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Anti-fatigue and Anti-oxidant Morchella Extract

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In order to study the effect of antioxidant and anti-fatigue activity of Morchella polysaccharide, determine the vitro antioxidant activity of Morchella polysaccharide (EPS), intracellular polysaccharide (IPS), and compare with the fermentation and mycelium extracts. The results showed that Morchella polysaccharides have a clear ability on superoxide anion free radical (O_2-·) and hydroxyl radical (·OH), but far less than that of fermentation and mycelium extracts with the same concentration. Through the mice gavage with Morchella polysaccharide, anti-fatigue performance index was determined, and by comparing the experimental group and the control group, it showed that Morchella polysaccharide has the physiological function of anti-fatigue.

1. Introduction

Edible (medicine) fungi, in the liquid deep fermentation process, can produce a variety of the metabolites with physiological activity. These substances have the function of preventing and curing diseases, and have anti-inflammatory, anti-bacteria, anti-tumour, anti-aging, improve the immunity and other health effects. At present, in the research field of molecular biology, medicine, and food science, researchers take edible (medicine) fungal polysaccharides as one of the most important research direction, in which the research of fungal polysaccharide biological activity is more. And the anti-tumour effect of fungal polysaccharide is one of the most important biological activities, but also the most active research part.
Morchella, as a kind of valuable edible and medicinal fungi, has good nutrition and health care, which is a very precious Chinese herbal medicines. In ancient times, it is used in the treatment of dyspepsia, shortness of breath, sputum and other diseases, without side effects, but significant efficacy. Its medicinal value has been proved by modern medical research (Ajmal et al, 2013). Polysaccharide is a common ingredient of Morchella. The mechanism of anti-oxidation of polysaccharide at home and abroad recognized is mainly divided into the following two types, one is direct effect on free radical of polysaccharides; the other antioxidant mechanism is polysaccharides indirect effects on free radicals. At the same time, Marcella polysaccharide has the function of anti-fatigue.

2. Experiment

2.1 Anti-oxidant research

2.1.1 Experimental materials

Morchella
1) Culture medium
PDA solid medium: potato 200g, glucose 20g, agar 15g, distilled water 1000mL, and pH natural.
Liquid seed medium: sucrose 30g, peptone 1g, yeast extract 5g, KH2PO41.5g, MgSO4, 7H2O1.5g, 1000mL of distilled water, and pH natural.
Fermentation medium: sucrose 40g, yeast extract 5g, NH_4NO_3 3g, KH_2PO_4 1.5g, $MgSO_4·7H_2O$ 1.5g, distilled water 1000mL, and pH 5.5.
2) Major instruments
UV VIS spectrophotometer, low speed centrifuge, constant temperature water bath and so on.
3) Major reagents
Salicylic acid, benzene three phenol, three tris (Tris), potassium ferricyanide, hydrochloric acid, ferrous sulfate, three chloroacetic acid, ferric chloride, glucose, peptone, yeast extract, potassium dihydrogen phosphate,

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Magnesium Sulfate, ammonium nitrate, sulfuric acid, phenol, etc. were analytically pure reagent, 95% ethanol, 30% hydrogen peroxide and so on (Chen et al, 2013).

2.1.2 Extraction of morchella polysaccharide

Take a certain amount of polysaccharide sample solution, and add 1/2 sample volume of chloroform: n-butanol (5:1). The mixture is placed in a separatory funnel, shake for 20~30min, remove water layer and white matter (variation of protein) between the organic layer, repeatedly, until the white matter disappeared, thus obtain crude polysaccharide protein (Sevage method to remove protein).

2.1.3 Determination of morchella polysaccharide reduction force

The aqueous solution of crude polysaccharides was diluted to form sample liquid with different concentration gradients. Add 2.0mL0.2mol/LpH6.6 phosphate buffer and 2.0mL1% potassium ferricyanide solution in per 1mL sample solution, and fully mixed. After heat preservation for 20min in 50 ~ C, 1.0mL10% three chloroacetic acid is added, fully mixed, and 3000r/min centrifugate for 15min. Then, 1mL supernatant was extracted, add with 1mL0.1% FeCl3, fully mixed, water volume to 7mL, and determine the absorbance after placing at room temperature for 10min, detection wavelength 700nm (Gang et al, 2015). The greater the absorbance value is, indicating that the higher the reduction will be.

2.1.4 Determination of morchella polysaccharide on O2- scavenging rate

The benzene three phenol oxidation is used to determine the effect of polysaccharide on O2-· scavenging : take 4.5mL50mmol/LpH8.2 Tris-HCl buffer solution in a test tube, pre-heat in 25 DEG C water bath for 20min, add 0.1mL of each sample solution, 2.5mmol/L benzene three phenol 0.5mL, mixed and react in 25 DEG C water bath for 6min, immediately add 0.1ml8mol/L HCl to terminate the reaction, and measure the absorbance under the wavelength of 320nm (Take distilled water as blank solution).

2.1.5 Etermination of morchella polysaccharide on - OH removal rate

Fenton system is used to determine the scavenging capacity of polysaccharides on·OH. The system consists of 16mmol/L H2O2, 18mmol/L FeSO4, 18mmol/L salicylic acid ethanol solution and 1mL sample solution with different concentration gradient. H2O2 is added to the mixture and react for 0.5h at 37 DEG C, and distilled water is used as the reference liquid (He et al, 2012). The absorbance value of each reaction solution is measured at the wavelength of 510nm (VC as reference).

2.2 Study on anti-fatigue movement

2.2.1 Experimental materials

1) Preparation for Morchella

Morchella crude polysaccharide preparation: according to the conditions determined in the experiments, extract Morchella extracellular and intracellular polysaccharide, prepare crude polysaccharide solution with Morchella polysaccharide extracted, the concentration of 2g/L.

2) Laboratory animals, instruments, and reagents

Body weight 18~229 male mice.

Spectrophotometer, swimming pool: 70cmX50cmX40cm, urea nitrogen, and blood lactic acid test kit.

2.2.2 Animal grouping, drug delivery, and statistical methods

The mice were randomly divided into Morchella polysaccharide group and control group, with 10 rats in each group. Polysaccharide group mice were given 100mg/kg Morchella polysaccharide gavage, and the control group was given the same volume of saline, continuous intragastric administration for 21d. All mice were served with conventional feed.

Experimental data uses SPSS13.0 software for statistical analysis.

2.2.3 Mouse loaded swimming test

The depth of water in the swimming tank is 30cm, water pump causes running water, and the water temperature is 25±0.5°C. 20 mice were selected and divided into two groups according to the design requirements, the drug was given, and with gavage continuously for 21d (Huang et al, 2012). After giving drug for 30min, the rat tail root is loaded with 5% body weight wire, placing in the swimming box to swim. Record the time required from the mice beginning to swim until they are exhaustive (submerged bottom until 105), namely the swimming time of mice.

2.2.4 Determination of serum urea nitrogen

Carry out eye blood extraction when the mice are not loaded and forced swimming for 30min, and determine the content of serum urea nitrogen according to the kit instructions.

2.2.5 Determination of blood lactic acid content

Carry out eye blood extraction before the mice swimming, swimming for 10min and 30min after swimming, and determine the content of blood lactic acid according to kit instructions.

3. Results

3.1 Antioxidant research

3.1.1 Comparison of morchella polysaccharide force

It can be seen from Figure 1 and Figure 2 that, the reduction force of intracellular polysaccharide (IPS), extracellular polysaccharide (EPS), fermentation broth without alcohol deposition, and mycelium extraction solution these four samples solutions increase with the increase of the concentration of polysaccharide.

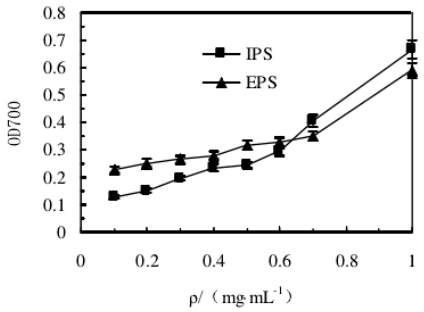

Figure 1: The relationship between the reduction force of IPS and EPS and polysaccharide concentration

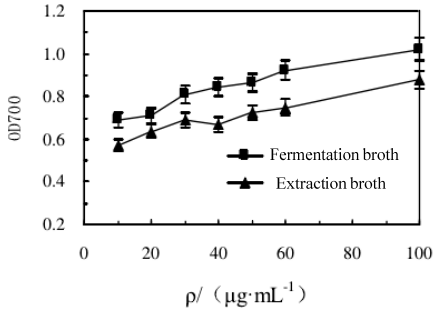

Figure 2: The relationship between the reduction force of fermentation broth and extract and polysaccharide concentration

3.1.2 Comparison of morchella polysaccharide on O2-· scavenging ability

Determine the scale of IPS, EPS, fermentation broth and extraction solution of O2-·scavenging capacity. The experimental results are shown in Figure 3 and Figure 4, from which it can be seen that there is an obvious doe effect relationship between the clearance ability of the 4 groups samples on the O2- scavenging capacity and the polysaccharide concentration.

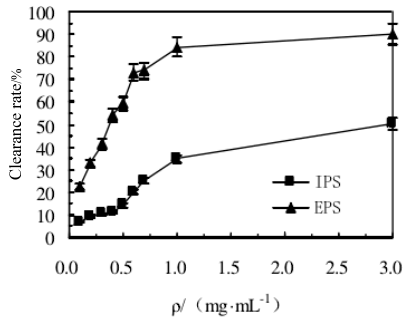

Figure 3: The clearance ability of IPS and EPS polysaccharide on O_2-·

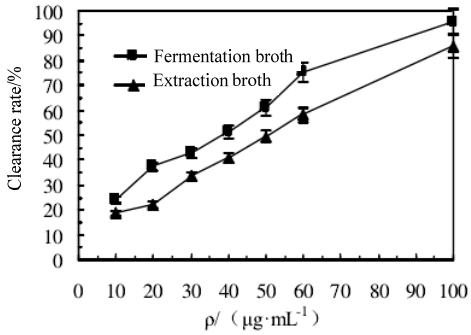

Figure 4: The scavenging ability of fermentation broth and extraction solution polysaccharide on O_2-·

3.1.3 Comparison of morchella polysaccharide scavenging ability on OH

Figure 5 and Figure 6 is the relationship between Morchella polysaccharide, fermentation broth and mycelia extracts scavenging activity of·OH and concentration of polysaccharide (Li et al, 2016). From Figure 5 and Figure 6, it can be seen that IPS, EPS, the fermentation liquid and extracts had scavenging activity on hydroxyl radicals, and the scavenging effect is strengthened with the increase of polysaccharide concentration.

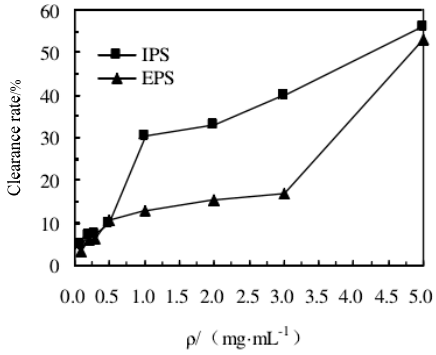

Figure 5: The scavenging ability of IPS and EPS polysaccharide of·OH

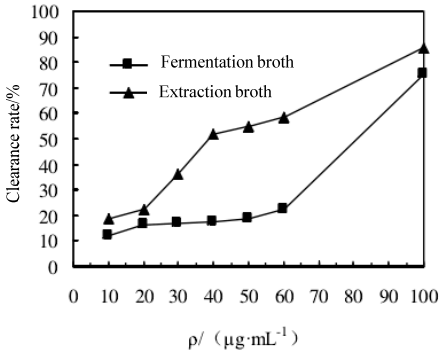

Figure 6: The scavenging ability of fermentation broth and extraction solution polysaccharide of ·OH

3.2 Study on anti-fatigue

3.2.1 Mouse loaded swimming test

Fatigue is caused by physical activity or muscle activity, which is mainly manifested as the decrease of exercise capacity. Exhaustive swimming time has been regarded as one of the important indexes to reflect the endurance of sport. Compared the average time of the mice beginning to swim until exhaustion after polysaccharide group movement with the normal control group, and the results were shown in Table 1.

Table 1: The effect of Morchella polysaccharide on exhaustive swimming time in mice

Test index	n	The control group	The polysaccharide group
Exhaustive swimming time(min)	10	15.7±3.5	22.0±3.2

Compared with the control group, P<0.05.

The result showed that there was a significant difference between the polysaccharide group and the control group. The experimental results show that Morchella polysaccharide can significantly prolong the exhaustive swimming time of the mice, that is to say, Morchella polysaccharide can significantly improve exercise tolerance in mice.

3.2.2 Determination of serum urea nitrogen

After a long-time exercise, sugar and fat metabolism cannot supply enough energy to the body, then the protein and amino acid decomposition and metabolism are strengthened, and urea nitrogen is also significantly increased (Su et al, 2013). The study found that the body blood urea nitrogen content was positively correlated with the exercise load, and negatively correlated with the body's adaptability to exercise load. Labour and exercise load increase, thus the body serum urea nitrogen content increases. The poorer the body's ability to the load, the more obvious the increase of serum urea. The results of the determination of urea nitrogen were analysed, and the results were shown in Table 2.

Table 2: The effect of Morchella polysaccharide on serum urea nitrogen of the mice after exercise

Test index	n	The control group	The polysaccharide group
Urea nitrogen (mmol/L)	10	5.5±0.40	5.08±0.38

Compared with the control group, P<0.05

The results showed that the serum urea nitrogen levels were significantly different between the control group and the polysaccharide group (P<0.05). The results of this study show that after forced swimming, the serum urea nitrogen content in experimental group mice was significantly lower than that of the control group, which indicates that Morchella polysaccharide can significantly reduce the degree of the decomposition of protein for energy, so as to achieve the purpose of saving the protein, thereby reducing the injury of the body caused by movement (Xiong et al, 2015).

3.2.3 Determination of blood lactic acid content

During intense exercise, due to the lack of oxygen supply, the main way to get energy is through the process of fermentation so that the final product of the fermentation - lactic acid largely accumulated in muscle and blood. Therefore, through the determination of blood lactic acid content of animal before and after strenuous exercise in different stages, the metabolism of lactic acid in the body can be known. As a result, the anaerobic metabolism and aerobic metabolism ability can be speculated, and evaluation of body fatigue and recovery situation is made. The results of blood lactic acid were analysed, as shown in Table 3.

Table 3: The effect of Morchella on blood lactic acid of the mice after exercise

Group	Blood lactic acid (mmol/L)		
	Before exercise	Exercising for 10min	30min after exercise
The control group	348±0.1	6.21±0.38	5.14±0.27
The polysaccharide group	3.5±0.34	5.57±0.64	4.02±0.37
F	0.6	7.366	61.78
P		0.014	0

Notice: P<0.05

The present study showed that the blood lactic acid of the mice after exercise in the control group were significantly different from that in the polysaccharide group (P<0.05). After the mice swim for 10min, the average rising rate of blood lactic acid in polysaccharide group is lower than that in the control group, indicating that Morchella polysaccharide can well restrain the improvement of lactic acid in the process of movement (Yang et al, 2016). 30min after swimming, the level of blood lactic acid in the polysaccharide group can faster recover to the pre exercise level than the control group does, suggesting that Morchella polysaccharide can enhance the anti-fatigue ability of the body during exercise.

4. Conclusion

Through the comparison of the common Morchella intracellular polysaccharide, extracellular polysaccharide, fermentation broth and mycelia extraction solution vitro antioxidant activity, it is known that the Morchella polysaccharide has stronger scavenging effect on O2-·than that on·OH, and free radical scavenging capacity of fermentation broth and mycelium extracts is far stronger than that of crude polysaccharide. It indicates that polysaccharide extracts in Morchella has a certain effect on oxidation resistance.

Mice blood urea nitrogen and blood lactic acid of the polysaccharide group and the control group are determined and analyzed. The results suggested that Morchella polysaccharide can significantly reduce the decomposition of protein energy-supplying degree, thereby reducing the movement damage to the body, which indicates that Morchella polysaccharide has resistance to high intensity exercise fatigue.

In conclusion, Morchella polysaccharide has the physiological function of anti-oxidation and anti-fatigue.

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