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# Anti-inflammatory and related pharmacological activities of cultured mycelia and fruiting bodies of *Cordyceps militaris*

So-Young Won, Eun-Hee Park*

*College of Pharmacy, Sookmyung Women's University, Seoul 140-742, South Korea*

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**Abstract**

This study aimed to elucidate pharmacological activities of *Cordyceps militaris*. The 70% ethanolic extracts of cultured mycelia (CME) and fruiting bodies (FBE) of *Cordyceps militaris* were prepared. CME was able to directly scavenge the stable free radical diphenyl-2-picrylhydrazyl (DPPH), indicating its antioxidant activity. Both CME and FBE showed topical anti-inflammatory activity in the croton oil-induced ear edema in mice. CME was found to contain acute anti-inflammatory activity, which was evaluated using the carrageenin-induced edema, and also strong antinociceptive activity in writhing test. CME and FBE contain potent inhibitory activity on the chick embryo chorioallantoic membrane (CAM) angiogenesis in a dose-dependent manner. Cordycepin, a metabolite of *Cordyceps militaris*, appeared to be at least partly responsible for its anti-inflammatory and anti-angiogenic activities. CME concentration-dependently inhibited the NO production and iNOS expression upon stimulation by lipoposaccharide in RAW 264.7, a murine macrophage cell line. In brief, we demontrate that *Cordyceps militaris* possesses anti-inflammatory and antinociceptive activites, and related antioxidant, anti-angiogenic, and NO production-inhibitory activities.


*Keywords:* Anti-inflammatory; Antinociceptive; Anti-angiogenic; Cordycepin; Antioxidant; Inducible nitric oxide synthase; *Cordyceps militaris*

## 1. Introduction

Various mushrooms have been traditionally used in many countries for the maintenance of health and for the prevention and treatment of variety diseases for several centuries. Dong-Chong-Xia-Cao in Chinese, which translates as winter worm and summer grass, is one of entomogenous fungi that forms a fruiting body mainly on pupae or larvae. It includes many different genera such as Cordyceps, Paecilomyces, Torrubiella and Podonectria. The entomopathogenic mycelia naturally grow in the intestine of pupae or larvae in the autumn and fruiting bodies of the fungus protrude from the body next summer (Nam et al., 2001). The pharmacological actions of *Cordyceps sinensis*, used as a tonic herb in Chinese traditional medicine, has been relatively well documented. They include: modulation of immune response (Kuo et al., 2001), inhibition of tumor growth (Kuo et al., 1994; Bok et al., 1999), induction of cell apoptosis (Yang et al., 2003), down-regulation of apoptotic and inflammatory genes (Shahed et al., 2001), hypotensive and vasorelaxant activities (Chiou et al., 2000) and secretion of adrenal hormone (Wang et al., 1998). It has also been shown to contain antifatigue and antistress effects against a stimulus in vivo using rats and mice (Koh et al., 2003).

More recently, *Cordyceps militaris* has been widely used due to its folkloric activities, which are not based on scientific studies. Only a few findings have been reported on the pharmacological actions of *Cordyceps militaris*. Extracellular biopolymers from mycelial liquid culture of *Cordyceps militaris* was reported to have on antifibrotic effect on fibrotic rats induced by a bile duct ligation and scission operation (Nan et al., 2001). *Cordyceps militaris* also inhibited proliferation of cultured human glomerular mesangial cell induced by low-density lipoprotein (Zhao-Long et al., 2000). Cordycepin (3′-deoxyadenosine), a metabolite of *Cordyceps*

* Corresponding author. Tel.: +82 2 7109565; fax: +82 2 32735847.
*E-mail address:* ehpark@sookmyung.ac.kr (E.-H. Park).

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*militaris*, has been showed to inhibit the growth of various tumor cells (Johns and Adamson, 1976; Muller et al., 1977; Kodama et al., 2000). Furthermore, it revealed potent growth-inhibiting activity toward some human intestinal bacteria such as *Clostridium paraputrificum* and *Clostridium perfringens* without adverse effects on the growth of bifidobacteria and lactobacilli, suggesting that cordycepin could be useful as a new preventive agent against various diseases caused by clostridia (Ahn et al., 2000). Cordycepin was also shown to contain larvicidal activity against *Plutella xylostella* via a direct effect rather than an inhibitory action on chitin synthesis (Kim et al., 2002). Therefore, the pharmacological actions of *Cordyceps militaris* remain largely unknown. In this article, we describe a few pharmacological actions of *Cordyceps militaris*.

## 2. Materials and methods

### 2.1. Microbiological materials

Fresh fruiting bodies of *Cordyceps militaris* grown on silkworm pupae were obtained from Gangwon Natural Farm (Chuncheon, Korea) and authenticated by Prof. Ki-Oug Yoo, Division of Life Sciences, Kangwon National University, Chuncheon, Korea. Host materials were completely removed before extraction. The fungus strain *Cordyceps militaris* (EFCC-C738) was from Dong-Chong-Xia-Cao Culture Collection (Director, Prof. Jaemo Sung), Kangwon National University, Chuncheon, Korea.

### 2.2. Mycelial growth

The fungus strain was inoculated into preculture medium (malt extract 30 g, yeast extract 5 g, and agar 15 g/l) with kanamycin and tetracycline. Precultures grown at 25 °C were cultivated as suspension cultures in potato dextrose broth (24 g/l, pH 5.2), and incubated at 25 °C without shaking until complete saturation. Mycelia were harvested by centrifugation.

### 2.3. Preparation of the ethanolic extract

Fresh fruiting bodies or mycelia of *Cordyceps militaris* were extracted with 70% ethanol at room temperature. The ethanolic extracts of fruiting bodies (FBE) and cultured mycelia (CME) were evaporated in vacuo to give solid extracts. The yields of FBE and CME were found to be 4.4 and 21.7%, respectively.

### 2.4. Animals

Male ICR mice (about 25 g) or female Sprague-Dawley rats (130–150 g) were housed in temperature-controlled rooms (22–23 °C) until use. Food and water were supplied ad libitum. The ethical guidelines described in the NIH Guide for Care and Use of Laboratory Animals was followed throughout the experiments.

### 2.5. Cell culture

RAW 264.7 cells, a murine macrophage cell line, were cultured in Dulbecco's modified Eagle's medium containing 10% fetal bovine serum (FBS), 25 mM HEPES (pH 7.5), 100 U/ml penicillin and 100 μg/ml streptomycin. RAT 264.7 cells were plated at a density of $1 \times 10^6$ cells and preincubated for 24 h at 37 °C, and maintained in a humidified atmosphere containing 5% $CO_2$. For all experiments, the cells were grown to 80–90% confluency, and were subjected to no more than 20 cell passages. RAW 264.7 cells were preincubated for 30 min with selected concentrations of CME.

### 2.6. Assay for DPPH radical scavenging activity

DPPH radical scavenging activities of CME were tested according to the method previously described (Song et al., 2003). In brief, reaction mixtures containing various concentrations of CME dissolved in saline and 100 μM DPPH solution in a 96-well microtiter plate were incubated at 37 °C for 30 min and absorbance was measured at 490 nm.

### 2.7. Croton oil-induced ear edema test

In order to test whether CME and FBE possess a plausible topical anti-inflammatory property, the croton oil-induced ear edema test was performed as previously described (Olajide et al., 2000b). Mice were anesthetized with ether. A total of 20 μl of an acetonic solution containing 0.4 μg of croton oil and CME or FBE at 0.5 mg/ear were applied to the inner surface of the right ear of each mouse. The left ear remained untreated. Control animals received only the irritant while indomethacin (0.5 mg/ear) served as the reference. The animals were sacrificed by cervical dislocation 5 h later and a plug (6 mm in diameter) was removed from both the treated and the untreated ear. The difference in weight between the two plugs was taken as a measure of oedematous response.

### 2.8. Carrageenin-induced rat paw edema test

To study the effect of CME and cordycepin on acute inflammation, the carrageenin-induced paw edema model was used (Winter et al., 1962). CME (12.5 and 50 mg/kg body weight, i.p.) and cordycepin (5 mg/kg body weight, i.p.) were administered 30 min before receiving 0.1 ml of carrageenin (1.5%, w/v) into subplantar area of the right hind paw. A control group received the vehicle only and a reference group was treated with indomethacin (5 mg/kg body weight, i.p.). The volume of the paw was measured before the injection carrageenin (time 0) and at 2, 4 and 6 h after injection of carrageenin using a plethysmometer (Ugo Basile, Milano, Italy). The edema was expressed as

the increase in paw volume, and edema inhibition was expressed as the reduction in volume with respect to the control group.

### 2.9. Chorioallantoic membrane (CAM) assay

Anti-angiogenic activity was measured using chorioallantoic membrane assay as previously described (Song et al., 2003). The fertilized chicken eggs used in this study were kept in a humidified egg incubator at 37 °C. After 3.5-day incubation, about 2 ml of albumin was aspirated from the eggs with 22-gauge needle through the small hole drilled at the narrow end of the eggs, allowing the small CAM and yolk sac to drop away from the shell membrane. The shell covering the air sac was punched out and removed by forceps, and the shell membrane on the floor of the air sac was peeled away. A sample-loaded Thermanox-coverslip was applied onto the CAM surface of the 4.5-day-old chick embryo, which was then returned to the incubator. Two days later, an appropriate volume of a 10% fat emulsion (Intralipose, 10%) was injected using a 33-gauge needle into a 6.5-day-old embryo chorioallantois. The eggs were then observed under a microscope.

### 2.10. Measurement of antinociceptive activity

Antinociceptive activity was measured as previously described (Olajide et al., 2000a). CME at doses of 25 and 50 mg/kg was orally administered, after an overnight fasting, to mice. One hour after the treatment, the mice were injected intraperitoneally with acetic acid solution to induce the characteristic writhings. The number of writhings occurring between 5 and 15 min after acetic acid injection was recorded.

### 2.11. Assay for nitrite concentration

Accumulated nitrite ($NO_2^-$) in the media of cell cultures was measured using an automated colorimetric assay based on the Griess reaction. Cells were plated in 12-well culture plates at a density of $8 \times 10^5$ cells and incubated with LPS (1 μg/ml) in the presence or absence of CME for 24 h. The supernatants were reacted with the Griess reagent at room temperature for 10 min, and then $NO_2^-$ concentration was determined by measuring the absorbance at 540 nm in a UV–vis spectrophotometer The standard curve was obtained using the known concentrations of sodium nitrite.

### 2.12. Immunoblot analysis

RAW 264.7 cells were incubated with LPS (1 μg/ml) in the presence or absence of CME for 24 h and then washed twice with ice-cold phosphate buffered saline (PBS). The cells were lysed in a buffer containing 20 mM Tris–Cl (pH 7.5), 1% Triton X-100, 137 mM NaCl, 2 mM EDTA, 1 mM sodium orthovanadate, 2 mM sodium pyrophosphate, 1 mM phenylmethylsulfonyl fluoride (PMSF), and 1 μg/ml leupeptin. Cell lysates were centrifuged at $10{,}000 \times g$ for 10 min to remove debris The supernatant was subjected to SDS–PAGE on 10% polyacrylamide gel and transferred to a poly vinylidene difluride membrane in 25 mM Tris, 20% methanol and 192 mM glycine. For immunoblotting, membranes were blocked with 5% non-fat milk dissolved in PBS containing 0.1% Tween-20 (PBST) at 4 °C overnight and then incubated at room temperature with 0.5 μg/ml anti-mouse iNOS antibodies (BD Biosciences) for 1 h. The blots were washed and incubated with goat anti-mouse IgG conjugated to peroxidase in PBST containing 3% non-fat milk for 1 h. Blots were washed in PBST two times, incubated in chemiluminescence reagents (Supersignal® detection system) and exposed to photographic film.

### 2.13. Statistical analysis

The data were analyzed for statistical significance using Student's *t*-test. *P* values less than 0.05 were considered to be significant

## 3. Results

### 3.1. Antioxidant activity

In an assay model for assessing an inhibitory ability to scavenge the stable free radical DPPH, the ethanolic extract of cultured mycelia (CME) of *Cordyceps militaris* was evaluated for its antioxidant activity. CME was shown to directly scavenge the DPPH radical in a concentration-dependent manner over a concentration range of 1–30 mg/ml (Fig. 1). The $IC_{50}$ value of CME was calculated to be 0.026 g/ml, whereas that of vitamine C, used as a positive control, was 51 μg/ml (Fig. 1). These results imply the weak antioxidant activity of CME.

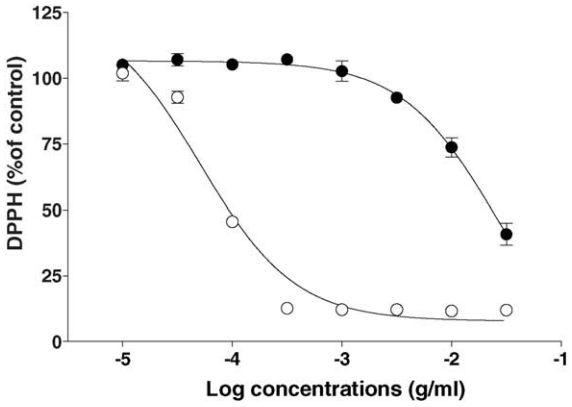


Fig. 1. Effect of the 70% ethanolic extract (CME, ●) prepared from cultured mycelia of *Cordyceps militaris* on scavenging DPPH free radical. Vitamine C (○) was used as a positive control. DPPH concentration used was 100 μM. The data represent three experiments.

Table 1
Topical anti-inflammatory activity of the 70% ethanol extracts prepared from fruiting bodies (FBE) and cultured mycelia (CME) of *Cordyceps militaris* on croton oil-induced ear edema in mice

| | Dose (mg/ear) | Edema[a] (mg) | Inhibition[b] (%) |
|---|---|---|---|
| Control | – | 11.07 ± 1.87 | – |
| FBE | 0.5 | 4.57 ± 1.20* | 58.7 |
| CME | 0.5 | 5.34 ± 2.44* | 51.8 |
| Indomethacin | 0.5 | 4.25 ± 0.45** | 61.6 |

[a] Edema weight of ear plug was measured. Each value represents mean ± S.E.
[b] Inhibition percentage with respect to the control treated only with croton oil.
* $P < 0.05$.
** $P < 0.01$.

### 3.2. Anti-inflammatory activity

Topical anti-inflammatory activity of CME and FBE was evaluated as the inhibition of the croton oil-induced ear edema in mice. Topical application of croton oil induced cutaneous inflammation at the ears of mice, which caused significant increase in ear plug weight of the right ear when compared to the vehicle-treated left ear (data not shown). As a positive control, indomethacin (0.5 mg/ear) gave rise to a significant inhibition of 61.6% in ear plug weight (Table 1). When CME and FBE were topically applied at 0.5 mg/ear, they provided an inhibition of 51.8 and 58.7%, respectively, in ear plug weight (Table 1).

Anti-inflammatory activity of CME was also evaluated using the carrageenin-induced edema, an acute inflammation model, in the rat hind paw. Intraperitoneal administration of CME (12.5 mg/kg body weight, 50 mg/kg body weight) significantly reduced carrageenin-induced paw swelling in a dose-dependent manner (Fig. 2). The reduction in edema was statistically significant as asssesed by Student's *t*-test. Taken together, it is shown that both fruiting bodies and cultured mycelia of *Cordyceps militaris* possess potent anti-inflammatory activity.

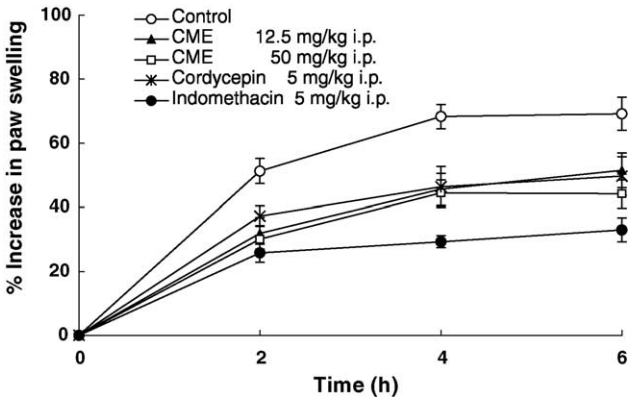


Fig. 2. Inhibitory effect of the 70% ethanolic extract (CME) prepared from cultured mycelia of *Cordyceps militaris* and cordycepin on the carrageenin-induced edema in the rat hind paw. Indomethacin was used as a positive control. Experiments were performed in triplicate. Each point represents mean ± S.E.

### 3.3. Antinociceptive activity

Antinociceptive activity of CME was examined using writhing test. Oral administration of CME at the doses of 25 and 50 mg/kg body weight gave rise to an inhibiton of 74.2 and 85.0% in the number of writhing, which was counted between 5 and 15 min, respectively (Table 2). Antinociceptive activity of CME appeared to be dose-dependent. Phenylbutazone (100 mg/kg body weight, p.o.), used as a positive control, gave 60.4% inhibition in the number of writhing. These data pharmacologically support the strong antinociceptive activity of *Cordyceps militaris*.

### 3.4. Anti-angiogenic activity

The CAM assay was used for examining the inhibitory activity of CME and FBE on vascular development, and retinoic acid was used as positive control for the assay. The disk weight did not give any changes in vascular density, indicating that it could not affect the growth of blood vessels (data not shown). When 0.1, 0.3, 1 or 3 μg/egg of CME was applied, the inhibition percentages in the CAM angiogenesis were measured to be 27.9, 50.0, 54.2 or 72.5%, respectively (Fig. 3). FBE gave 34.3, 50.5, 57.9 or 68.3% inhibition in the CAM angiogenesis, when it was applied at the doses of 0.01, 0.1, 1 or 10 μg/egg, respectively (Fig. 4). Anti-angiogenic activity of CME and FBE at higher doses was comparable to that of retinoic acid (1 μg/egg). Taken together, the extracts of *Cordyceps militaris* possess potent anti-angiogenic activity.

### 3.5. Anti-inflammatory and anti-angiogenic activities of cordycepin

Since cordycepin has been known to be a component contained in *Cordyceps militaris* cells, its anti-inflammatory and anti-angiogenic activities were examined in this study. In the carrageenin-induced edema in the rat hind paw, the intraperitoneal administration of cordycepin at the doses of 5 mg/kg body weight gave rise to a significant inhibition in the paw swelling (Fig. 2). Anti-angiogenic activity of cordycepin was

Table 2
Antinociceptive activity of the 70% ethanolic extract (CME) of cultured mycelia of *Cordyceps militaris* on writhing induced by acetic acid in mice[a]

| | Concentration (mg/kg, p.o.) | No. of writhing (times/10 min)[b] | Inhibition (%) |
|---|---|---|---|
| Control | | 24.94 ± 7.27 | – |
| CME | 25 | 6.43 ± 1.83* | 74.2 |
| CME | 50 | 3.00 ± 0.05** | 85.0 |
| Phenylbutazone | 100 | 7.88 ± 3.13* | 60.4 |

[a] Acetic acid (0.7%) was intraperitoneally injected in a volume of 0.1 ml per 10 g body weight.
[b] Number of writhing was counted for 10 min. Each value represents the mean ± S.E.
* $P < 0.05$.
** $P < 0.01$.

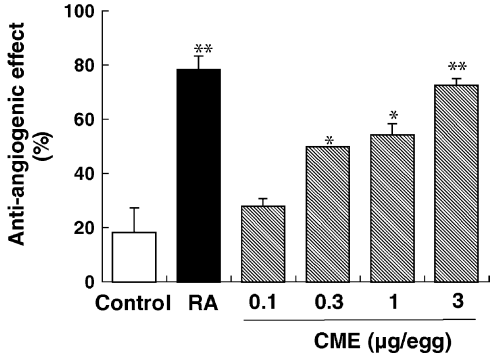


Fig. 3. Dose-dependent anti-angiogenic activity of the 70% ethanolic extract (CME) prepared from cultured mycelia of *Cordyceps militaris* in the chick embryo chorioallantoic (CAM) assay. Retinoic acid (RA, 1 μg/egg) was used as a positive control. The dose for half-maximal inhibition ($IC_{50}$) of CME was 0.236 μg/egg. Experiments were performed in triplicate. Each column represents mean ± S.E. (*), $P < 0.05$; (**), $P < 0.01$.

also identified using the CAM assay. When cordycepin was applied at the doses of 0.1, 0.3, 1 or 3 μg/egg, it provided 28.2, 44.2, 52.8 or 58.6% inhibition in the CAM angiogenesis (Fig. 5). Through this work, cordycepin, known as an inhibitor in transcription, is convinced to contain anti-inflammatory and anti-angiogenic activities.

### 3.6. Effect of CME on iNOS expression

To assess the effect of CME on LPS-induced NO production in RAW 264.7 macrophages, cell culture medium was harvested, and the concentration of accumulated nitrite, the oxidative product of NO, was determined by the Griess method (Fig. 6). The accumulated nitrite, estimated by the Griess method, in the culture medium was used as an index for NO synthesis from these cells. After treatment with LPS for 24 h, nitrite concentration markedly increased about six-fold (~44 μM). When cells were treated with various concentrations of CME, nitrite production induced by LPS was

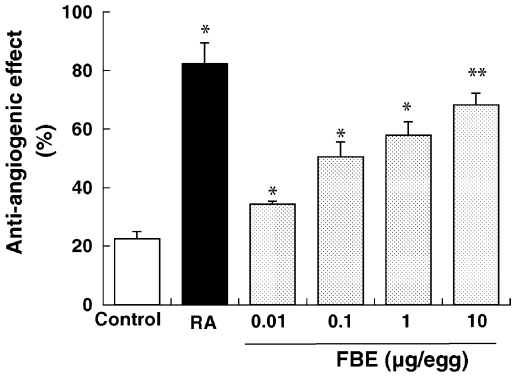


Fig. 4. Dose-dependent anti-angiogenic activity of the 70% ethanolic extract (FBE) from *Cordyceps militaris* fruiting bodies in the chick embryo chorioallantoic (CAM) assay. Retinoic acid (RA, 1 μg/egg) was used as a positive control. The dose for half-maximal inhibition ($IC_{50}$) of FBE was 0.089 μg/egg. Experiments were performed in triplicate. Each column represents mean ± S.E. (*), $P < 0.05$; (**), $P < 0.01$.

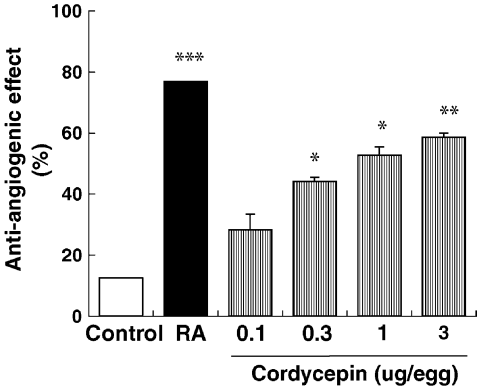


Fig. 5. Anti-angiogenic activity of cordycepin, a component of *Cordyceps militaris*, in the chick embryo chorioallantoic (CAM) assay. Retinoic acid (RA, 1 μg/egg) was used as a positive control. The dose for half-maximal inhibition ($IC_{50}$) of Cordycepin was 0.114 μg/egg. Experiments were performed in triplicate. Each column represents mean ± S.E. (*), $P < 0.05$; (**), $P < 0.01$; (***), $P < 0.001$.

significantly inhibited at concentrations from 1 to 5 mg/ml in a concentration-dependent manner. No effects on cell viability were observed in used concentrations of CME as determined by MTT assay (data not shown). With the assumption that the inhibition of NO production by CME in RAW 264.7 cells would be caused by a decrease especially in the iNOS protein, the effect of CME on the iNOS protein expression was examined in cells treated with LPS for 24 h. As shown in Fig. 7, treatments with CME inhibited iNOS protein expression induced by LPS in a concentration-dependent manner without changes in the amount of β-actin protein, an internal control, indicating the specific inhibition of iNOS protein expression by CME. The results suggest that CME contributes to the decreased iNOS protein and NO production.

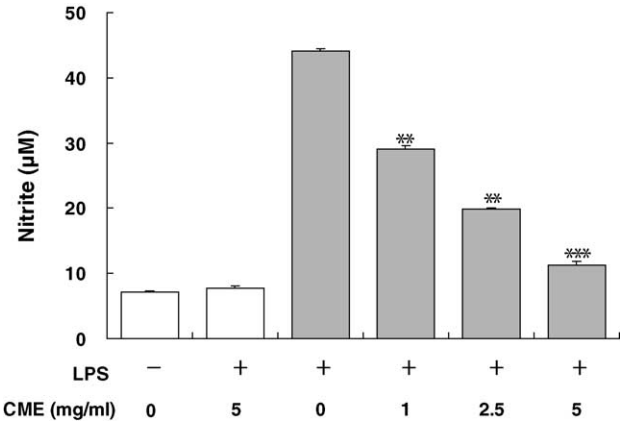


Fig. 6. Inhibitory effect of the 70% ethanolic extract (CME) prepared from cultured mycelia of *Cordyceps militaris* on LPS-induced NO production in RAW 264.7 cells were incubated for 24 h with LPS (1 μg/ml) in the presence or absence of indicated concentrations of CME. Accumulated nitrite in the culture medium was determined by the Griess reaction. The values are mean ± S.E. of three independent experiments. (***), $P < 0.001$; (**), $P < 0.01$.

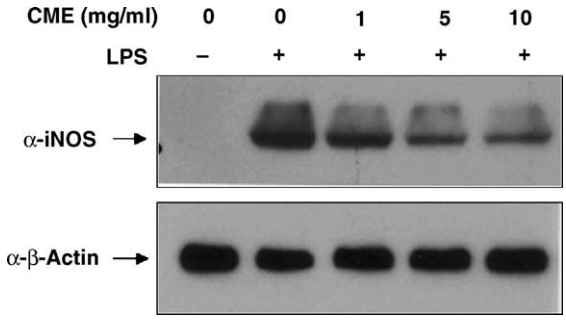


Fig. 7. Inhibitory effect of CME on LPS-induced expression of iNOS protein. RAW 264.7 cells were incubated with LPS (1 μg/ml) in the presence or absence of indicated concentrations of CME. After 24 h incubation, cell lysates were separated by SDS–PAGE, transferred to a polyvinylidene difluoride membrane and blotted with a mouse monoclonal anti-iNOS antibody. β-Actin was used as an internal control. This blot is a representative of three independent experiments.

## 4. Discussion

*Cordyceps militaris* is one of the well-known medicinal entomopathogenic fungi, and has been widely used for the treatment of various diseases. However, its pharmacological and biochemical actions have not been clearly elucidated. As shown in Fig. 1, the ethanolic extract of *Cordyceps militaris* cultured mycelia was able to scavenge the stable free radical DPPH, indicating that it contains antioxidant activity. When compared with the antioxidant activity of vitamin C, it shows relatively low scavenging activity. The presence of various components in the ethanolic extract might imply the existence of strong antioxidant compounds. Until recently, antioxidant activity has never been identified in *Cordyceps* species.

The ethanolic extract of *Cordyceps militaris* cultured mycelia shows strong inhibitory activity on the croton oil-induced ear edema in mice (Table 1), which is comparable to that of indomethacin, used as a positive control. The ethanolic extract prepared from the fruiting bodies also contain a high degree of anti-inflammatory activity. These data suggest that the strong anti-inflammatory of *Cordyceps militaris* dose not depend on growth conditions. The ethanolic extract of *Cordyceps militaris* cultured mycelia was found to possess potent anti-inflammatory activity in the carrageenin-induced edema, an acute inflammation model (Fig. 2). These findings are the first pharmacological evidences on the anti-inflammatory of *Cordyceps* species, which proposes the application of *Cordyceps militaris* for the treatment of inflammatory diseases. It seems that cordycepin is partly responsible for the anti-inflammatory activity of *Cordyceps militaris* (Fig. 2).

Angiogenesis contributes to the development and progression of various pathological conditions including tumor growth and metastasis, cardiovascular diseases, inflammatory diseases and psoriasis. Down-regulation of angiogenesis would be very advantageous during periods of neoplastic growth and chronic inflammation. Many researchers are trying to screen anti-angiogenic principle from various natural products. Up to the present, angiogenesis inhibitors such s fumagillin and minocycline have been identified from microbes (Ingber et al., 1990; Tarmago et al., 1991). Recently, potent anti-angiogenic activity has been convinced in *Gardenia jasminoides* fruits and fruiting bodies of the mushroom *Phellinus linteus* (Park et al., 2003; Song et al., 2003). As shown in Figs. 3 and 4, both the two *Cordyceps militaris* extracts show significant anti-angiogenic activity, which was detected using the CAM assay. Their low $IC_{50}$ values may suggest the presence of very strong anti-angiogenic principle in *Cordyceps militaris*. Cordycepin appears to contain anti-angiogenic activity although relatively weak (Fig. 5). Anti-angiogenic activity of *Cordyceps militaris* would be closely linked with its anti-inflammatory and antinociceptive activities.

Nitric oxide (NO) synthesis by inducible nitric oxide synthase (iNOS) is increased in inflammatory diseases and leads to cellular injury. The present studies also demonstrate that the ethanolic extract of *Cordyceps militaris* cultured mycelia markedly decreases inducible nitrite oxide synthase (iNOS) expression at a low concentration in RAW 264.7 macrophages. Its inhibition on iNOS expression, together with its antioxidant activity, may support its anti-inflammatory and anti-angiogenic activities. The extract may exert beneficial effects in the prevention or treatment of the oxidative stress-induced inflammatory diseases.

In conclusion, we have demonstrated that *Cordyceps militaris* extract possesses anti-inflammatory and anti-angiogenic activities, which are supported by its inhibitory activity on iNOS expression. These novel findings provide an additional pharmacological background on its efficacies of *Cordyceps militaris*.

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## References

Ahn, Y.J., Park, S.J., Lee, S.G., Shin, S.C., Choi, D.H., 2000. Cordycepin: selective growth inhibitor derived from liquid culture of *Cordyceps militaris* against *Clostridium* spp. Journal of Agricultural and Food Chemistry 48, 2744–2748.

Bok, J.W., Lermer, L., Chilton, J., Klingeman, H.G., Towers, G.H., 1999. Antitumor sterols from the mycelia of *Cordyceps sinensis*. Phytochemistry 51, 891–898.

Chiou, W.F., Chang, P.C., Chou, C.J., Chen, C.F., 2000. Protein constituent contributes to the hypotensive and vasorelaxant acttvtties of *Cordyceps sinensis*. Life Sciences 66, 1369–1376.

Ingber, D., Fujita, T., Kishimoto, S., Sudo, K., Kanamaru, T., Brem, H., Folkman, J., 1990. Synthetic analogues of fumagillin that inhibit angiogenesis and suppress tumor growth. Nature 348, 555–557.

Johns, D.G., Adamson, R.H., 1976. Enhancement of the biological activity of cordycepin (3′-deoxyadenosine) by the adenosine deaminase inhibitor 2′-deoxycoformycin. Biochemical Pharmacology 25, 1441–1444.

Kim, J.R., Yeon, S.H., Kim, H.S., Ahn, Y.J., 2002. Larvicidal activity against *Plutella xylostella* of cordycepin from the fruiting body of *Cordyceps militaris*. Pest Management Science 58, 713–717.

Kodama, E.N., McCaffrey, R.P., Yusa, K., Mitsuya, H., 2000. Antileukemic activity and mechanism of action of cordycepin against terminal deoxynucleotidyl transferase-positive ($TdT^+$) leukemic cells. Biochemical Pharmacology 59, 273–281.

Koh, J.H., Kim, K.M., Kim, J.M., Song, J.C., Suh, H.J., 2003. Antifatigue and antistress sffect of the hot-water fraction from mycelia of *Cordyceps sinensis*. Biological and Pharmaceutical Bulletin 26, 691–694.

Kuo, Y.C., Lin, C.Y., Tsai, W.J., Wu, C.L., Chen, C.F., Shiao, M.S., 1994. Growth inhibitors against tumor cells in *Cordyceps sinensis* other than cordycepin and polysaccharides. Cancer Investigation 12, 611–615.

Kuo, Y.C., Tsai, W.J., Wang, J.Y., Chang, S.C., Lim, C.Y., Shiao, M.S., 2001. Regulation of bronchoalveolar lavage fluids cell function by the immunomodulatory agents from *Cordyceps sinensis*. Life Sciences 68, 067–082.

Muller, W.E.G., Seihard, G., Beyer, R., Breter, H.J., Maidhof, A., Zahn, R.K., 1977. Effects of cordycepin on nucleic acid metabolism in L5178Y cells and on nucleic acid-synthesizing enzyme systems. Cancer Research 37, 3824–3833.

Nam, K.S., Jo, Y.S., Kim, Y.H., Hyun, J.W., 2001. Cytotoxic activities of acetoxyscirpenediol and ergosterol peroxide from *Paecilomyces tenuipes*. Life Sciences 69, 229–237.

Nan, J.X., Park, E.J., Yang, B.K., Song, C.H., Ko, G., Sohn, D.H., 2001. Antifibrotic effect of extracellular biopolymer from submerged mycelial cultures of *Cordyceps militaris* on liver fibrosis induced by bile duct ligation and scission in rats. Archives of Pharmacol Research 24, 327–332.

Olajide, O.A., Awe, S.O., Makinde, J.M., Ekhelar, A.I., Olusola, A., Morebise, O., Okpako, D.T., 2000a. Studies on the anti-inflammatory, antipyretic and analgesic properties of *Alstonia boonei* stem bark. Journal of Ethnopharmacology 71, 179–186.

Olajide, O.A., Makinde, J.M., Okpako, D.T., Awe, S.O., 2000b. Studies on the anti-inflammatory and related pharmacological properties of the aqueous extract of *Bridelia ferruginea* stem bark. Journal of Ethnopharmacology 71, 153–160.

Park, E.H., Joo, M.H., Kim, S.H., Lim, C.J., 2003. Anti-angiogenic activity of Gardenia jasminoides. Phytotherapy Research 17, 961–962.

Shahed, A.R., Kim, S.I., Shoskes, D.A., 2001. Down-regulation of apoptotic and inflammatory genes by *Cordyceps sinensis* extract in rat kidney following ischemia/reperfusion. Transplantation Proceedings 33, 2986–2987.

Song, Y.S., Kim, S.H., Sa, J.H., Jin, C.B., Lim, C.J., Park, E.H., 2003. Anti-angiogenic, antioxidant and xanthine oxidase inhibition activities of the mushroom *Phellinus linteus*. Journal of Ethnopharmacology 88, 113–116.

Tarmago, R.J., Bok, R.A., Brem, H., 1991. Angiogenesis inhibition by minocycline. Cancer Research 51, 672–675.

Wang, S.M., Lee, L.J., Lin, W.W., Chang, C.M., 1998. Effects of a water-soluble extract of *Cordyceps sinensis* on steroidogenesis and capsular morphology of lipid droplets in cultured rat adrenocortical cells. Journal of Cellular Biochemistry 69, 483–489.

Winter, C.A., Risley, E.A., Nuss, G.W., 1962. Carrageenan-induced edema in hind paw of the rats as an assay for anti-inflammatory drugs. In: Proceedings of the Society for Experimental and Biological Medicine 111, 544–547.

Yang, L.Y., Huang, W.J., Hsieh, H.G., Lin, C.Y., 2003. H1-A extracted from *Cordyceps sinensis* suppresses the proliferation of human mesangial cells and promotes apoptosis, probably by inhibiting the tyrosine phosphorylation of Bcl-2 and Bcl-XL. Journal of Laboratory and Clinical Medicine 141, 74–83.

Zhao-Long, W., Xiao-Xia, W., Wei-Ying, C., 2000. Inhibitory effect of *Cordyceps sinensis* and *Cordyceps militaris* on human glomerular mesangial cell proliferation induced by native LDL. Cell Biochemistry and Functions 18, 93–97.