle (1.5 g/kg) and low (0.5 g/kg) once a day for 14 days. At the end of drug intragastric gavage, following endurance running test, rotating rod test, normobarie hypoxia test and sodium nitrite poisoning test were performed.
Result: 14-day Tremella extract administration fails to influence mouse horizontal and vertical movement indicating little neurotoxicity at chosen doses. Through rotating rod, forced running test, the anti-fatigue activity of Tremella extract was demonstrated. Via normobarie hypoxia test, sodium nitrite poisoning test, Tremella extract was confirmed to possess anti-hypoxia effect. Tremella extract treatment significantly enhanced the liver levels of superoxide dismutase (SOD), and glutathione peroxidase (GSH-Px), increased the expressions of ATP and hepatic glycogen, muscle glycogen in tissue.
Conclusion: We conclude that Tremella extract efficiently enhances mouse fatigue endurance and anti-hypoxia capability partly through energy reserves and antioxidant enzyme activity.

0. Introduction

With the progress of society and accelerating of pace of life of residents, fatigue and hypoxia have become extremely common and increasingly valued. Fatigue is a complex physiological and biochemical process of the body, which is a physiological phenomenon that occurs when the brain or physical strength of the body reaches a certain stage (Zhong et al., 2015). Delaying the occurrence of fatigue and promoting the recovery of fatigue is a research hotspots of military medicine and sports medicine. Studies have shown that the mechanism of fatigue is extremely complex, mainly divided into central fatigue (localized to the central nervous system) and peripheral fatigue (localized to spinal motor nerves, neuromuscular junctions and skeletal muscle). According to the "energy depletion theory", the decrease of blood sugar and glycogen content caused by exercise is the main cause of fatigue (Wang and Zhang, 2015); according to the "oxygen radical - lipid peroxidation theory", exercise and other stress can lead to excessive oxygen radical production in the body, causing cell metabolism disorder, drop of work efficiency and fatigue. With the deepening of research, the current understanding of fatigue goes deep into the microscopic level of organization, cells and molecules (Liu et al., 2016).

At present, the measures and drugs for eliminating fatigue are not satisfactory. In particular, some biochemical drugs can improve the locomotivity, but contain ingredients similar to stimulants, causing damage to the body. Screening anti-fatigue and anti-hypoxia drugs from plants is the research direction of researchers. Rhodiola, which enhances immunity, regulates dysfunction and anti-oxidation, has received extensive attention in anti-fatigue research. *Tremella*, also known as white fungus, snow fungus, etc., was originated in Sichuan. *Tremella* can improve liver detoxification ability and protect liver function (Xue, 2014). It can not

* Corresponding author at: School of Pharmacy and Food Science, Zhuhai College of Jilin University, Jinwan District, Zhuhai 519041, China.
E-mail address: yds@jluzh.edu.cn (W. Hu).
Peer review under responsibility of King Saud University.

Production and hosting by Elsevier

https://doi.org/10.1016/j.sjbs.2019.08.014
1319-562X/

only enhance the body's anti-tumor immunity, but also enhance the tolerance of cancer patients to radiotherapy and chemotherapy (Woong and Sod, 2008). *Tremella* is also a nourishing remedy, which can replenish spleen appetite, tonify qi, clear intestines, help sleep, invigorate stomach and boost brain (Pan, 2015). But research on the anti-fatigue and anti-anoxia activity of *Tremella* has rarely been reported.

This study uses KM mice as a model to demonstrate the anti-fatigue and anti-anoxia activity of *Tremella* in animals. By examining the biochemical parameters of the liver in mice, whether the anti-fatigue and anti-hypoxia activity of *Tremella* is related to its oxidation regulation is investigated.

1. Experiment materials

1.1. Animals

Equal numbers of male and female KM mice, no specific pathogen (SPF) grade, 18–22 g, purchased from Changchun Institute of Biological Products, license number: SCXK-(Ji) 2008-0003.

1.2. Experiment reagents

Superoxide dismutase (SOD) assay kit, glutathione peroxidase (GSH-Px) assay kit, adenosine triphosphate (ATP) assay kit and muscle glycogen assay kit were purchased from Nanjing Jiancheng Bioengineering Institute. Other reagents are of analytical grade.

2. Experiment Methods

2.1. Preparation of Tremella extract

Take 100 g dry *Tremella*, add 1 L of water, heat to reflux for 1.5 h, cool to room temperature, filter through a 120 mesh screen to obtain *Tremella* aqueous extract, extract twice, combine the extracts, and then store at −20 °C after concentration.

2.2. Administration methods

5 days of adaptive feeding were conducted on the healthy KM mice before the tests, and then divided into 5 groups randomly (n = 12/group; half male and female). Respectively: low, medium and high *Tremella* extract dose groups at doses of high (3.0 g/kg), middle (1.5 g/kg) and low (0.5 g/kg), a rhodiola control group at a dose of 0.6 g/kg, and a blank control group at a saline dose of 10 mL/kg. Intragastric gavage was conducted on the groups for 14 days, during which free diet and water was allowed. New mice for experimental use were replaced in each set of tests.

2.3. Locomotor activity test

After 30 min of the last intragastric gavage, mice in the high (3.0 g/kg), the middle (1.5 g/kg) and the low (0.5 g/kg) *Tremella* extract groups, 0.6 g/kg rhodiola group and blank group were placed in the mouse locomotor activity tester. After the caps were covered, the mice were acclimated for 2 min, and the number of locomotor activities of the mice within 5 min was automatically recorded three times in parallel, including the number of standings and the number of locomotor activities.

2.4. Forced running test

The mice were intragastrically administered for 14 days, and after 30 min of the last intragastric gavage, mice in the high (3.0 g/kg), the middle (1.5 g/kg) and the low (0.5 g/kg) *Tremella* extract groups, 0.6 g/kg rhodiola positive group and blank group were placed in the treadmill. The mice were trained 3 times for 1 min each time to understand how to escape from the charged area, the rotation speed was set to 20 r/min, and current to 30 A, and the number of times the mice were shocked within 5 min of each dose group was recorded.

2.5. Rotating rod test

The mice were intragastrically administered for 14 days, and after 30 min of the last intragastric gavage., mice in the high (3.0 g/kg), the middle (1.5 g/kg) and the low (0.5 g/kg) *Tremella* extract groups, 0.6 g/kg rhodiola positive group and blank group were put on the rotating rod, leaving muscles in a state of static tension. The speed of the rotating rod was adjusted to 20 r/min, and the time for each group of mice to fall from the glass rod due to muscle fatigue was recorded. The training was performed 3 times before the test for 1 min, and the residence time of the mice on the rotating rod was recorded. The test was performed three times in parallel.

2.6. Normobarie hypoxia test

The mice were intragastrically administered for 14 days, and after 60 min of the last intragastric gavage, each group of KM mice was placed in a 250 mL jar with 20 g of sodium lime, and the bottlenecks were covered with vaseline to keep it tight and air proof. Respiratory arrest was taken as an indicator to death of mice, and the time from when the mouse was placed in the bottle to the death of the mouse was observed and recorded.

2.7. Sodium nitrite poisoning test

The mice were intragastrically administered for 14 days, and after 60 min of the last intragastric gavage. Each mouse was counted intraperitoneally injected with 240 mg/kg sodium nitrite ($NaNO_2$) and immediately, and the survival time after mouse poisoning was recorded in turn.

2.8. Collection of tissues and determination of relevant indicators

The KM mice were intragastrically administered for 14 days, and after 30 min of the last intragastric gavage. Lead brick accounting for 5% of body mass were loaded at the tails heel of the mice, and the mice were placed in plastic buckets with a water depth of 25 cm, a diameter of 15 cm and a water temperature of 23 ± 1 °C. After swimming for 20 min, the mice were sacrificed, and the livers and muscle tissues of the mice were collected.

The livers and muscle tissues were homogenized, and the contents of ATP, SOD and GSH-Px in the liver and the content of muscle glycogen in the muscle were measured.

2.9. Statistical methods

The *t*-test using two-sample means to compare the data of each group was performed by SPSS statistical software. The data was expressed by Mean ± S.D., $P < 0.05$ was a significant difference.

3. Results and discussion

3.1. Effects of Tremella extract on locomotor activity of mice

After 14 days of intragastric gavage of 0.5–3.0 g/kg *Tremella* extract and 0.6 g/kg rhodiola, there was no significant change in the number of standings and the number of locomotor activities of the mice contrasted with the blank group (Fig. 1, $P > 0.05$). The

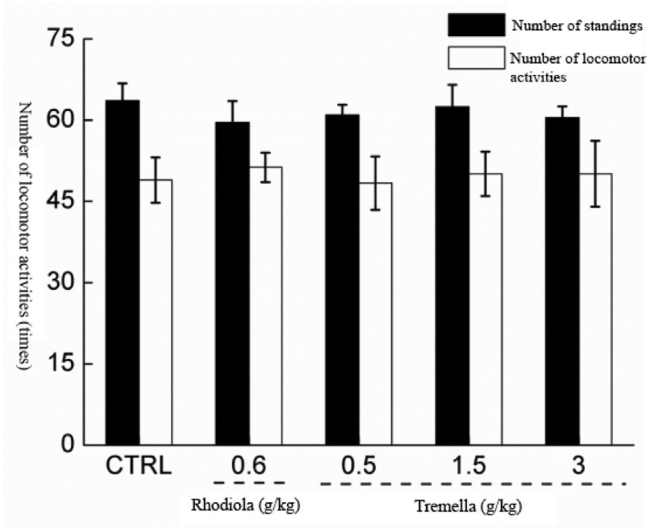

Fig. 1. *Tremella* and rhodiola extract have no significant effect on locomotor activity of mice.

test results show that *Tremella* extract is not irritating to the central nervous system of mice and has good safety.

3.2. Anti-fatigue activity of Tremella extract

Similar to previous studies, rhodiola has good anti-fatigue activity, and can reduce the number of electric shocks by 38.8% in the forced running test; in the rotating rod test, it can increase the residence time by 59.% ($P < 0.01$; Fig. 2). Similar to rhodiola, the high-dose *Tremella* extract group can significantly increase the locomotivity of mice ($P < 0.05$; Fig. 2). The most direct and objective manifestations of fatigue is the decline in exercise endurance, and the test data obtained from forced running and rotating rod has been used to reflect important indicators of exercise endurance (Xu, 2014). This study demonstrates that *Tremella* extract has good anti-fatigue activity.

3.3. Anti-hypoxia activity of Tremella extract

Similar to the anti-anoxia activity of rhodiola, 3 g/kg *Tremella* extract can prolong the survival time of mice in $NaNO_2$ poisoning by 33% ($P < 0.05$; Fig. 3A), and prolong the survival time of mice in normobarie hypoxia by 43.9% ($P < 0.05$; Fig. 3B) contrasted with the blank group. The results show that *Tremella* extract has a good ability to increase hypoxia tolerance.

3.4. Regulation of ATP and muscle glycogen in tissues by Tremella extract

After 14 days of *Tremella* extract administration, after 20 min of swimming, 3.0 g/kg *Tremella* extract increased ATP content in the liver by 47.4% ($P < 0.05$; Fig. 4), increased hepatic glycogen by 64.0% ($P < 0.05$; Fig. 5A), and increased muscle glycogen reserve in the muscle by 91.2% ($P < 0.05$; Fig. 5B) contrasted with the blank group. ATP molecules are the most direct source of energy in living organisms, which can provide energy for cell metabolism, and are

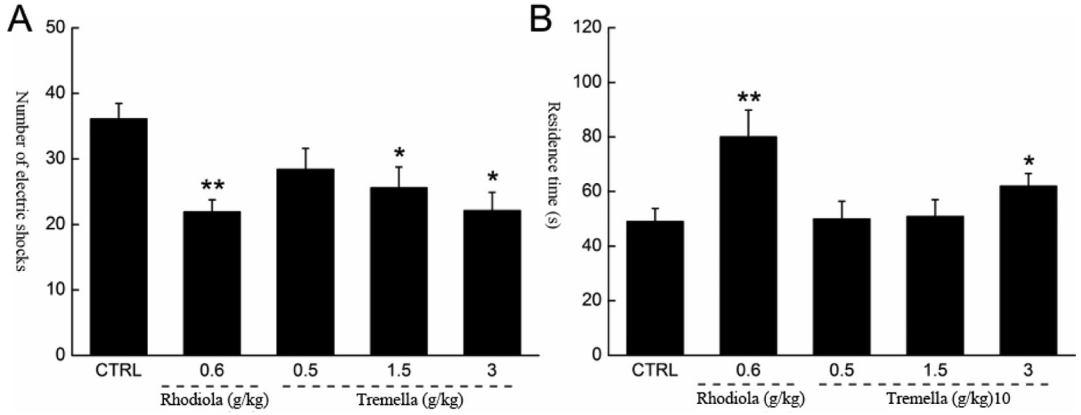

Fig. 2. *Tremella* and rhodiola extract significantly increase the anti-fatigue activity of mice: (A) reduce the number of electric shocks on mice in the forced running test; (B) increase the residence time of the mice on the rotating rod. *$p < 0.05$ and **$p < 0.01$ contrasted to the blank group (n = 12).

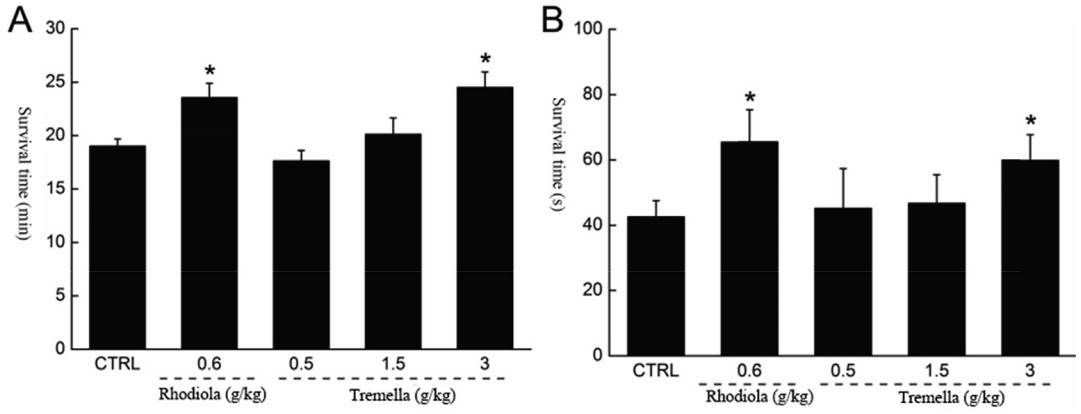

Fig. 3. *Tremella* and rhodiola extract significantly increase hypoxia tolerance in mice: (A) increase survival time of mice in sodium nitrite poisoning test; (B) prolong survival time of mice in normobarie hypoxia test. *$p < 0.05$ contrasted to the blank group (n = 12).

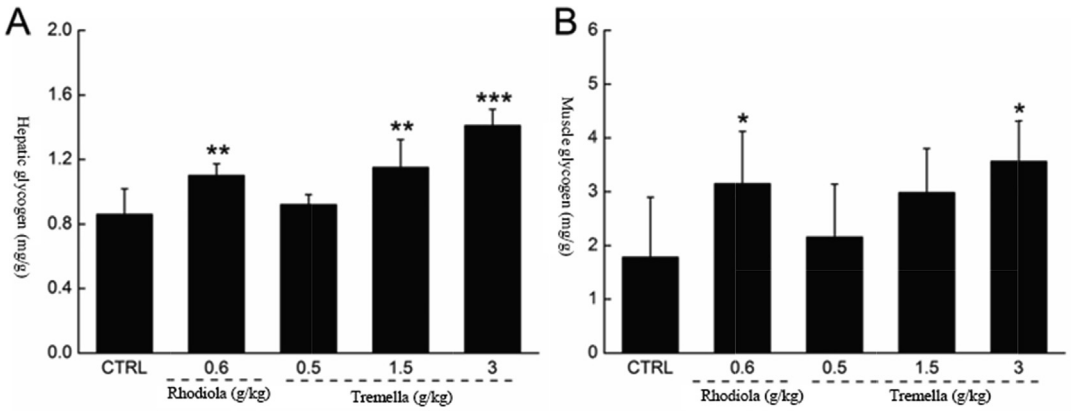

Fig. 5. *Tremella* and rhodiola extract significantly increase hepatic glycogen (A) and muscle glycogen (B) levels in mice after exercise. *p < 0.05 contrasted to the blank group (n = 12).

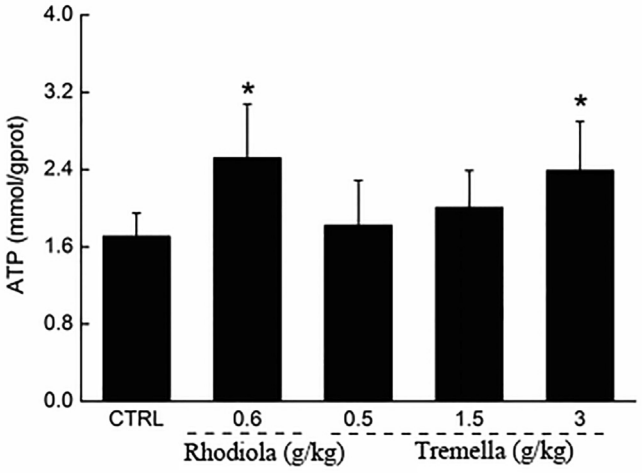

Fig. 4. *Tremella* and rhodiola extract significantly increase ATP content in the liver of mice after exercise. *p < 0.05 contrasted to the blank group (n = 12).

responsible for storing and transferring chemical energy (Miao et al., 2014). Glycogen is an important source of energy for mouse body tissues. When the body performs large-intensity exercise, it first uses sugar to provide energy, and when sugar is depleted, it uses fat and protein. Muscle glycogen, liver glycogen and blood sugar are the three major sugar reserves in the body. When the glycogen is depleted, it will affect the endurance of exercise, so the glycogen content can be used as an important indicator to reflect the degree of fatigue (Zhen and Zhu, 2015). The test results show that the anti-fatigue and anti-hypoxia activity of *Tremella* is based on its regulation of ATP, hepatic glycogen and muscle glycogen in the liver.

3.5. Antioxidant activity of Tremella extract

After 20 min of swimming, the 3.0 g/kg *Tremella* extract increased the SOD content in the liver by 34.5% ($P < 0.01$; Fig. 6A) and the GSH-Px level increased by 18.4% ($P < 0.05$; Fig. 6B) compared with the KM mice in non-administered blank group. The peroxide free radical can cause damage to the body, and SOD is a free radical scavenger that is widely present in various tissues of the organism to scavenge oxygen free radicals and eliminate harmful substances produced by the organism in metabolism (Deng, 2015). The vigor of GSH-Px can reflect the selenium level of the body, and selenium can catalyze the conversion of GSH to GSSG, which can restore toxic peroxides and protect the normal function of cells (Chen et al., 2015). Sub-limit or high-intensity exercise, especially after exhaustive exercise, can lead to a decrease in antioxidant enzyme activity and a reduction in the efficiency of scavenging free radicals, which in turn causes fatigue damage in the body (Yang et al., 2011). The results of this study indicate that the anti-fatigue and anti-anoxia effects of *Tremella* are associated with its resistance to oxidation and regulation of oxidase activity.

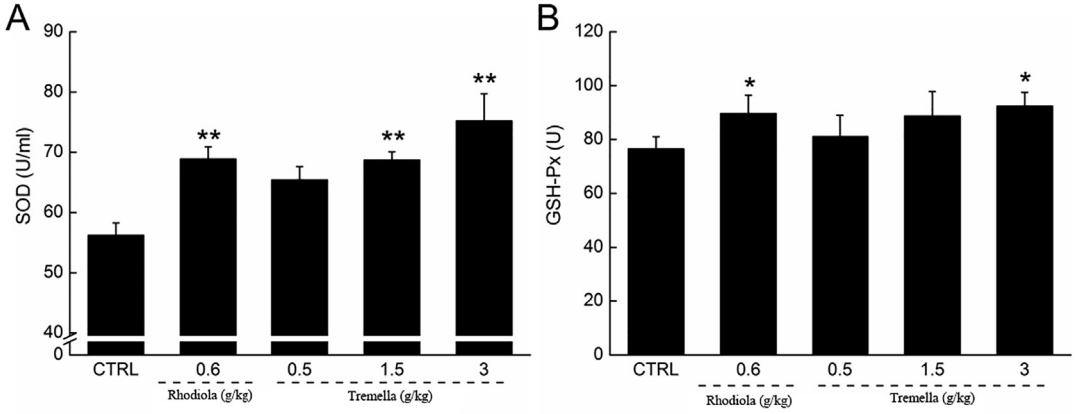

Fig. 6. *Tremella* and rhodiola extract significantly increase (A) SOD and (B) GSH-Px levels in the liver of mice after exercise. *p < 0.05 and **p < 0.01 contrasted to the blank group (n = 12).

4. Conclusion

The occurrence of exercise fatigue is the decrease of muscle contraction force caused by a series of biochemical changes from exercise, that is to say, the most direct and most objective manifestation of exercise fatigue is the decrease of body exercise endurance (Bao et al., 2014). At the beginning of exercise, the ATP and glucose in the cells are consumed, blood sugar is lowered, liver glycogen is decomposed into glucose into the blood, and muscle glycogen is decomposed for anaerobic respiration, resulting in a decrease in tissue's ATP and glycogen content. In the present study, the rotating rod time and number of electric shocks can be used as objective indicators for judging the strength and anti-fatigue ability of mice. From the results, it can be seen that *Tremella* extract can significantly improve the locomotivity of mice and increase their forced running ability and residence time on the balanced rotating rod, while significantly increasing liver's ATP and muscle's glycogen content, indicating that *Tremella* extract can improve exercise tolerance and delay the occurrence of fatigue, and its role may be related to increase the body's energy reserves.

Hypoxia is an inferior stimulus to the body, affecting the normal oxidative decomposition function of the body. Although the brain only accounts for 2% of the body mass, the oxygen consumption accounts for 25% of the whole body's oxygen consumption. Once the oxygen supply is limited, it will directly cause brain damage, which can lead to brain death in severe cases (Xu Y., 2014). In the state of hypoxia, the body can only perform anaerobic respiration, temporarily maintaining its life activities, and anaerobic respiration causes the body's organic matter to be incompletely oxidized, and the body's incomplete oxidation products accumulate, leading to increased tissue oxidative damage and increased hypoxia capacity in mice. The improvement of anti-hypoxia ability of mice mainly relies on the enhancement of the adaptability of body cells to the hypoxic environment, the improvement of the body's ability to reserve and utilize oxygen, and the corresponding increase in the locomotivity of the body. In this study, *Tremella* extract significantly increased the survival time of mice under hypoxia and poisoning, enhanced the body's anti-hypoxia ability, and significantly increased the activity of SOD and GSH-Px in mice liver, reduced the accumulation of free radicals, accelerated the elimination of lipid peroxides in the body and increased the body's antioxidant capacity.

In this study, the anti-fatigue and anti-oxidation activities of *Tremella* extract were demonstrated by in vivo experiments in mice, and their effects were directly related to the regulation of energy reserve and antioxidant enzyme activity. In this context, this study provides a scientific basis for the widespread use of *Tremella* as a health food.

Acknowledgements

This research was financially supported by Key Platform and Major Research Program of Guangdong Provincial University (2017GXJK221). This research was financially supported by Key Platform and Major Research Program of Guangdong Provincial University (2017KTSCX212).

References

Bao, L., Chen, H., Bian, J., et al., 2014. Anti-fatigue and anti-hypoxia ability of compound ganoderma lucidum spore essential oil soft capsule. Chin. J. Exp. Formulas 30 (14), 130–133.

Chen, L., Zhao, L., Bai, C., et al., 2015. Antioxidant activity and anti-fatigue effect of grass carp polypeptide. J. Henan Univ. Technol. 36 (6), 74–82.

Deng, B., 2015. Experimental Study on Anti-Fatigue Effect and Related Mechanism of Soybean Isoflavones. Chinese People's Liberation Army Academy of Military Medical Sciences.

Liu, Y. et al., 2016. Research progress and new ideas on detection and evaluation of anti-fatigue functional foods. Food Res. Dev. 37 (2), 209–213.

Miao, M., Jiang, M., Zhang, X., 2014. Effects of Shenqi Pollen tablets on exercise-induced fatigue in rats. Chin. J. Exp. Formulas 20 (5), 177–179.

Pan, C., 2015. Crown Fungus, Tremella. Green Food. 09, 42.

Wang, M., Zhang, H., 2015. Physical holism of TCM in sports fatigue. Chin. J. Sports Med. 34 (12), 1211–1219.

Woong, L., Sod, L., 2008. Antitumor and immuno-modulatory effect of crude polysaccharides from fruiting body of tremella aurantialba against mouse sarcoma 180. Korean J. Mycol. 36 (1), 66–74.

Xu, Y., 2014. Anti-Fatigue and Hypoxia Tolerance of Paecilomyces Batmoth Mycelium and its Mechanism. Chinese Academy of Military Medical Sciences.

Xue, L., 2014. The protective effect of tremella fuciformis extract on alcoholic liver injury. Mycosystema 33 (5), 1112–1118.

Yang, B., Ren, X., Li, X., 2011. Effects of quercetin on free radical metabolism in serum of rats with exhaustive exercise fatigue. Chin. J. Prev. Med. 29 (1), 19–21.

Zhen, J., Zhu, R., 2015. Effects of aerobic exercise on skeletal muscle glycogen and exercise endurance in rats with chronic heart failure. Chin. Rehabil. Theory Pract. 21 (4), 426–431.

Zhong, Y., Yan, M., Zhang, Z., et al., 2015. Study on anti-sports fatigue effect of water extract of Okra. Food Sci. Tech.-Brazil 40 (07), 221–225.