Enhancement of Neuromuscular Activity by Natural Specimens and Cultured Mycelia of *Cordyceps sinensis* in Mice

K. P. SINGH*, H. S. MEENA AND P. S. NEGI
Defence Institute of Bio-Energy Research (DIBER), DRDO, Field Station, Pithoragarh-262 501, India

Singh, *et al*.: Enhancement of Neuromuscular Activity by *Cordyceps sinensis*

The present study was aimed to evaluate the effect of natural specimen and laboratory cultured mycelia of *Cordyceps sinensis* on neuromuscular activity in mice. The powder of natural specimen and laboratory cultured *Cordyceps sinensis* was orally administered at the dose rate of 100, 300 and 500 mg/kg for 30 days. Natural specimen and *in vitro* propagated *Cordyceps sinensis* showed significant ($P<0.05$) enhancement in neuromuscular endurance and antidepressant activity at 300 and 500 mg/kg as compared to the control group. However, the fungus did not proved to be as effective as fluoxetine in exhibiting antidepressant action. Muscular endurance was determined on a Rota rod apparatus while antidepressant (mood elevating) activity was measured on a photoactometer in Swiss albino mice. The effects produced by both natural specimens and laboratory cultured *Cordyceps sinensis* were comparable and showed almost equal potency.

Key words: Enhancement, Neuromuscular, *Cordyceps sinensis*, antidepressant, mice

***Address for correspondence**
E-mail: kpvet@rediffmail.com

Cordyceps sinensis is a fungus of Ascomycetes family closely related to the mushroom. The fungus is parasitic in nature, which grows on an insect larval host (*Hepialis armoricanus* family Hepialidac). It grows in high-altitude regions of about 11 000–15 000 ft height, in cold, grassy, alpine meadows of the central Himalayan mountains in India[1]. The fungus has also been found and used extensively in china and Tibet for many medicinal properties. *C. sinensis* has been known to have so many medicinal properties preventing or curing a number of diseases in the local regions. Local people have been found to use the fungus locally known as *Yarsha Gamboo* for enhancing stamina, respiratory efficiency, immunomodulation, and treatment of liver, renal, respiratory and cerebrovascular diseases for a very long time. The fungus has also been used for increasing athletic power[1,2]. Because of the high medicinal properties of the *C. sinensis*, its market price is very high in India as well as in international market. During the last decades, its medicinal effects have been intensively investigated and efforts have been made to culture the fungus in laboratory conditions.

Owing to high medicinal value and high market price of *C. sinensis,* the present study was aimed to evaluate and compare the neuromuscular activity of the laboratory-cultured mycelia (LCM) as an alternative to wild-harvested *C. sinensis* in mice.

The natural specimens of *C. sinensis* (CS) were collected from high altitude areas (13 000 ft) Dharchula-Munsyari region of Pithoragarh, Uttarakhand (India) during May–June 2011. Powder of laboratory-cultured mycelia (LCM) of *C. sinensis* was obtained from Medicinal Mushroom Laboratory of the Institute. All samples were freeze dried at –72° in a lyophilizer (Model No. 038, NU Labcare, New Delhi) to prepare a fine powder with the help of a mechanical grinder. The samples were stored in an air-tight container at 4° till further use. Both types of powder were freshly suspended in distilled water just before administration to the mice.

Swiss albino mice (20-25 g, age 10-12 weeks) were obtained from the Experimental Animal House of the Institute. They were kept in the polycarbonate plastic cages under standard conditions (22±3°, RH 50-70%, and 12 h/12 h dark/light cycle) for laboratory animals. The animals had free access to chow food and drinking water. The experimental protocol was approved by the Institutional Animal Ethics Committee and animal care was taken as per the guidelines of CPCSEA (Registration No. 1306/c/09/CPCSEA, Dated 23rd Nov 2009), Government of India.

For locomotor activity, 48 mice of either sex were randomly divided in 8 groups (6 in each) including one control and standard (fluoxetine 20 mg/kg, p.o.). Natural and LCM of *C. sinensis* were administered at three nearby doses of 100, 300 and 500 mg/kg, orally for 30 days in rest 6 groups[3]. Locomotor activity of the animal using digital photoactometer (M/S Orchid Scientifics, India) was measured by the interceptions in the photobeams because of animal's movement in the defined arena.

Neuromuscular endurance (motor coordination) of mice was measured by digital Rota rod apparatus (Orchid Scientifics, Pune). The mice capable of remaining on the revolving rod rotating at the speed of 20 rpm for 3 min or more, in three successive trials, were selected for the study and were divided into one control and six test groups with three doses of 100, 300 and 500 mg/kg, orally for each natural and LCM of *C. sinensis* to evaluate the activity. The fall off time from the Rota rod for each animal was considered as the activity score[4].

The results in the study are expressed as mean±SEM. Statistical analysis of the results was done by using one way analysis of variance followed by Dunnet's multiple comparison test[5]. The results are considered significant at P values <0.05.

Fig. 1 depicts the effect of natural specimens and laboratory cultured mycelia of *C. sinensis* (100, 300 and 500 mg/kg, oral), on neuromuscular performance in mice. *C. sinensis* at 300 and 500 mg/kg significantly increased the time spent by mice on Rota rod revolving at 20 rpm as compared to control group. However, lower dose (100 mg/kg) failed to exhibit significant enhancement in muscular performance in mice. Natural specimens and laboratory cultured mycelia of *C. sinensis* produced almost comparable effects. The findings of the present study shows that oral administration of both natural and laboratory-cultured *C. sinensis* for 30 days enhances neuromuscular endurance and have mood elevator activity in mice. *C. sinensis* has been shown to have improvement in metabolic threshold, antifatigue and antistress activity[6-11]. In the present study we observed

the increased performance of mice on Rota rod for significantly more time than control at the dose rate of 300 and 500 mg/kg. This increased performance can be attributed mainly to increased muscular activity or motor coordination and to improved metabolic and ventilatory response to some extent. This effect of *C. sinensis* may be because of the presence of carbohydrates (45-51% of dry weight) and polysaccharides (210 KD) in the Indian isolate of *C. sinensis*[12], which has been earlier reported to promote endurance and energy metabolism[10-13]. Our findings are well supported with those of Kumar *et al.* where they have reported increased skeletal muscle enduring cellular markers by *C. sinensis* because of the activated skeletal muscle metabolic regulators[3]. Enhanced muscular performance and antifatigue can also be correlated with proper utilization of glucose as *C. sinensis* has been reported as hypoglycemic agent[14] and increases insulin sensitivity[15]. *C. sinensis* has also been found to improve exercise performance even in healthy older subjects[11]. In contrary, a number of human studies using trained cyclists have reported the inefficiency of *C. sinensis* and *C. sinensis*-based commercial supplements[16,17] in improving muscular performance. However, these studies have been conducted with athletes who attained their maximum metabolic and ventilatory response and the scope of endurance improvement was minimum. Our study also showed almost similar types of results by natural and LCM of *C. sinensis*, which can be correlated with the findings of Singh *et al.* who reported minimal genetic variability between *in vitro* cultured whole mycelia and natural specimens of *C. sinensis*[18]. One more reason for increased skeletal muscle activity may be the antioxidative property of *C. sinensis*, as *C. sinensis* whether natural or *in vitro* cultured, possesses antioxidative properties[1,2,19].

Fig. 2 depicts the effect of both types (natural and laboratory cultured) of *C. sinensis* on locomotor activity in mice. Oral administration of laboratory cultured and natural *C. sinensis* at higher doses (300 and 500 mg/kg, orally) increased locomotor activity significantly as compared to control mice. However, *C. sinensis* at the lower dose (100 mg/kg, orally) could not increase photoactometer score significantly as compared to control group of mice. Both types of *C. sinensis* showed almost similar potency to affect the locomotor activity however, the effect was not so potent as with fluoxetine.

Locomotor activity of mice following oral administration of natural and *in vitro* cultured

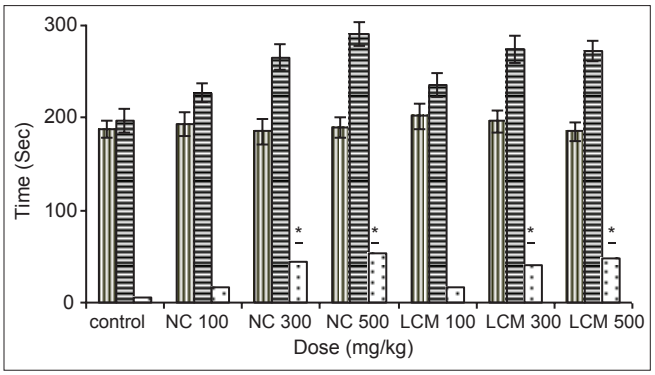

Fig. 1: Effect of natural and LCM of *C. sinensis* on Rota rod activity of mice.

Data represented as mean±SEM (*n*=6), *significant (*P*<0.05) as compared to control. NC is natural *Cordyceps sinensis*, LCM is laboratory-cultured myecelia of *Cordyceps sinensis*. 0th day, 30th day, %increase.

mycelia of *C. sinensis* was recorded to evaluate the antidepressant effect or mood elevating effect in terms of increased locomotor activity. Both natural and LCM samples enhanced locomotor activity at 300 and 500 mg/kg significantly as compared to control, however a dose of 100 mg/kg did not show significant effect to enhance the motor activity in mice. Nishizawa has reported that administration of supercritical fluid extract of *C. sinensis*, shortened immobility times dose dependently in mouse in tail suspension test showing antidepressant action of the fungus as fatigue is closely related to depression[20]. The findings of the present study are also in accordance with Liang *et al.* who reported the decreased immobility time in force swim test by *C. sinensis*[21]. Prolonged swimming time by hot water fraction of *C. sinensis* in mice has also been reported[18], which is indicative of antidepressant and antifatigue effect. Antidepressant effect of *C. sinensis* has been reported in the diabetic rats[22]. Some authors have reported the antidepressant-like effect of *C. sinensis* by both noradrenergic and dopaminergic neurotransmissions, but not by serotoninergic neurotransmission[20]. Our study also support the less involvement of serotonin in antidepressant effect as the photoactometer score by *C. sinensis* is significantly less than fluoxetine, however the possibility of involvement of noradrenaline and dopamine can not be ruled out. Another reason for antidepressant like effect of *C. sinensis* may also be elicited due to inhibition of monoamine oxidase (MAO), which is involved in catabolism of excitatory neurotransmitters. However, a further investigation is still required to find out the mechanism involved for antidepressant like activity of *C. sinensis*.

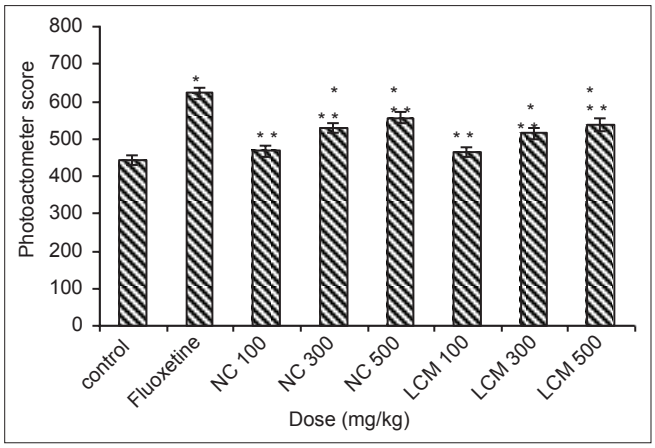

Fig. 2: Effect of natural and LCM of *C. sinensis* on locomotor activity of mice.
Data represented as mean±SEM (*n*=6), *significant (*P*<0.05) as compared to control. **significant as compared to fluoxetine administered group, NC is natural *Cordyceps sinensis*, LCM is laboratory-cultured myecelia of *Cordyceps sinensis*.

In the view of above findings, it can be concluded that natural as well as LCM of *C. sinensis* have capacity to increase the motor coordination in form of increased muscle endurance or antifatigue like activity and mood elevator or antidepressant like activity as a result of decreased endogenous depression. The neuromuscular effect of LCM of *C. sinensis* was almost similar to that of natural samples. Hence, *in vitro* propagated *C. sinensis* can be used in development of product formulation for improving human neuromuscular activity and quality of life.

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