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Effection of Anti-Fatigue of Selenium-Rich Cordyceps Militaris in Mice

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Purpose to probe into the anti-fatigue ability of selenium-rich Cordyceps militaris. Research methods: (1) Determination of mycelium components: The polysaccharides, organic selenium and superoxide dismutase of mycelium were measured; (2) Determination of anti-fatigue ability: The mice were treated with different dosages of selenium-rich mycelium per day. Each group was tested separately of the mice's exhaustive swimming time and the content of blood lactate and blood urea nitrogen. Results: the content of mycelia polysaccharide and the activity of superoxide dismutase (SOD) in Cordyceps militaris enriched selenium mycelia was increased. The mice treated with mid-dosage obviously prolonged the weight loading swimming exhaustive time. The mid-dosage of selenium-rich Cordyceps militaris could prolong or reduce the production of lactate acid, quicken the clearance of lactate acid. Conclusions: The polysaccharides content, organic selenium content and the activity of SOD enzyme in the selenium-rich mycelium powder increased significantly. selenium-rich Cordyceps militaris can increase the anti-fatigue ability of the exercise in the mice.
Key words: selenium, polysaccharides, SOD, Cordyceps militaris, antifatigue

1. Introduction

Cordyceps genus, the name given to the fungi on insects and its existence has been known since 2000 B.C(Shonkor Kumar Das et al.2010).Cordyceps militarris also is termed cordyceps, is a complex composed by varieties, which are stroma (grass) and sclerotium (corpse) (Kirk PM et al.2001). The chemical components of Cordyceps militarris are very similar to that of aweto. Some components of Cordyceps militarris are higher than that of aweto.The results of research show that Cordyceps militarris contains various bioactivity components such as cordycepin, Cordyceps acid, polysaccharides, nucleoside, etc. (Gu YX et al.2007;Nag TB and Wang HX. 2005) , which have revealed functions of reducing blood lipid, preventing from atherosclerosis(Jing Z. Dong et al. 2012;), protecting the tissue of heart and brain, sedation and hypnosis, enhancing the activity of macrophage, anti-inflammation activities(Das et al.2010), anti cancer, anti bacterium(Ahn et al.2000), anti-co senescence(Khan et al.2010), anti-fatigue(Jung et al.2004), and so on.
Selenium is one of the essential trace elements for human beings, which participates in physiological metabolism of life organism(Anonymous.2005). Lack of selenium will lead to Keshan disease, cardio-cerebrovascular disease, tumor, body immunity decrease, co senescence, and so forth(Fairweather-Tait et al. 2011; Agbor et al. 2007). The results of research have proved that organic selenium is superior to the inorganic selenium because it has the characteristics of higher absorptivity and little toxic side effects, etc(Kirk et al.2001;Panter et al. 1996). Cordyceps militarris can transform organic selenium into inorganic selenium to increase the medicinal and economic value, and are perfect organic food of supplementing selenium for human beings(Chun et al.2006). The experiment determined the main chemical components of selenium-rich Cordyceps militarris, studied that the selenium-rich Cordyceps militarris influenced on the anti-fatigue ability of mice, and provided references of exploitation in functionality production and application in sports medical field.

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2. Material and Method

2.1 Reagent, materials and instruments

Heparin sodium injection for medical application, soda lime, whole-blood lactic acid reagent kit and blood urea nitrogen reagent kit, UV-Vis spectrophotometer, atomic absorption spectrophotometer,water-bath cauldron, centrifuge, electronic balance, thermometer, stopwatch, glass-making swimming cylinder (φ50cm×50cm), lead wire, mycelium powder of selenium-rich Cordyceps militarris.Mice: female and healthy (avoirdupois 20±2g).

2.2 Experiment Methods

2.2.1 Extraction and Measure of Intracellular Polysaccharides of Mycelium

(1) Extraction method of polysaccharides: weigh 1g of dried mycelium, put 15ml water into sample, use ultrasonic waves to clean up it, extract clear liquid from mixture, repeat it three times and combine the liquid together, then concentrate it to 20ml, add 3 times volume of grain alcohol (95%). After that it was put into refrigeratory for standing and alcohol-precipitation, and centrifuged 4800r/min 10min. Precipitate was washed by appropriate acetone, acetone was centrifuged. Appropriate ether was added to wash it, and then was centrifuged. Precipitate was dried to constant weight, namely, intracellular polysaccharides.

(2) Measuring method of polysaccharides: polysaccharides were measured by phenol-sulfuric acid method.

(3) Sugar standard curve: weigh 200mg of analytically pure glucose and put it into a 100mL volumetric flask; make it to scale and shake it up, then extract 1mL into 50mL volumetric flask, put it to scale and shake it up, extract 0.4mL, 0.6mL, 0.8mL, 1.0mL, 1.2mL, 1.4mL, 1.6mL and 1.8mL into test tubes respectively, add water to 2.0mL. After that, 1.0mL phenol with concentration of 6% and 5.0 mL oil of vitriol were also added to the test tubes. After standing for 10 min, shake it up. After standing for 20 min at room temperature, use 2.0mL water to make it blank, measure the value of absorbency at 490nm, obtain the regression equation relating the concentration of polysaccharides and value of absorbency.

Y=0.0157x-0.0397　R2=0.998

Y is the value of absorbency; x is the content of polysaccharides.

(4) Sample determination: After dissolving certain amount of intracellular polysaccharides, put it to 200mL and extract 10mL from the mixture to 100mL, extract 1 mL from it and add deionized water to 2mL, and quickly add 1mL phenol and 5mL vitriol, shake it up and standing for 20 min, then measure the value of absorbency at 490 nm by means of spectrophotometer. According to regression equation y=0.157x-0.0397, the content of polysaccharides was obtained.

2.2.2 Determination of the Content of Organic Selenium

The values of absorbency of sample and standard solution were measured by atomic absorption method (wavelength was 196.1nm; the electric current of light was 5mA; the rate of propane flow was 2000mL/min; the height of burning was 5.0mm; the bandwidth of action spectrum was 0.4nm), the regression curve was constructed based on the standard liquid of selenium and absorbency. Regression equation for the standard curve was y=0.0275x+0.0292, R^2=0.9911, y was absorbency and X was the concentration of selenium.

2.2.3 Determination of SOD Enzyme Activity in the Mycelium

Measuring Method of SOD

Put Phosphate buffer solution (PBS)(0.05 mol·L^{-1}K2HPO4-KH2PO4，pH 8.37) and pyrogallol （50 mmol·L^{-1} ）into 25℃water bath boiler for 25 min respectively. Then, according to Table 1, mixed reagent was made.

Table 1 Dosage of each reagent

	Blank	Control	Sample
PBS	4.7mL	4.7mL	4.5mL
pyrogallol	0	15μL	15μL
hydrochloric acid	10μL	0	0
SOD	0	0	0.2mL
Total volume	4.7mL	4.7mL	4.7mL

Notice: hydrochloric acid is 10 mmol·L^{-1}.

The mixture was shook up quickly and made it at 325nm of wavelength. The value of A was measured for 0.5 min interval (properly dilute the mixture in order to control the autoxidation speed at 0.07). The changing speed △A325o of absorbency per min in the contrasting pipe was determined, and the changing value △A325 o of absorbency of sample solution was also determined per min.

Calculation of enzyme activity:

SOD activity (U·mL^{-1})= (△A325o -△A325)×100%×Vtotal volume÷(△A325o×50%·Vdefining volume· V sample volume)

2.2.4 Experiment Animals and grouping

50 mice were randomly divided into pure blank control group, nonselenium-rich blank control group, low-dosage group, mid-dosage group and high-dosage group. The number of mice of each group was 10. The mice in the pure blank control group drank and ate freely every day; the mice in the nonselenium-rich blank control group were treated with the aqueous solution of mycelium powder of normal Cordyceps militarris at a dose of 0.2mL (37.5mg/kg bw) per day. The three dosage groups were treated with the aqueous solution of selenium-rich mycelium powder at a dose of 0.2mL which was different in concentrations. The concentrations of the three groups were 37.5mg/kg bw, 187.5mg/kg bw and 375mg/kg bw respectively (which were equal to 1, 5 and 10 times of human body.). Each group was treated one time per day, totally 14d.

2.2.5 Determination of Serum Lactic Acid Content and Urea Nitrogen Index

The mice ended the need for the aqueous solution. After that about 30 min, the tails of mice were cut off, the blood was first extracted in still state. The wound was tied up and then the mice were put into swimming cylinder.After swimming 30, the blood of mice was extracted in the second time (30min swimming moment). After 30min of recovery, the blood was extract in the third time (swimming and recovery). The blood of extraction is 50μL each time. According to the instructions of the whole- blood lactate acid (BLA) reagent kit and blood urea nitrogen (BUN) reagent kit respectively, the contents of whole- BLA and BUN were determined.

2.2.6 Experiment of the Weight Loading Swimming Exhaustively

The mice ended the need for the aqueous solution. After that about 30 min, According to 5% of mice' avoirdupois, the lead wire was tied in the tail of the mice (naturally twist in the tail using lead wire). Then the mice were put into glass-making cylinder for swimming, the water temperature was 30±2℃, the water depth was 30cm. The time was recorded when the mice began to swim until they could float upward under the 5s of water surface.

2.3 Data Processing

The data from the experiment was statistically analyzed using SPSS v18.0.

3. Results and Analysis

3.1 The Intracellular Polysaccharides Content of Selenium-Rich Mycelium of Cordyceps Militarris

The intracellular polysaccharides content of selenium-rich mycelium of Cordyceps militarris is shown in Figure1

As the evident from Figure 1 and one-way analysis of variance, there is more intracellular polysaccharides content in the selenium-rich mycelium than the normal mycelium（P<0.05）.When the concentration of selenium was 20μg/mL, the intracellular polysaccharides content was 41.39 mg/g, higher (13.7%) than that in normal mycelium.

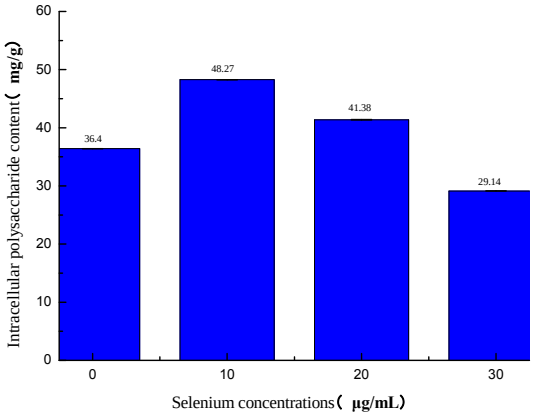

Figure1: The intracellular polysaccharides content of selenium-rich mycelium of Cordyceps militarris

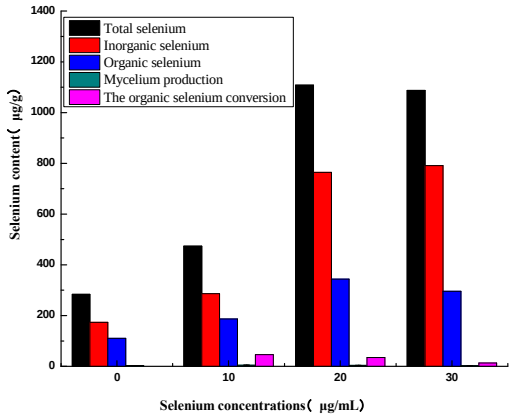

Figure2: Selenium Content of Selenium-Rich Mycelium of Cordyceps Militarris

3.2 Organic Selenium Content of Selenium-Rich Mycelium of Cordyceps Militarris

It is obvious from Figure 2 and one-way analysis of variance that the difference is highly significant in organic selenium content between normal mycelium and selenium-rich mycelium. The concentration of selenium had significant influence on the organic selenium content of mycelium. When the concentration of selenium was 20μg/mL, the organic selenium content in mycelium reached 343.6μg/mL, and was 3.11 times more than that in normal mycelium.

3.3 SOD Enzyme Activity in the Selenium-Rich Mycelium of Cordyceps Militarris

From Figure 3 and analysis of variance, we can know there was a tendency for an increase in the concentration of selenium with the increase in SOD enzyme activity. When the concentration of selenium was 20μg/mL, the enzyme activity reached 24.5 U/mL, higher (60.9%) than that in normal mycelium. After one-way analysis of variance, there was obviously different between the blank contrasting group and the three dosage groups (P<0.01).The concentration of selenium had significant influence on SOD enzyme activity of mycelium.

3.4 Selenium-Rich Cordyceps Militarris Influencing on the Weight Loading Swimming Exhaustive Time of the Mice

The experiment results that the rich-selenium Cordyceps militarris influenced on the weight loading swimming time of the mice are illustrated in Figure 4.

As shown in Figure 4, exhaustive time of the mice in selenium-rich-dosage group was higher than that in nonselenium-rich blank contrasting group. There was significant difference among the pure blank contrasting group, the nonselenium-rich blank contrasting group and mid-dosage group with rich selenium (P＜0.01). there were differences among mid-dosage group and other three groups. The weight loading swimming exhaustive time of the mice in the mid-dosage group was 3.18 times more than that in nonselenium-rich blank contrasting group.

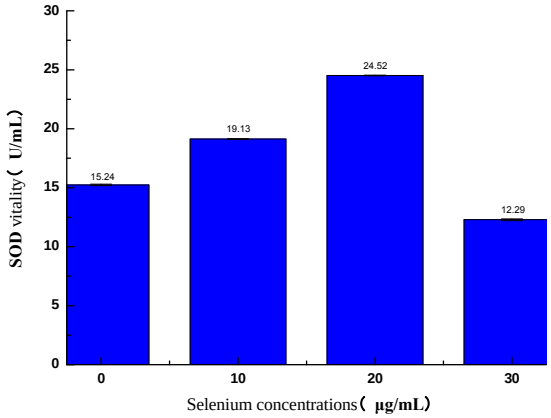

Figure3: SOD enzyme activity in the selenium-rich mycelium of Cordyceps militarris

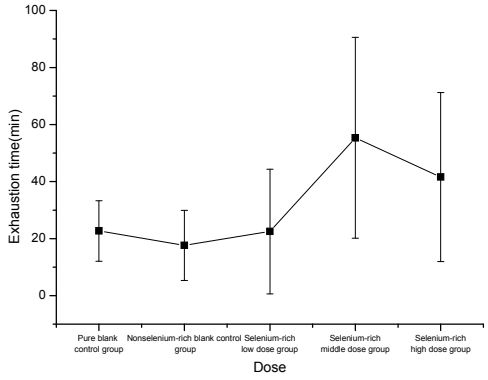

Figure4: Weight Loading Swimming Time of the Mice(x±SD)

3.5 Selenium-Rich Cordyceps Militarris Influencing on the BLA Content of the Mice

Selenium-rich Cordyceps militarris influenced on the BLA content of the mice at different motion states, the experiment results is shown in Figure 5.

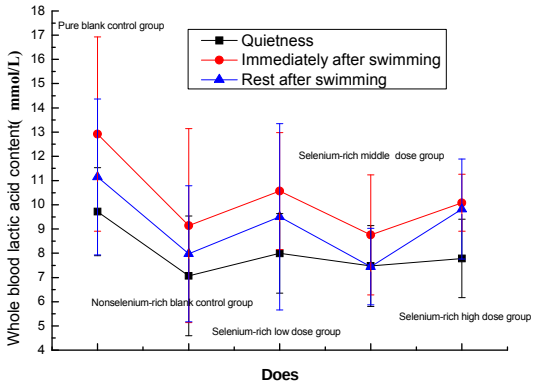

Figure5: BLA content at different dosages of selenium-rich mycelium of Cordyceps militarris

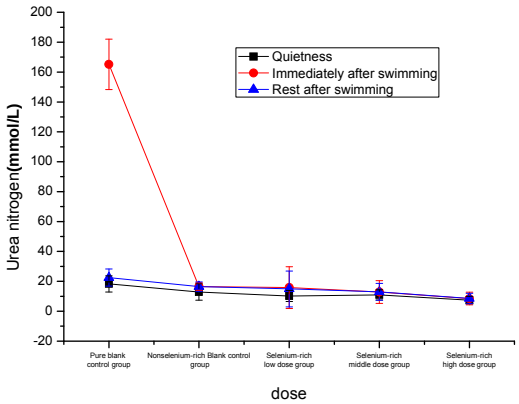

Figure6: The urea nitrogen content of the mice(x±SD)

There is significant difference between each experiment group (P＜0.05). The whole BLA content of the rest groups is significantly lower than the pure blank contrasting group (P＜0.05). After swimming, BLA content in mice's body was immediately measured; differences were significant between each experiment group. The pure blank contrasting group had significant difference with the nonselenium-rich blank contrasting group (P＜0.05) and the mid-dosage group (P＜0.01).After recovery, differences were significant between each group (P＜0.05). The pure blank contrasting group had significant differences with the mid-dosage group (P＜0.01).

3.6 The Urea Nitrogen Content of the Mice Influenced by Selenium-Rich Cordyceps Militarris

The urea nitrogen content of the mice at different motion states was influenced by selenium-rich Cordyceps militarris, the results are shown in Figure 6.

According to Duncan's new-range multiple comparisons test, the pure blank contrasting group in the still state had significant difference with each one of other groups in the urea nitrogen content ($P < 0.05$). The nonselenium-rich blank contrasting group had significant difference with high-dosage group ($P < 0.05$). After swimming, the urea nitrogen content in the pure blank contrasting group had obviously differences with that in each one of other groups ($P < 0.01$). After swimming and recovery, the urea nitrogen content in the pure blank contrasting group showed significant differences with that in each one of other groups ($P < 0.05$).

4. Discussions

The experiment results showed that when the concentration of selenium was 20 μg/mL, the intracellular polysaccharides content was higher (13.7%) than that in normal mycelium. The organic selenium content in mycelium was 3.11 times more than that in normal mycelium. The SOD enzyme activity was higher (60.9%) than that in normal mycelium. Selenium plays an important role in organism metabolism and electron transfer, takes part in the synthesis of protein, adjusts the expression of gene, promotes the organism metabolism, and has an important regulatory effect on life activity(Arthur 2003; Anonymous.2005). The polysaccharides content, the organic selenium content and the SOD enzyme activity in mycelium increased obviously in the selenium-rich mycelium powder of Cordyceps militarris compared to that of normal mycelium. The reason is that selenium in mycelium combines polysaccharides and protein together and forms organic selenium. This can improve the enzyme activity of glutathione synthetase, make the synthesis and metabolism increase, and consequently strengthen the synthesis ability of the substance(Jing Z. Dong et al. 2012).

Length of swimming time can reflect the degree of fatigue. The experiment results show that selenium-rich Cordyceps militarris of mid dosage can significantly prolong the weight loading swimming exhaustive time of the mice. The reason is that selenium participates in metabolic activity, improves the SOD enzyme activity, and eliminates the excessive free radicals in mice's body. That is able to keep the activity of the cell function higher and delay the production of fatigue. Although mycelium powder of selenium-rich Cordyceps militarris in low- and high-dosage groups could improve the weight loading swimming exhaustive time of the mice, this had no statistically difference compared to the contrasting groups. Low-dosage selenium has a benefit for body, while high-dosage selenium is harmful to body(Panter et al. 1996). The results have shown that the scope of safety measurement is relatively small; the effective dosage is close to poisoning dosage(Food and Nutrition Board 2000). The low-dosage mycelium powder of selenium-rich Cordyceps militarris could not obviously prolong the weight loading swimming exhaustive time of the mice. The possible reason is that the content of selenium is lower. The high-dosage mycelium powder of selenium-rich Cordyceps militarris could not significantly increase the weight loading swimming exhaustive time. It is possible that excessive selenium has influence on the composition of enzymes, destroys metabolism balance in normal state, reduces the activity of metabolism.

The statistical results showed that the differences were significant in the lactate content of the pure blank group and the blank contrasting group at the three different stages: in the still state, after swimming immediately, and after swimming and recovery. This demonstrates that Cordyceps militarris had an ability to eliminate the lactate acid in mice's body. This is also the case in Jung，K. study on the anti-fatigue ability of Cordyceps militarris(Jung et al. 2004). The differences were significant in the lactate content of the selenium-rich with mid dosage group and the nonselenium-rich blank contrasting group at the three different stages: in the still state, after swimming immediately, and after swimming and recovery. This demonstrates that the ability of selenium-rich Cordyceps militarris to eliminate the lactate acid is higher than that of normal Cordyceps militarris. The reseasrch results of Tara(selenium can improve the deformability of red blood cells, reduce the aggregation of red blood cells, and improve the ability of blood carrying oxygen. Thus, aerobic metabolism is promoted, the production of anaerobic respiration and lactate acid is reduced(Tara et al.2010) .) also prove this conclusion. The lactate acid in the mid-dosage group increased the least amount after exercise.

Urea nitrogen is also the sensitive index of appraising exercise fatigue. Also, since excess ammonia has a toxic effect on the central nervous system, exercise-induced ammonia accumulation may contribute to the induction of central fatigue (Pathak 1969) . According to Mosso, there exist peripheral and central fatigue, and the latter causes disadvantageous results in the maintenance of exercise(Mosso 1997). As the data shows in Figure6, the low-dosage, mid-dosage and high-dosage mycelium of selenium-rich Cordyceps militarris can all reduce the urea nitrogen in exercise mice obviously in still state, exercise state and after exercise state. The high-dosage group with rich selenium effectively delayed the production of BUN.The main reason may be that the effective components of selenium-rich Cordyceps militarris, such as selenium polysaccharides, selenium protein, etc., acted on organism.

5. Conclusion

The polysaccharides content, organic selenium content and the activity of SOD enzyme in the selenium-rich mycelium powder of Cordyceps militarris increased significantly compared to that of normal mycelium. Selenium-rich mycelium powder with mid-dosage could obviously prolong the weight loading swimming exhaustive time of the mice, evidently delay and decrease the production of lactate acid in mice's body when the mice do exercises, and quicken the clearance of lactate acid in mice's body after exercise. Selenium-rich mycelium powder with high dosage effectively delayed the production of BUN when the mice are in still state. The effect of relieving the production of BUN was better with the increasing in the dosage of selenium-rich mycelium powder. Therefore, selenium-rich mycelium powder could also increase the exercise ability, delay the exercise fatigue, and have the anti-fatigue effect. However, further study is needed to elucidate the more exact mechanism of the effect of the selenium-rich Cordyceps militarris on fatigue and/or exercise durability.

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