

Effect of *Cordyceps militaris* on Testosterone Production in Sprague-Dawley Rats

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Some of men have been suffered from the insufficient secretion of testosterone causing by physical factors, social and psychological factors. Testosterone is an essential steroid hormone controlling male reproductive function. Alternative medicines in plants, fungi, and insects have been studied to enhance sexuality. *Cordyceps* species including *Cordyceps sinensis* (CS) and *C. militaris* (CM) has been used as for the enhancement of sexual function for hundreds of years in Far East Asian. In the present study, we determined the effect of fruiting bodies of *C. militaris* which cultured on bee drone medium (CMD) and brown rice medium (CMB) on testosterone concentration in Sprague-Dawley rats. Eighteen rats per group were housed to regular diet or diet supplemented with CMB and CDD, respectively for 4 weeks. Serum was collected from 6 rats per group. Results showed that changes of the body weight, food and water intake of the rats were not observed in this study. However, both CMB and CDD increased the serum testosterone concentration in rats. Furthermore, CMD significantly stimulated testosterone production ($p < 0.05$) compared to the control. Hence, it suggests that *C. militaris* fruiting body might be developed as a complementary medicine to improve sexual hormones.

Key words: Brown rice, *Cordyceps militaris*, Drone, Testosterone

Introduction

The average life expectancy has continuously increased with the development of medicine. However, some of men have been suffered from the insufficient secretion of testosterone, which is caused by physical factors, social and psychological factors (Sinclair, 2000; Roscoe *et al.*, 2001). Testosterone is an essential steroid hormone controlling male reproductive function (Saez, 1994). Alternative medicines in plants, fungi, and insects have been studied to enhance sexuality (Crimmelet *et al.*, 2001). In fact, the injection of testosterone to restore the reproductive function has been applied to treat men with insufficient testosterone secretion for decades (Huff *et al.*, 2001). The scientific evidence related to the mechanisms or efficacy of these alternatives is scarce or often unconvincing. Recently Korean researchers have been studying the complementary medicines to improve sexual hormones in silkworm or bee drone (Ryuet *et al.*, 2010, 2011; Hong *et al.*, 2011). *Cordyceps* species including *Cordyceps sinensis* (CS) and *C. militaris* (CM) has been used as Chinese traditional herb for centuries (Zhu *et al.*, 1998). It has been reported that CS can induce the steroidogenic enzyme estradiol-17 (E2) expression in human granulosa-lutein cells (GLC) and testosterone in primary mouse Leydig cells and MA-10 mouse Leydig tumor cells (Hsu *et al.*, 2003; Huang *et al.*, 2004). CS is comparatively rare in nature and cannot be easily grown in culture, whereas CM occurs worldwide and forms fruiting bodies well. CM also contains higher concentration of cordycepin than CS (Yu *et al.*, 2006). Cordycepin from *C. militaris* has been reported to have acute anti-inflammatory, anti-nociceptive, anti-angiogenesis and immunoregulatory activities (Kim *et al.*, 2006).

In this study, we investigated the effect of fruiting bod-

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ies of *C. militaris* which cultured on bee drone medium (CMD) and brown rice medium (CMB), respectively on testosterone concentration in Sprague-Dawley rats.

Materials and Methods

Fungal strain

C. militaris, Cmb233, the mating strain between the single ascospores of Cm186 and Cm209 preserved in the Rural Development Administration, was used for fruiting body production.

Materials

C. militaris was inoculated into vegetable medium (brown rice medium) and animal medium (bee drone medium), respectively, and cultivated in National Academy Agricultural Science (NAAS). Fruiting bodies of *C. militaris* grown on brown rice medium (CMB) and bee drone medium (CMD) were harvested. The harvested fruiting bodies were lyophilized and ground into powder.

Animals

Six-week-old male rats of Sprague-Dawley (SD) strain were purchased from SAMTAKO Bio Korea (Osan, Korea). Rats were housed 6 per cage in Polysulfone rat cage. The animal room was maintained at 22°C and 50% of room temperature and humidity under 12L: 12 D. Animals were allowed to access on Rodent diet (SAMTAKO Bio Korea) and sterilized water ad libitum. After 1 week, animals were randomly divided into three groups based on dietary categories: the control rats fed with diet, the rats fed with CMB of 250 mg/kg/day (body weight) and basal diet, and the rats fed with CMD of 250 mg/kg and basal diet. Average body weight of SD rats were 200~250 g at the beginning of the experiment. Each group contained 6 rats was fed on diet of various additives for 4 weeks.

Testosterone concentration

Animals were sacrificed after fasting for 16 hours before the end of experiment. The blood serum was taken at abdominal vein after incised the abdomen with ether treatment. This serum was separated by centrifuge for 15 min at 2500 rpm. The serum concentrations of testosterone were detected using ELISA method at the level of testosterone was analyzed at Green Cross Clinical Laboratory (Yongin, Korea).

Statistical analysis

The data from animal experiments are presented as the mean \pm S.E. and were analyzed using one way analysis of

Table 1. Body weight change (Mean \pm SD) of SD rats treated with or without *Cordyceps militaris* for 4 weeks

Weeks	Body weight (g)		
	NC ¹⁾	CMB	CMD
1	272 \pm 14	272 \pm 10	272 \pm 11
2	309 \pm 18	305 \pm 23	302 \pm 20
3	350 \pm 23	345 \pm 30	340 \pm 21
4	375 \pm 23	364 \pm 31	355 \pm 23

¹⁾NC, Normal control; CMB, Fruiting body of *Cordyceps militaris* grown on brown rice medium, CMD, Fruiting body of *Cordyceps militaris* grown on bee drone medium

Table 2. Food consumption of SD rats treated with or without *Cordyceps militaris* for 4 weeks

Weeks	Food consumption (g)		
	NC	CMB	CMD
1	23.27	20.30	22.40
2	25.78	24.83	26.58
3	24.68	28.27	24.37
4	23.58	23.13	23.54

variance (ANOVA), with the differences analyzed using the Duncan's new multiple-range test. A p value <0.05 was accepted as being a statistical significance of difference.

Results and Discussion

Change in body weight

Our analysis was focused on the comparison of change in body weights between the treatment group and the control group. There was no difference in bodyweight in rats before, during and after the experiment among rats between the treatment group and the control group (Table 1). Chang *et al.* (2008) reported that change of body weights in rats fed with CM supplementation was not observed.

Change of feed intake

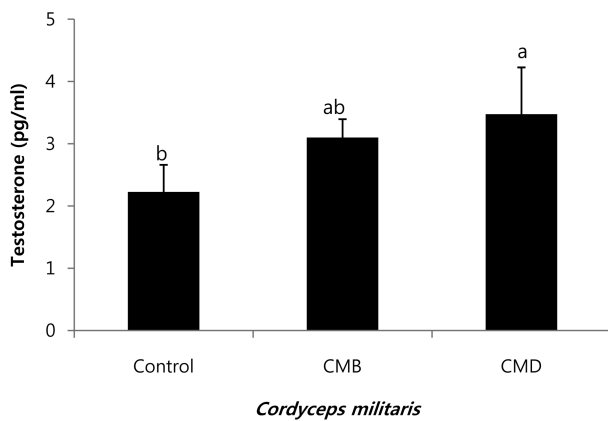
The daily diet for the experiments was 200 g per cage. After 24 hours of food supply we subtract the remainder from 200 g per cage. Then, we divided the amount by the number of animals in the cage to obtain the quantity of feed intake. The result shows no significant difference in feed intake between the groups (Table 2).

Change of water intake

We prepared 500 ml water the day before water intake for

Table 3. Water consumption of SD rats treated with or without *Cordyceps militaris* for 4 weeks

Weeks	Water consumption (ml)		
	NC	CMB	CMD
1	30.00	35.00	40.00
2	38.33	35.83	36.67
3	36.67	30.00	35.00
4	38.33	31.67	31.67

**Fig. 1.** Serum testosterone concentration in SD rat fed with CM for 4 weeks. Different letters above the bars are significantly different ($p < 0.05$).

water intake quantity. The remainder of water after 24 hours from given water 500 ml leaves the water intake quantity. The water intake quantity of each rat is value which divided the total water intake quantity per animals of cage. There was no difference in water intake among rats between the treatment group and the control group (Table 3).

Change of testosterone concentrations

We compared the testosterone secretion effect of the lyophilized powders from CMB and CMD. The result is provided in Fig. 1. The increased serum testosterone concentration was found in rats fed with CMB (3.100 ± 0.294 pg/ml) and CMD (3.475 ± 0.750 pg/ml) as compared to the control (2.225 ± 0.435 pg/ml). Furthermore, Fig. 1 illustrates that CMD significantly stimulated testosterone production ($p < 0.05$) compared to the control. We determined that the serum testosterone concentration was increased in rats fed with the fruit bodies of CM. It was reported that the sperm production is enhanced significantly by supplementation with CM mycelium even to the subfertile adult pigs (Lin *et al.*, 2007). Our present result was in agreement with previous reports (Hsu *et al.*, 2003; Huang *et al.*, 2004; Chang *et al.*, 2008).

In conclusion, the present study shows that the CM increased serum testosterone concentration in SD rats. It suggested that CMD might be developed as a complementary medicine to improve sexual hormones.

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References

- Chang Y, Jeng KC, Huang KF, Lee YC, Hou CW, Chen KH, Cheng FY, Liao JW, Chen YS (2008) Effect of *Cordyceps Militaris* supplementation on sperm production, sperm motility and hormones in Sprague-Dawley rats. *Am J Chin Med* 36, 849-859.
- Crimmel AS, Conner CS, Monga M (2001) Withered Yang: a review of traditional Chinese medical treatment of male infertility and erectile dysfunction. *J Androl* 22, 173-182.
- Hong IP, Choi YS, Woo SO, Han SM, Kim HK, Lee MR, Nam SH, HaNG (2011) Stimulatory effect of *Cordyceps militaris* on testosterone production in male mouse. *Kor J Mycol* 39, 148-150.
- Hsu CC, Huang YL, Tsai SJ, Sheu CC, Huang BM (2003) *In vivo* and *in vitro* stimulatory effects of *Cordyceps sinensis* on testosterone production in mouse Leydig cells. *Life Sci* 73, 2127-2136.
- Huang YL, Leu SF, Liu BC, Sheu CC, Huang BM (2004) *In vivo* stimulatory effect of *Cordyceps sinensis* mycelium and its fractions on reproductive functions in male mouse. *Life Sci* 75, 1051-1062.
- Huff DS, Snyder III HM, Rusnack SL, Zderic SA, Carr MC, Canning DA (2001) Hormonal therapy for the subfertility of cryptorchidism. *Horm Res* 55, 38-40.
- Kim GY, Ko WS, Lee JY, Lee JO, Ryu CH, Choi BT, Park YM, Jeong YK, Lee KJ, Choi KS, Heo MS, Choi YH (2006) Water extract of *Cordyceps militaris* enhances maturation of murine bone marrow-derived dendritic cells *in vitro*. *Biol Pharm Bull* 29, 354-360.
- Lin WH, Tsai MT, Chen YS, Hou RC, Hung HH, Li CH, Wang HK, Lai MN, Jeng KC (2007) Improvement of sperm production in subfertile boars by *Cordyceps militaris* supplement. *Am J Chin Med* 35: 637-647.
- Roscoe WA, Barr KJ, Mhawi AA, Pomerantz DK, Kidder GM (2001) Failure of spermatogenesis in mice lacking connexin 43. *Biol Reprod* 65, 829-838.
- Ryu KS, Lee HS, Kim KY, Kim MJ, Lee KG (2010) Testosterone secretion effect according to the growth stage of silkworm (*Bombyx mori* L.). *Int J Indust Entomol* 20, 75-77.
- Ryu KS, Lee HS, Kang PD, Kim KY, Kim MJ, Oh HG, Kang

- PD (2011) Secretion effect of estrogen of different growth stages of silkworm (*Bombyx mori* L.). *Int J IndustEntomol* 22, 17-20.
- Saez JM (1994) Leydig cells: endocrine, paracrine and autocrine regulation. *Endocrine Review* 15, 574-626.
- Sinclair S (2000) Male infertility: nutritional and environmental considerations. *Altern Med Rev* 5, 28-38.
- Won SY, Park EH (2005) Anti-inflammatory and related pharmacological activities of cultured mycelia and fruiting bodies of *Cordyceps militaris*. *J Ethnopharmacol* 96, 555-561.
- Yu HM, Wang BS, Huang SC, Duh (2006) Comparison of protective effects between cultured *Cordyceps militaris* and natural *Cordyceps sinensis* against oxidative damage. *J Agric Food Chem* 54, 3132-3138.
- Zhu, JS, Halpern GM, Jones K (1998) The scientific rediscovery of a precious ancient Chinese herbal regimen: *Cordyceps sinensis*: Part I. *J Altern Complem Med* 4, 289-303.