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*Full Length Research Paper*

# Impact of polysaccharides from *Cordyceps* on anti-fatigue in mice

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**In this study, the anti-fatigue effect of the purified polysaccharides from *Cordycep* were evaluated in mice. Swimming endurance experiment of mice were carried out after 21 days of polysaccharides from *Cordyceps* administration. And Body weight, blood lactic acid, serum blood urea nitrogen and tissue glycogen levels of mice were determined. Results showed that polysaccharides from *Cordyceps* extended the swimming endurance time of mice, effectively delayed the increasing of blood lactic acid, increasing the storage of liver and muscle glycogen. The dosage of 200 mg/kg was more effective than any other dosage. The results suggested that polysaccharides from *Cordyceps* had significant anti-fatigue effects on mice.**

**Key words:** Polysaccharides, *cordyceps*, anti-fatigue, mice.

## INTRODUCTION

*Cordyceps,* is one of the most valued traditional Chinese medicines (Paterson 2008), is the dry complex composed of the sclerotium of the fungus *Cordyceps sinensis* (Berk.) Sacc. (Clavicipitaceae) and the larva corpses of insects of the family Hepialidae, on which the fungus is parasitic (Leung et al., 2000). *Cordyceps* have long been used in Chinese society to treat many illnesses or to maintain health (Ng and Wang, 2005). It is commonly used to replenish the kidney and soothe the lung, for the treatment of fatigue, night sweating, hyposexualities, hyperglycemia, hyperlipidemia, asthenia after severe illness, respiratory disease, renal dysfunction and renal failure, arrhythmias and other heart diseases, and liver disease (Zhu et al., 1998; Li et al., 2006). A number of bioactive constituents from *Cordyceps* species have been reported. These include cordycepin (3′-deoxyadenosine) and its derivative, mannitol, ergosterol, glycoproteins, peptides containing α-aminoisobutyric acid and polysaccharides (Ng and Wang 2005). Polysaccharides have been reported to account for the anti-inflammatory, antioxidant, steroidogenic, hypolipidemic and immunomodulatory effects (Kuo et al., 2007). But the anti-fatigue effects of polysaccharides haven't been reported. The present study was designed to investigate the anti-fatigue effects of polysaccharides from *Cordyceps.*

## MATERIALS AND METHODS

### Animals

The study protocol was approved by the Institutional Animal Care and Use Committee of China West Normal University and was carried out according to the "Principles of Laboratory Animal Care" (World Health Organization (WHO) Chronicle, 1985). Kunming male mice were obtained from medical scientific academy in Sichuan (Sichuan, China). Kunming male mice were housed in environmentally controlled conditions with a 12-h light/dark cycle. All animals had free access to standard rodent pellet food and water *ad libitum*, except when fasted before experiments. Animals of 10-15 weeks of age were used in the study.

### Preparation of polysaccharides fraction from *Cordyceps* extract

*Cordyceps* were purchased from a local drug market (Nanchong, China) and authenticated by Mr Feiwan Huang, a biologist of China West Normal University. Preparation of polysaccharides fraction from *Cordyceps* extract was performed based on previous reports (Li et al., 2001; Li et al., 2006). One kg dry *Cordyceps* was boiled in 10L water for 2 h. After centrifugation, the supernatant was concentrated and treated with 4 volumes of ethanol for precipitation. The

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precipitation. The precipitate was suspended in water, dialyzed and lyophilized to yield the crude polysaccharide-enriched fraction. The polysaccharide was then dissolved in 10 mM Tris-HCl buffer (pH 8.4) for fast performance liquid chromatography (FPLC) with DEAE-cellulose column.

The partially purified polysaccharide was used subsequently for further purification. The concentrated fractions were applied onto a Sephacryl S-300 column equilibrated with 0.2 M NaCl in 10 mM Tris-HCl pH 8.0. Elution was carried out with the same solution with a flow rate of 0.2 ml/min, and the amount of polysaccharides was determined. The fractions of first-peak were pooled and dialyzed extensively against 10 mM Tris-HCl pH 8.0. The purified polysaccharide was obtained.

#### Group division of mice

After bred adaptively for 7 days, thirty male mice were randomized into 4 groups based on applied dose: Control group (CG), polysaccharides from *Cordyceps* low dose group (CLG, 100 mg/kg), polysaccharides from *Cordyceps* middle dose group (CMG, 200 mg/kg), and polysaccharides from *Cordyceps* high dose group (CHG, 400 mg/kg).

Polysaccharides from *Cordyceps* were dissolved in distilled water and were fed by gavage to mice once a day. The control group was given distilled water and the treated groups were given different doses of polysaccharides from *Cordyceps* (100, 200, 400 mg/kg) for 21 consecutive days.

#### Swimming endurance experiment

Ten mice were taken out from each group to make swimming endurance experiment after being administrated with different dose of polysaccharides from *Cordyceps* for 21 days. Each mouse's tail was loaded with galvanized wire, which was 5% of its body weight, then they were pulled into different acrylic plastic pool (90 × 45 × 45 cm), respectively, which were filled with water to a depth of 35 cm (Kamakura et al., 2001). The temperature of the water was maintained at 25 ± 0.5℃. The swimming endurance of the mouse was observed. The endurance time was defined as the time mice kept swimming activity until they sank into the bottom of swimming boxes and stopped moving forat least 10 s (Abe et al., 1995; Yu et al., 2008).

#### Determination of body weight

To understand the effect of the polysaccharides from *Cordyceps* on body weight, the mice were weighed every day. After all mice had been administrated with the different dose of polysaccharides from *Cordyceps* for 21 days, the increase in body weight of different group was analyzed.

#### Analysis of blood biochemical parameters and tissue glycogen contents

Ten mice were taken out from each group for blood lactic acid analyses. The blood samples were collected from eye sockets of mice 30 min after administration of polysaccharides from *Cordyceps* and 30 min after weight loading swimming (2% body weight), respectively. The levels of blood lactic acid was determined using commercial diagnostic kit obtained from Jiancheng Diagnostic Systems (Nanjing, China).

Ten mice were taken out from each group for liver glycogen, muscle glycogen and serum Blood Urea Nitrogen (BUN) analyses. The mice made swimming exercise for 90 min without a load. After an hour's resting, the mice were killed to collect liver, gastrocnemius muscle and plasma samples. The levels of liver glycogen and muscle glycogen were determined using commercial diagnostic kit obtained from Jiancheng Diagnostic Systems (Nanjing, China). The levels of serum Blood Urea Nitrogen (BUN) were determined by an SABA/18 automatic biochemistry analyzer from Italy.

#### Statistical analysis

Data are expressed as mean ± standard error. Statistical signify-cance between vehicle-treated mice vs. drug treated mice and between before treatment and after treatment were determined by paired Student's *t* test. A value of $P < 0.05$ was considered statistically significant.

## RESULTS AND DISCUSSION

### Effect of polysaccharides from *Cordyceps* on the body weight of mice

Change of body weight, during the experimental period are shown in Figure 1. Results showed that the increase weights in the experimental groups were of no significant difference compared with the CG ($P > 0.05$). So the polysaccharides from *Cordyceps* had no significant effect on body weight.

### Effect of polysaccharides from *Cordyceps* on the swimming endurance time of mice

The swimming experiment was employed in this study to evaluate the effects of polysaccharides from *Cordyceps* on exercise durability of mice. It is commonly accepted that swimming is an experimental exercise model (Orlans, 1987; Lapvetelainen et al., 1997; Jung et al., 2004). Swimming endurance time of mice are shown in Figure 2. The loaded-weight swimming time of mice in CLG, CMG, CHG were significantly prolonged compared with that in CG ($P<0.05$), which was 1.43, 2.51 and 1.70 times longer that in CG, respectively. These results indicated that polysaccharides from *Cordyceps* had significant effect on the endurance of the mice in the experimental. In addition, after the dose of 200 mg/kg was supplied to mice, the loaded-weight swimming time was 1.75 times that of the dose of 100 mg/kg and 1.47 times that of the dose of 400 mg/kg, so the dosage of 200 mg/kg was more effective.

### Effect of polysaccharides from *Cordyceps* on the blood lactic acid level of mice

Blood lactic acid is the glycolysis product of carbohydrate under an anaerobic condition and glycolysis is the main energy source for intense exercise in a short time.

Therefore, the blood lactic acid is one of the important indicators for judging the degree of fatigue (Yu et al., 2008; Lin et al., 2005). It has been reported that blood lactic acid accumulate during exercise (Dawson et al.,

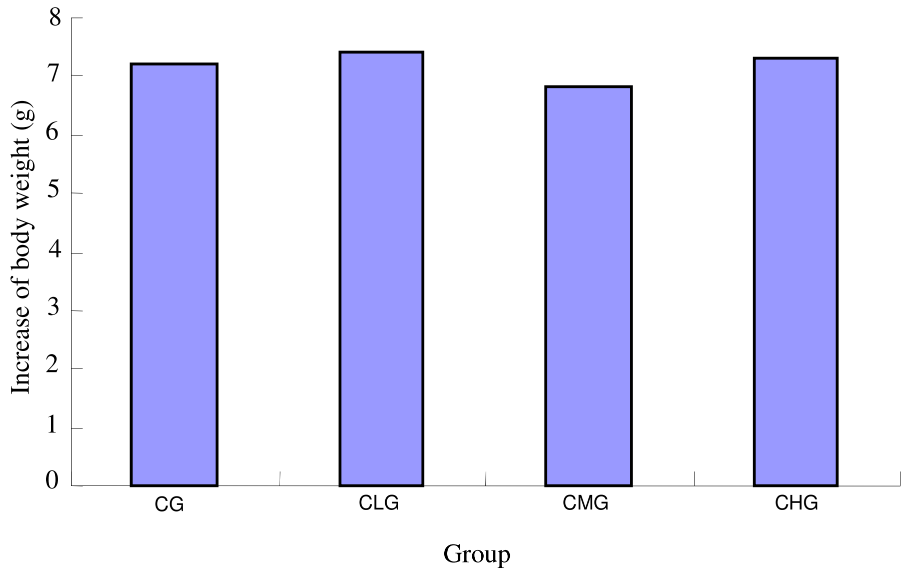


**Figure 1.** Effect of polysaccharides from *Cordyceps* on the body weight of mice (mean ± SD, n=30)

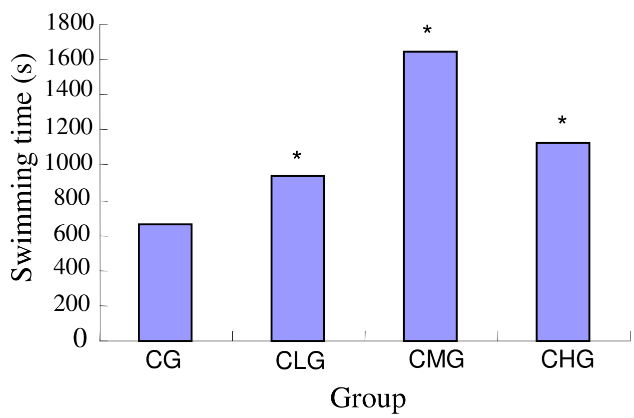


**Figure 2.** Effect of polysaccharides from *Cordyceps* on the swimming endurance time of mice (mean ± SD, n=10).
*P < 0.05 as compared with CG.

**Table 1.** Effect of polysaccharides from *Cordyceps* on the blood lactic acid of mice (Mean ± SD, n=10).

| Groups | The levels of blood lactic acid (mmol/l) | |
|---|---|---|
| | Before swimming | After swimming |
| CG | 4.87±0.49 | 12.09±1.61 |
| CLG | 4.92±0.23 | 9.32±1.53* |
| CMG | 4.78±0.56 | 8.15±1.76* |
| CHG | 5.04±0.42 | 8.04±1.42* |

*P < 0.05 as compared with CG.

1971; Banister et al., 1983; Mutch and Banister, 1983). The levels of blood lactic acid level of mice were measured 30 min after administration of polysaccharides from *Cordyceps* and 30 min after weight loading swimming (2% body weight). The data of blood lactic acid were shown in Table 1, there was no significant difference in the levels of blood lactic acid between the treated groups and the control group before swimming (P>0.05). After swimming, the level of blood lactic acid of CLG, CMG and CHG were significantly lower than that of CG (P<0.05). The results suggested that polysaccharides from *Cordyceps* could inhibit the production of blood lactic acid during exercise. The dosages of 200 mg/kg and 400 mg/kg were more effective.

## Effect of polysaccharides from *Cordyceps* on the serum blood urea nitrogen of mice

Serum blood urea nitrogen is the other important biochemical parameter related to fatigue (Lin et al., 2005) and the levels of serum BUN raise with the exercise load increasing (Yu et al., 2008). Change of serum BUN of mice were measured after swimming exercise for 90 min without a load, the data were shown in Figure 3. In this experiment, the serum BUN of the treated groups were lower than that of the control group, but there wasn't any significant difference (P>0.05).

## Effect of polysaccharides from *Cordyceps* on the tissue glycogen of mice

Energy for exercise is derived initially from the breakdown of glycogen and later, from circulating glucose released by the liver (Suh et al., 2007). So liver and muscle glycogen are sensitive parameters related to fatigue (Ma et al., 2008). Wilber (1959) reported that severe

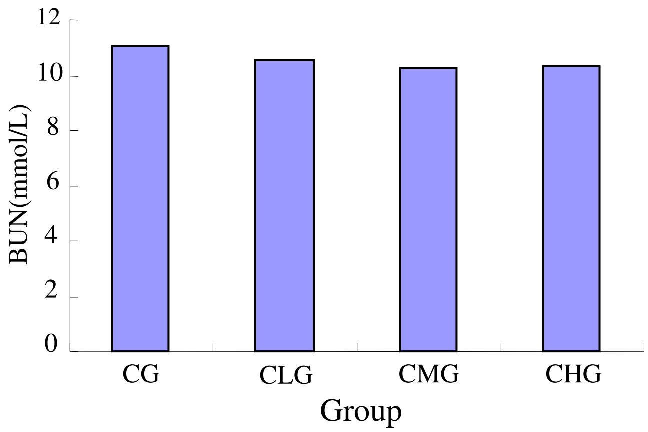


**Figure 3.** Effect of polysaccharides from *Cordyceps* on the serum blood urea nitrogen of mice (mean ± SD, n=10).

**Table 2.** Effect of polysaccharides from *Cordyceps* on the tissue glycogen of mice (Mean ± SD, n=10).

| Group | The levels of glycogen (mg/g) | |
|---|---|---|
| | Liver | Muscle |
| CG | 7.26 ± 1.46 | 1.21 ± 0.74 |
| CLG | 11.45 ± 1.23* | 1.84 ± 0.43* |
| CMG | 16.75 ± 1.58* | 2.41±0.37* |
| CHG | 14.27±1.72* | 2.15±0.848* |

*P < 0.05 as compared with CG.

depletion of liver glycogen was noted in all guinea pigs that swam to exhaustion. Liver glycogen depletion might be an important factor in the development of fatigue because as liver glycogen is depleted during exercise there is an inability to maintain blood glucose level and the ensuing hypoglycemia could result in impaired nervous function (Dohm et al., 1983).

Dohm et al. (1983) also demonstrated the importance of muscle glycogen levels in endurance exercise and suggested that depletion of muscle glycogen was a factor in fatigue and exhaustion. In the present study, the data of glycogen were shown in Table 2. After swimming, the levels of liver glycogen and muscle glycogen of the treated groups were higher than that of control group ($P<0.05$). These data indicated that polysaccharides from *Cordyceps* could significantly increase the levels of liver and muscle glycogen of mice after swimming. The dosage of 200 mg/kg was more effective.

## Conclusions

Polysaccharides from *Cordyceps* extended the swimming endurance time of mice, effectively delayed the increaseing of blood lactic acid, increasing the storage of liver and muscle glycogen. This suggested that polysaccharides from *Cordyceps* had significant anti-fatigue effects on mice. Compared with the dosage of 400 mg/kg, the dosage of 200 mg/kg could cause a little higher level of blood lactic acid of mice after swimming, but there was no obvious difference. Besides, the dosage of 200 mg/kg had the most powerful effect on tissue glycogen and swimming endurance time of mice. So when choosing the dosage, we supposed that the dosage of 200 mg/kg was more effective. However, further study is needed to elucidate the more exact mechanism of the effect of the polysaccharides from *Cordyceps* on anti-fatigue.

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