

## Antifatigue and Antistress Effect of the Hot-Water Fraction from Mycelia of *Cordyceps sinensis*

Jong-Ho KOH,<sup>a</sup> Kyung-Mi KIM,<sup>a</sup> Jin-Man KIM,<sup>b</sup> Jae-Chul SONG,<sup>c</sup> and Hyung-Joo SUH<sup>\*,b</sup>

<sup>a</sup> Obesity Research Center, Dongduk Women's University, Wolgok-dong, Sungbuk-ku, Seoul, 136–714, Korea; <sup>b</sup> Department of Food and Nutrition, College of Health Sciences, Korea University; 1 Jeongneung-dong, Sungbuk-ku, Seoul, 136–703, Korea; and <sup>c</sup> Department of Food and Nutrition, University of Ulsan, Ulsan, 680–748, Korea.

Received December 2, 2002; accepted February 5, 2003

This study was conducted to investigate the chemical component of the hot water (HW) fraction of mycelia of *Cordyceps sinensis* and its antifatigue and antistress effect against a stimulus *in vivo* using rats and mice. The growth of mycelia reached a maximum level of 31.6 g/l after 120 h of incubation. The main chemical composition of the HW fraction of mycelia of *C. sinensis* was found to be carbohydrate (78.9%) with 5% moisture. The swimming endurance capacity of mice orally administered with the HW fraction (150 and 300 mg/kg/d, respectively) was significantly prolonged from 75 to 90 min with a lessening of fatigue. When the HW fraction (150 mg/kg/d) was given to rats for 8 d including a 48 h stress period, the weight changes of the adrenal gland, spleen, thymus, and thyroid, which is an index of stress, were suppressed. The HW fraction also significantly inhibited the increase in total cholesterol and the decrease in alkaline phosphatase levels as biochemical parameters of immobilization stress in rats.

**Key words** antifatigue; antistress; *Cordyceps sinensis*

Higher *Basidiomycetes* mushrooms have been used in folk medicine throughout the world since ancient times. Edible higher *Basidiomycetes* are well known for their nutritional value and acceptability as well as their pharmacological properties. Mushrooms are a nutritionally functional food and a source of physiologically beneficial and noninfective medicines.<sup>1)</sup> Various physiological properties (pharmacological effects) such as bioregulation (immunological enhancement), maintenance of homeostasis, regulation of biorhythm, cure of disease, prevention and improvement of diseases such as cancer, cerebral stroke, and heart disease are affected by mushrooms. It was also confirmed that mushrooms contain effective substances for decreasing serum cholesterol, improving hyperlipemia, antithrombotic effects, reduction of blood pressure, hypoglycemic action, and various other applications.<sup>2)</sup>

It was also found that the medicinal properties of cultured mycelia of *Cordyceps* sp. were as effective as those of the wild species.<sup>3)</sup> The typical *Cordyceps* used in traditional oriental medicine is *Cordyceps sinensis* which forms the fruiting body on the larvae of moths. *C. sinensis* is also known to have an effect on physiological systems: inhibition of the proliferation of human glomerular mesangial cells,<sup>4)</sup> significant contribution to hypotensive activity,<sup>5)</sup> and enhancement of Kupffer cell function.<sup>6)</sup>

However, since most of these physiologically active substances in various *Cordyceps* sp. were extracted from natural or solid cultured stromata, only a few active substances have been developed for pharmaceutical use. Recently, artificial media for high mass production have been developed in Korea to provide many opportunities for practical use. According to Koh *et al.*,<sup>7)</sup> the hot-water (HW) fraction of submerged cultured mycelia of *C. sinensis* stimulates the proliferation of bone marrow cells through Peyer's patch cells and has bioactivity.

Therefore we studied the chemical components and the effects of the HW fraction, the most potent fraction in enhancing immune activity, against stimulus-induced fatigue and

stress *in vivo* using rats and mice.

### MATERIALS AND METHODS

**Microorganism and Materials** *C. sinensis* from Sanning Microbiological Institute in China was used in this experiment. The medium used for liquid culture contained the following per liter: 100 g of molasses, 1 g of yeast extract, and 3 g of K<sub>2</sub>HPO<sub>4</sub>. The submerged cultivation of *C. sinensis* was carried out at 150 rpm, 25 °C, pH 5, and an airflow rate of 1.0 vvm for 7 d in a 5-l jar fermenter using liquid culture medium. The enzymatic kits to estimate the levels of cholesterol, aspartate transaminase (AST), alanine transaminase (ALT), lactate dehydrogenase (LDH), alkaline phosphatase (ALP) were purchased from Sigma Chemical Co. (St. Louis, MO, U.S.A.).

**Animals** Male ICR mice (6–8 weeks old) and Sprague-Dawley rats (6–8 weeks old) were purchased from Daihan-Biolink Co. (Korea). Before adaptation, these animals were fed a commercial chow pellet diet (Samyang Co., Korea) containing the following diet (g/kg diet): moisture, 80; protein, 230; fat, 35; fiber, 50; carbohydrate, 600, and water *ad libitum*. Then the animals were fed this commercial chow pellet and the HW fraction of cultured mycelia of *C. sinensis* for the experiments.

**Preparation of the HW Fraction** Samples were prepared to investigate the physiological effects *in vivo* of the HW fraction of cultured mycelia of *C. sinensis*. The freeze-dried mycelia (50 g) of cultured *C. sinensis* were blanched at 100 °C for 5 min and homogenized at 7000 rpm for 20 min with an Ultra-turrax T-50 (Janke Kunkel IKA-Laborotechniker, Germany). After centrifugation (8000×g, 30 min), the obtained residues were initially fractionated with ethylacetate (1 l), followed by fractionating with MeOH (1 l) and then with hot-water (1 l), in increasing order of polarity. The hot-water extract was centrifuged to remove the insoluble material, and the resulting supernatant was lyophilized to give the HW fraction (yield: 29.3%).

\* To whom correspondence should be addressed. e-mail: suh1960@unitel.co.kr

**Antifatigue Assay** To determine antifatigue activity, the swimming capacity of male ICR mice was studied with an adjustable-current water pool. The details of this apparatus were reported by Matsumoto *et al.*<sup>8)</sup> and it was an acrylic plastic pool (90×45×45 cm) filled 35 cm deep with water maintained at 34 °C. The current in the pool was generated by circulating water with a pump, and the strength of the current was adjusted to 8 l/min with a water flow meter (type F45500, Blue White Co., Westminster, CA, U.S.A.). Mice were judged to be fatigued when they failed to rise to the water surface to breathe within a 7 s period as the index of swimming capacity. Mice were fed the commercial chow pellet diet during the preliminary period. They were forced to swim for 30 min in the current with a flow rate of 6 l/min. On the last day of the preliminary period, the mice were subjected to exhaustion in a current strength of 8 l/min, and the swimming capacity was measured. Then those mice were divided into the control and sample groups with the same mean swimming capacity (6 mice/group). After an adaptation period, the mice groups were orally administered the HW fraction (150 and 300 mg/kg/d, respectively) or placebo solution (0.9% NaCl) once daily *via* a stomach tube during the experiment. Then the chronic swimming time was estimated as an index of antifatigue effect.

**Immobilization-Stress Assay** To investigate the anti-stress effects, male Sprague-Dawley rats (about 250 g) were fed the commercial pellet diet and water *ad libitum* for 8 d. The immobilized-stress technique was carried out by the modified method of Brekhman and Dardymov.<sup>9)</sup> The rats were then divided into three groups, nonstress control, stress control, and stress groups, with oral administration of the HW fraction (150 mg/kg/d) for 8 d (6 rats/group). The immobilized stress was given for the last 48 h of the experiment. Then each organ was weighed and whole blood was obtained by cardiac puncture after anesthetization with ether. To analyze biochemical parameters, blood serum was prepared by centrifugation at 3000 rpm and 4 °C for 10 min. The internal organs (liver, adrenal gland, spleen, thymus and thyroid) were immediately rinsed with ice-cold 0.9% NaCl solution, dried with paper towels, and weighed. Blood serum was prepared by centrifugation at 250×g and 4 °C for 20 min. LDH, ALP, AST, ALT, and cholesterol levels of blood serum induced by stress were measured as stress indicators according to the methods of Jern *et al.*<sup>10)</sup> and Hu *et al.*<sup>11)</sup>

**Analytical Methods** Samples collected at various intervals from the fermenter were centrifuged at 10000×g for 20 min, and the supernatant was filtered through a membrane filter (0.45 μm, Millipore). Then the culture filtrate was mixed with four times the volume of absolute ethanol, stirred vigorously, and stored overnight at 4 °C. The precipitated *exo*-biopolymer was centrifuged at 10000×g for 20 min and the supernatant was discarded. The precipitate of *exo*-biopolymer was lyophilized and the weight of the polymer was measured. The dry weight of the mycelial pellet was obtained by drying overnight at 70 °C.

**Statistical Analysis** All results are expressed as the mean±S.E. Differences between the control and the treatments in experiments were tested for statistical significance using Duncan's multitest, values of  $p < 0.1$  and  $p < 0.05$  were considered to indicate statistical significance.

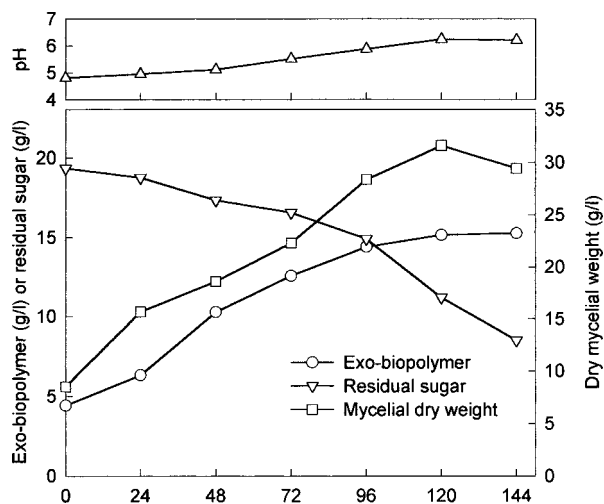


Fig. 1. Mycelial Growth of and *Exo*-Biopolymer Production by *C. sinensis* in a 5-l Fermenter

The submerged culture of *C. sinensis* was fermented at 150 rpm, 25 °C, pH 5 with an air flow rate of 1.0 vvm for 7 d.

## RESULTS AND DISCUSSION

**Mycelial Growth** Figure 1 shows the mycelial growth and *exo*-polymer production in the 5-l jar fermenter under optimal culture conditions. The growth of mycelia increased continuously as the fermentation time increased up to 120 h, but after that the growth began to decrease to 29.4 g/l after 144 h of fermentation. The *exo*-polymer concentration increased as the fermentation time increased and reached a maximum level of 15.27 g/l after 144 h of fermentation with corresponding depletion of the sugar concentration. The residual sugar concentration decreased during the entire period of the fermentation process. The initial pH value of the fermentation slowly increased from 4.8 to 6.2.

Lee *et al.*<sup>12)</sup> reported that maximum polysaccharide production and mycelial biomass of *Ganoderma lucidum* were 7.51 and 13.9 g/l, respectively. Kim *et al.*<sup>13)</sup> reported that maximum polysaccharide production and mycelial biomass of *Pleurotus linteus* were 3.5 and 14.2 g/l, respectively. Our results showed that the maximum polysaccharide production and mycelial biomass were 15.3 and 29.4 g/l, respectively. The mycelium was lyophilized for further studies.

The fermented mycelia of *C. sinensis* were composed of protein (9.2%), fat (7.1%), carbohydrate (67.1%), ash (3.6%) and moisture (10.4%). The HW fraction (14.7 g) was obtained from the freeze-dried mycelia (50 g) of *C. sinensis*. The general chemical composition of the HW fraction (14.7 g) from the dried mycelia (50 g) of fermented *C. sinensis* consisted of 78.9% carbohydrate, 11.8% protein, 1.9% lipid, and 2.4% ash with 5.0% moisture. The carbohydrate content is higher than the protein content of the fermented mycelia of *C. sinensis*, which is contrary to the natural fruiting body of *C. sinensis*. In the same species of the edible mushroom *Agrocybe cylindracea*, a carbohydrate content higher than the protein content of strain M, contrary to strain B, has been described.<sup>14)</sup>

The HW fraction was mainly composed of glucose, mannose, galactose, and a little amount of arabinose with a molar ratio of 1.0 : 0.8 : 0.5 : 0.1. Although the carbohydrate compo-

sition of mycelia from submerged cultured *C. sinensis* is rarely known, the present result showed that HW consisted mainly of glucose, mannose and galactose.

**Antifatigue Activity** The antifatigue activity of the HW fraction was measured as the swimming endurance capacity of mice using an adjustable current swimming pool, which is a forced swimming apparatus for measuring maximal swimming time. The mice administered the HW fraction were divided into two test groups (HW fraction 150 and 300 mg/kg/d, respectively). The experiment was carried out with two test groups and one placebo group (0.9% NaCl) by measuring the swimming time to fatigue at a flow rate of 8 l/min. The swimming time of each test group (150 and 300 mg/kg/d, respectively) increased significantly ( $p < 0.05$ ) compared with that of the control group after 7 d (Fig. 2). It is suggested that the effect of the HW fraction on the recovery from exhaustion might be related to the resistance to stress-induced intensive exercise and enhanced immune system. Ahmad *et al.*<sup>15</sup> reported that a germ-containing Unani formulation (a traditional medicine) had antistress activity against the physical stress of forced swimming, and Moriura *et al.*<sup>16</sup> also reported that the ethanol extract of the dried whole body of *Agkistrodon blomhoffii* BOIE (a venomous snake, mamushi in Japanese) given orally (0.5 g/kg/d) for 3 successive days enhanced the swimming capacity of rats and prolonged the swimming time. Liang *et al.*<sup>17</sup> reported that the oral administration (8 g/kg/d) for 5 d of the water extract of dried *C. sinensis* (Berk.) Sacc. improved the swimming time of mice from 56 to 81 min.

From these results, the HW fraction of *C. sinensis* has an antifatigue effect and also prolongs the swimming time of

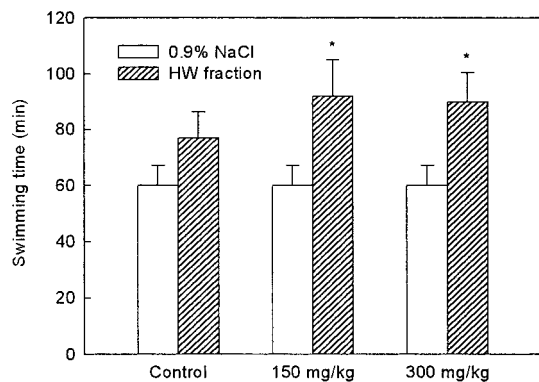


Fig. 2. Effect of the Orally Administered the Hot-Water (HW) Fraction of Cultured Mycelia of *Cordyceps sinensis* on the Swimming Time of Mice

Water was maintained at 34 °C, the current in the pool was generated by circulating water with a pump, and the strength of the current was adjusted to 8 l/min by controlling the voltage supplied to the pump and monitored with a water flow meter. \* $p < 0.05$ , significance between the control and samples.

mice with less fatigue. These effects are related to the enhancement of the immunity.<sup>18</sup>

**Immobilization-Stress Activity** Stress represents the reaction of the body to stimuli that disturb its normal physiological equilibrium or homeostasis, often with detrimental effects, and therefore the weight of internal organs related to the immune system is changed by immobilized stress.<sup>9,19</sup> As shown in Table 1, the weight of the liver, spleen, thymus, and thyroid decreased, but that of the adrenal gland increased significantly ( $p < 0.1$ ) with the immobilization stress. Therefore it was assumed that the immobilized stress method used in this study was suitable. When the HW fraction (150 mg/kg/d) was given to the rats for 8 d, including the 48 h stress period, the HW fraction had a remarkable antistress effect as it suppressed the weight changes of the adrenal gland, thymus, and thyroid (Table 1).

As stress indicators, the serum levels of total cholesterol, LDH, ALP, AST, and ALT were measured according to the methods of Jern *et al.*,<sup>10</sup> Hu *et al.*,<sup>11</sup> and Bowers and McComb,<sup>20</sup> and the results are shown in Table 2. Immobilization stress induced a marked increase in serum LDH, AST and total cholesterol levels, but the levels of total cholesterol and ALP recovered to those in the nonstress group with the oral administration of the HW fraction (150 mg/kg/d). These results are consistent with those of the antistress activity of ginseng, the oriental medicine (*Samul-tang*), and cholic acid derivatives in immobilized stress-induced rats.<sup>21,22</sup> Hu *et al.*<sup>11</sup> reported that repeated immobilization stress (2 h/d for 60 d) resulted in a significant inhibition (25%) of body weight gain, a significant increase in adrenal gland weight, an increase in glucocorticoid receptor (GR) in the liver, thymus, and spleen, decreased plasma triacylglycerol levels, and increased lipid peroxidation in the liver and heart in a study using rats. However, the administration of 5 mg i.p. of dehy-

Table 2. Effect of the Orally Administered Hot-Water (HW) Fraction of Cultured Mycelia of *C. sinensis* on the Blood Biochemical Parameters of Immobilization Stress in Rats

Blood biochemical parameters	Nonstress	Stress control	HW
AST (Karmen unit)	149.3 ± 16.4	179.7 ± 18.2	196.3 ± 20.7
ALT (Karmen units)	50.4 ± 5.9	51.9 ± 3.3	47.5 ± 5.8
TC (mg/dl)	86.5 ± 5.9	139.9 ± 15.4*	90.1 ± 6.7†
LDH (units)	942.6 ± 285.4	1159.1 ± 206.6	1082.6 ± 343.5
ALP (units)	53.3 ± 1.8	13.5 ± 0.8**	21.9 ± 1.8†

Rats were orally administered with the HW fraction (150 mg/kg) for 8 consecutive days. Stress was induced by immobilization for 48 h before removal of internal organs. Data are expressed mean ± S.E. of 6 rats in each group. \* $p < 0.1$ , \*\* $p < 0.05$ , significantly different from nonstress control, † $p < 0.05$ , significantly different from stress control. AST, aspartate transaminase; ALT, alanine transaminase, TC, total cholesterol, LDH, lactate dehydrogenase; ALP, alkaline phosphatase.

Table 1. Effect of the Orally Administered Hot-Water Fraction (HW) of Cultured Mycelia of *C. sinensis* on Some Indices of Immobilization Stress in Rats

Group	Liver	Adrenal	Spleen (mg/100 g body weight)	Thymus	Thyroid
Nonstress	4389.8 ± 108.1	15.82 ± 0.75	294.6 ± 12.9	282.4 ± 129.5	5.94 ± 0.81
Stress control	3516.6 ± 142.9**	21.09 ± 0.73**	179.6 ± 14.4**	169.1 ± 16.4*	4.31 ± 0.17*
HW fraction	3842.6 ± 43.7†	16.25 ± 3.30†	207.3 ± 12.4	239.0 ± 27.6††	6.45 ± 0.58††

Rats were orally administered the HW fraction (150 mg/kg) for 8 consecutive days. Stress was induced by the immobilization for 48 h before the removal of internal organs. Data are express mean ± S.E. of 6 rats in each group. \* $p < 0.1$ , \*\* $p < 0.05$ , significantly different from nonstress control, † $p < 0.1$ , †† $p < 0.05$ , significantly different from stress control.

droepiandrosterone (DHEA) resulted in a significant reversal of stress-induced inhibition of body weight gain, adrenal gland weight, GR levels in the liver, thymus, and spleen, and lipid peroxidation levels in the liver and heart. Therefore they concluded that DHEA acted as an antistress hormone in rats. Some researchers,<sup>23,24)</sup> using immunological approaches, observed a significant increase in immunoactivity in hippocampal and spiral ligament tissues of chronically stressed rats. DHEA inhibited a major change of the weight of the adrenal, thymus, spleen, and thyroid.

From these results, it can be concluded that the HW fraction has antifatigue and antistress effects. To evaluate the physiological or pharmaceutical effects of the HW fraction *in vivo*, we need a more detailed understanding of the factors that enable the HW fraction to exert antifatigue and antistress effects. Therefore more research on the characteristics of the HW fraction will be carried out.

## REFERENCES

- 1) Feofilova E. P., *Appl. Biochem. Microbiol.*, **34**, 597—608 (1997).
- 2) Wasser S. P., Weis A., *Crit. Rev. Immunol.*, **19**, 65—96 (1999).
- 3) Wang S. Y., Shiao M. S., *J. Food Drug Anal.*, **8**, 248—257 (2000).
- 4) Wu Z. L., Wang X. X., Cheng W. Y., *Cell Biochem. Funct.*, **18**, 93—97 (2000).
- 5) Chiou W. F., Chang P. C., Chou C. J., Chen C. F., *Life Sci.*, **66**, 1369—1376 (2000).
- 6) Nakamura K., Yamaguchi Y., Kagota S., Shinozuke K., Kunitomo M., *Jpn. J. Pharmacol.*, **79**, 505—508 (1999).
- 7) Koh J. H., Yu K. W., Suh H. J., Ahn T. S., *Agric. Chem. Biotechnol.*, **44**, 77—83 (2001).
- 8) Matsumoto K., Ishihara K., Tanaka K., Inoue K., Fushiki T., *J. Appl. Physiol.*, **81**, 1843—1849 (1996).
- 9) Brekhman I. I., Dardymov I. V., *Lloydia*, **32**, 46—51 (1969).
- 10) Jern C., Wadenvik H., Mark H., Hallgren J., Jern S., *Brit. J. Haematol.*, **71**, 153—159 (1989).
- 11) Hu Y., Cardounel A., Gursoy E., Anderson P., Kalimi M., *Biochem. Pharmacol.*, **59**, 753—762 (2000).
- 12) Lee S. Y., Kang T. S., Lee M. C., *Kor. J. Biotech. Bioeng.*, **13**, 547—553 (1998).
- 13) Kim D. H., Yang B. K., Jeong S. C., Park J. B., Cho S. P., Das S., Yun J. W., Song C. H., *Biotechnol. Lett.*, **23**, 513—517 (2001).
- 14) Mau J. L., Tseng Y. H., *J. Agric. Food Chem.*, **46**, 2071—2074 (1998).
- 15) Ahmad G., Amin K. M. Y., Khan N. A., Tajuddin, *J. Ethnopharmacol.*, **59**, 187—193 (1998).
- 16) Moriura T., Matsuda H., Kubo M., *Biol. Pharm. Bull.*, **19**, 62—66 (1996).
- 17) Liang Y. L., Liu Y., Yang J. W., Liu C. X., *Phytother. Res.*, **11**, 237—239 (1997).
- 18) Salman H., Bergman M., Weizman A., Bessler H., Weiss J., Strausberg R., Djaldetti M., *Biomed. Pharmacother.*, **54**, 311—315 (2000).
- 19) Khansari D. N., Murgo A. J., Faith R. E., *Immunol. Today*, **11**, 170—175 (1990).
- 20) Bowers G. N., Jr., McComb R. B., *Clin. Chem.*, **21**, 1988—1995 (1975).
- 21) Park I., Kim Y. I., Lee S. M., Cho T. S., *J. Appl. Pharmacol.*, **4**, 162—166 (1996).
- 22) Cho B. W., Lee K. S., Song B. K., *J. Oriental Gynecol.*, **12**, 41—74 (1999).
- 23) Biagini G., Pich E. M., Carani C., Marrama P., Gustafsson J. A., Fux K., Agnati L. F., *Biol. Psychiat.*, **33**, 712—719 (1993).
- 24) Curtis L. M., Rarey K. E., *Heart. Res.*, **92**, 120—125 (1995).