Sub-acute toxicity of cultured mycelia of Himalayan entomogenous fungus
*Cordyceps sinensis* (Berk.) Sacc. in rats

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Received 25 July 2012; revised 15 February 2013

Oral administration of laboratory cultured mycelia powder of *C. sinensis* did not show any sign of toxicity as no significant change was observed in organ weight and serological parameters in rats. However, there was a significant increase in food intake, body weight gain and hematological parameters like WBC, RBC, Hb and lymphocytes in treated groups. Histopathology of vital organs also supported the non toxic effect of *C. sinensis*. The results conclude that laboratory cultured mycelia powder of *C. sinensis* is safe and non toxic up to 2g/ kg body weight dose.

Keywords: *Cordyceps sinensis*, Sub-acute toxicity

*Cordyceps (C.) sinensis* (Berk.) Sacc. or Caterpillar Mushroom is a high value medicinal fungus, which is being used as physical stamina enhancer and traditional medicine for preventing or curing a number of diseases such as cancer, diabetes, hepatic, renal, respiratory diseases etc. since a long time. The market price of *C. sinensis* is Rs. 4,00,000-5,00,000 per kg in India, while it costs more than 25,000 US$ per kg in international market. The natural habitat of this medicinal fungus is high altitude areas 11,000 ft. to 14,000 ft. of Nepal, China, Tibet and India. In India, *C. sinensis* is found in high altitude areas of central Himalayan region. This fungus is parasitic on larvae of a small moth, Chongcao bat (*Hepialus armoricans*, family Hepialidae). It is called ‘winter-worm and summer-grass’ or ‘worm-grass’ in China. In India, it is locally known as Yarsha Gamboo or Kira Ghas. In China, it is used as traditional Chinese medicine for the treatment of liver, renal, respiratory and cerebro-vascular diseases and immunomodulatory function. Moreover, it is also useful as an antioxidant and anti-tumor agent. *C. sinensis* contains various bioactive compounds, including cordycepin, adenosine, adenine, guanosine, ergosterol, uridine, uracil, hypoxanthine, mannitol, and polysaccharides. Multiple compound-based drugs may provide important combination therapies that simultaneously affect multiple pharmacological targets and provide clinical efficacy beyond the reach of single compound-based drugs. Now a days various *Cordyceps* based products are available in the markets as health supplement or neutraceuticals.

Owing to its high medicinal value and market price, Defence Institute of Bio-Energy Research, Field Station, Pithoragarh (India) has developed a protocol for laboratory culture of mycelia (LCM) with an objective to develop health supplements or neutraceuticals from it. Although naturally grown *C. sinensis* is widely studied for its efficacy and safety, LCM needs to be tested for the same parameters. Present study has been aimed to determine the sub-acute toxicity of LCM of *C. sinensis* in rats. Sub-acute toxicity study will provide the information on long term toxic effects for further chronic and clinical studies.

Materials and Methods

Animals—Adult female Wister rats (180-220 g body weight, 7-8 weeks old) were used. For the study animals were assigned to control and test groups, each group having 5 animals of the same sex according to Organization for Economic Co-operation and Development (OECD) guidelines. Different groups of rats were placed in acrylic cages with appropriate space for clear behavioural observation without any interference. Cages were arranged in such a way that possible effect due to cage placement was minimized. Animals were used.

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were kept in the cages for at least 7 days prior to the start of the study to allow them to acclimatize under the prevailing laboratory conditions. During the whole experimental period, all the animals were kept under constant room temperature (22±3 °C), relative humidity (50–70%), and 12/12 h dark/light cycle. Rat chow food and drinking water were provided ad libitum. All procedures followed in the present study were carried out under strict compliance of approved directions of Institutional Animal Ethics Committee, DIBER, field station, Pithoragarh (Uttarakhand) India.

Biological material—The fungus mycelium of *C. sinensis* was cultured in the laboratory broth culture medium. LCM was freeze dried at -72 °C in lyophilizer (Model No. 038, NU LABCARE, New Delhi) then finely powdered with the help of mechanical grinder and stored in an air-tight container at -4 °C. This test substance (powder of LCM) was suspended in distilled water before administration to rats.

Sub-acute oral toxicity study—Rats with minimum weight variation were randomly divided into following three groups of five animals in each: T1, T2 and control. All animals were subjected to fasting for 12 h prior to test substance administration. LCM of *C. sinensis* was orally administered at the dose rate of 0.5 and 2 g/kg body weight to T1 and T2 groups, respectively. Water was given to control group by oral route through 18 G metallic cannula.

Effect of LCM on general behaviour, food intake and body weight—Animals were observed for toxic effects and general behavioural changes at an interval of 1, 2.5 and 4 h after administration of LCM and then at least once in a day during the whole study period of 28 days. Mortality of animals was recorded during the entire study period. Food intake and body weight of individual animal was recorded on 0, 7th, 14th, 21st and 28th day prior to administration of test substance. At the end of the experiment, blood samples were collected for hematological and serological examination. Animals were kept under overnight fasting before taking blood samples from the heart in sterilized disposable syringes (22 gauge needle) after anaesthetizing the rat with anesthetic ether. Blood samples were transferred to heparinized and non-heparinized tubes for haematological and serological examination respectively. The serum was stored at -20 °C in clean vial for further biochemical analysis. All the animals were sacrificed for removal of their vital organs viz. liver, kidney, heart and brain.

Different organs were examined for macroscopic and weight changes and were preserved in 10% formalin for microscopic examination. Separate record was maintained for each animal.

Biochemical studies—Total protein, albumin, globulin, cholesterol, triglycerides, serum glutamate oxaloacetate transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT), creatinine, bilirubin (total, direct and indirect), glucose, calcium, and urea were determined at the end of the study in all groups of animals. The study was carried out with the help of semi auto chemical analyzer (RA-50, BAYAR) and specific diagnostic kits from SIEMENS (AUTOPAK), India.

Hematological studies—The hematological parameters like total count (TC) of RBC and WBC, differential leukocyte count (DLC) and hemoglobin percentage were recorded in all groups of animals. Total erythrocyte count and total leukocyte count were determined according to the method of Natt and Herrick using Hayem blood diluting fluid and WBC diluting fluid, respectively. Pack Cell Volume (PCV %) was estimated as per the method of Jain and hemoglobin concentration (Hb %) was estimated using Sahli’s hemometer. DLC was carried out under compound microscope using Leishman stain.

Observation on vital organ weight, gross necropsy and histopathology—Isolated organs were weighed separately for the change in weight of individual organ of each animal of all groups. All animals in the study were subjected to a full and detailed gross necropsy which included careful examination of the external surface of the body, all orifices and the cranial, thoracic and abdominal cavities and their contents. Isolated tissues were separately sliced in pieces and fixed in 10% buffered formalin for histopathology. Tissue sections (4-5 µm thick) were stained in hematoxyline and eosine on glass slides and examined for histopathological changes, under high power microscope.

Statistical analysis—The results are presented as mean ± SD. Statistical analysis of the data was done using Student’s *t*-test for comparison among groups. Statistically significant difference was considered at 5% level.

Results

Effect of LCM of *C. sinensis* on general signs, food intake, and body weight—No mortality and significant changes in general and psychological behaviour of
animals were observed during the entire study period. Both food intake and body weight gain increased significantly in test groups as compared to control (Fig. 1 and Table 1).

Effect of LCM on biochemical parameters—Total protein, albumin, globulin, cholesterol, triglycerides, SGPT, SGOT, creatinine, bilirubin (total, direct and indirect), glucose, calcium and urea level were estimated in serum of all groups. The glucose level in test groups was slightly decreased as compared to control group. Total protein, albumin, SGOT and SGPT contents were slightly increased in test group T-2, whereas no significant change was observed in the case of total and indirect bilirubin, cholesterol, triglycerides and urea. All biochemical parameters remained within normal range in all groups of animals (Table 2).

Effect of LCM on hematological parameters—Hematological parameters including RBC, WBC, Hb, and PCV were found significantly higher in test groups as compared to control. Total WBC and lymphocyte percent increased significantly while there was a significant decreased in percent neutrophil as compared to control group. RBC, WBC, Hb, PCV and different leucocytes were found within normal range in the blood of all groups (Table 3).

Effect of LCM on vital organ weight, gross necropsy and histopathology—No significant difference in the

![Graph showing food intake of rats](image)

**Fig. 1**—Effect of LCM of *C. sinensis* on food intake of rats. [Values mean ± SD from 5 animals in each group, *P* value: *< 0.05 significant value]

<table>
<thead>
<tr>
<th>Name of parameters</th>
<th>Control</th>
<th>Test-1 (0.5 g/kg, PO)</th>
<th>Test-2 (2 g/kg, PO)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body wt. (g)</td>
<td>231±7.43</td>
<td>248.6±12.5193*</td>
<td>253.75±10.04573*</td>
</tr>
<tr>
<td>Brain wt. (g)</td>
<td>1.2025±0.0058</td>
<td>1.139±0.0583</td>
<td>1.103±0.0909</td>
</tr>
<tr>
<td>Liver wt. (g)</td>
<td>9.3086±0.5996</td>
<td>8.528±0.5952</td>
<td>8.725±0.4680</td>
</tr>
<tr>
<td>Heart wt. (g)</td>
<td>0.9543±0.0878</td>
<td>0.9745±0.3881</td>
<td>0.935±0.0977</td>
</tr>
<tr>
<td>Kidney (L+R) wt. (g)</td>
<td>1.8364±0.20151</td>
<td>1.772±0.0245</td>
<td>1.761±0.1118</td>
</tr>
</tbody>
</table>

*P* *< 0.05 significant value

<table>
<thead>
<tr>
<th>Biochemical Parameters</th>
<th>Control</th>
<th>Test-1 (0.5 g/kg, PO)</th>
<th>Test-2 (2 g/kg, PO)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Protein (g/dl)</td>
<td>6.575±0.6122</td>
<td>6.951±1.3895</td>
<td>7.4904±1.2775</td>
</tr>
<tr>
<td>Albumin (g/dl)</td>
<td>3.8077±0.4348</td>
<td>3.9922±0.1765</td>
<td>4.3908±0.0770</td>
</tr>
<tr>
<td>Globulin (g/dl)</td>
<td>2.7397±0.4283</td>
<td>2.933±0.3515</td>
<td>3.042±0.4707</td>
</tr>
<tr>
<td>Total Bilirubin (mg %)</td>
<td>0.4719±0.0470</td>
<td>0.4507±0.0754</td>
<td>0.4678±0.0410</td>
</tr>
<tr>
<td>Direct Bilirubin (mg %)</td>
<td>0.1907±0.0151</td>
<td>0.1759±0.0758</td>
<td>0.204±0.2210</td>
</tr>
<tr>
<td>Indirect Bilirubin (mg %)</td>
<td>0.2863±0.0418</td>
<td>0.2748±0.0864</td>
<td>0.263±0.0882</td>
</tr>
<tr>
<td>SGOT (IU/L)</td>
<td>36.7525±5.7411</td>
<td>35.926±3.7817</td>
<td>36.5000±5.4354</td>
</tr>
<tr>
<td>SGPT (IU/L)</td>
<td>19.8325±0.52316</td>
<td>20.12±1.9690</td>
<td>19.9925±2.3820</td>
</tr>
<tr>
<td>Glucose (mg/dl)</td>
<td>111.254±8.2544</td>
<td>108.542±7.5941</td>
<td>102.78±11.2798</td>
</tr>
<tr>
<td>Cholesterol (mg/dl)</td>
<td>69.377±3.0261</td>
<td>68.087±3.6468</td>
<td>66.959±6.2409</td>
</tr>
<tr>
<td>Triglycerides (mg/dl)</td>
<td>44.1609±29.6062</td>
<td>35.195±11.5345</td>
<td>34.399±3.1807</td>
</tr>
<tr>
<td>Urea (mg/dl)</td>
<td>30.382±8.2990</td>
<td>31.250±8.3497</td>
<td>27.679±6.3760</td>
</tr>
<tr>
<td>Creatinine (mg/dl)</td>
<td>0.614±0.2247</td>
<td>0.6369±0.1522</td>
<td>0.6193±0.1360</td>
</tr>
<tr>
<td>Calcium (mg/dl)</td>
<td>9.980±0.5956</td>
<td>10.417±0.4062</td>
<td>10.1049±1.6142</td>
</tr>
</tbody>
</table>

*P* *< 0.05 significant value
weight of vital organs (liver, brain, heart and kidney) was recorded in treated groups as compared to control (Table 1). Texture of external surface of the organs, all orifices and the cavities of cranial, thoracic and abdominal region with their contents were found normal in all animals. Histopathological study revealed almost similar microscopic findings in control and treatment groups (Fig. 2). However, presence of inflammatory cells in hypertrophied Juxta Glomerular apparatus appears spontaneous in nature and did not show any dose dependent drug induced toxicity.

Discussion

Behavioural observation of animals during 28 days period of study provide the information on short and long term effects, level of toxicity, dose regimens and safe use of herbal products in animals for further studies. Since, changes in general behaviour, body weight and food intake are critical for the evaluation of any product on test animals as first signs of toxicity, during sub-acute studies, no mortality and no signs of toxicity were observed at any stage of study during 28 days period in any group of animals. Significant changes were observed in the food intake ($P<0.05$) and body weight ($P<0.05$) of the animals in the test groups as compared to control group. The increase in body weight can be attributed to the increase in food consumed by the rats. Increased food intake may result from increased energy production in form of ATP in treated animals. Zhu and Rippe have suggested that the *C. sinensis* significantly increases energy output and oxygen capacity. Increased food intake and body weight gain are indicative of the enhanced growth of the animals. The present findings are in agreement with those of Mounnissamy et al. who have reported progressive increase in body weight and organ weight of rats during 28 days study, resulting from administration of ethanol extract of *Cansjera rheedit* indicating the improvement in the nutritional state of the animal.

Haematopoietic system is the most sensitive target for toxicity of the compounds and an important index of physiology and pathology status in man and animals. Adeyemi et al. have reported that increased WBC count and eosinophil proportion indicates immune system boosting potential. Lymphocytes increase is due to the increased WBC production and growth factors production. The Hb level increase in test groups may result from increased production of RBC and growth factors. In the present study, hematological parameters like RBC, Hb, WBC and PCV were found significantly increased in test groups as compared to control group which are indicative of the growth of body and boosting to the immune system. This finding is well supported by a previous study reporting immunostimulating activities in mice inoculated with sarcoma 180 tumor cells, when treated with an ethanol extract of *C. sinensis*.

The liver and kidney are the major vital organs in the body which perform several important functions. Clinical blood chemistry examination was performed in animals to evaluate the functions of liver and kidney. Biochemical parameters like total proteins, albumin, bilirubin (total, direct and indirect), creatinine and urea in test groups were found within normal range with no significant difference as compare to control group. SGOT and SGPT are the most important and common liver enzymes used for the evaluation of functions of liver. Generally, increased SGOT and SGPT content imply

<table>
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<tr>
<th>Hematological Parameters</th>
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<th>Test-2 (2 g/kg, PO)</th>
</tr>
</thead>
<tbody>
<tr>
<td>RBC (10$^6$ cells/mm$^3$)</td>
<td>7.3450±0.5288</td>
<td>8.0633±0.3400*</td>
<td>8.2233±0.3743*</td>
</tr>
<tr>
<td>WBC (10$^3$ cells/mm$^3$)</td>
<td>8.3625±0.4904</td>
<td>9.3125±0.4535*</td>
<td>11.1167±0.4252**</td>
</tr>
<tr>
<td>Hemoglobin (g %)</td>
<td>12.650±0.6928</td>
<td>13.760±1.0526*</td>
<td>13.9667±0.3512*</td>
</tr>
<tr>
<td>PCV (%)</td>
<td>54.750±0.9574</td>
<td>59.000±1.6330*</td>
<td>62.670±1.5275*</td>
</tr>
<tr>
<td>Lymphocytes (%)</td>
<td>69.800±1.3038</td>
<td>71.400±1.1402*</td>
<td>74.800±1.3038*</td>
</tr>
<tr>
<td>Monocytes (%)</td>
<td>4.2500±0.5000</td>
<td>4.2000±0.8367</td>
<td>4.5000±0.5774</td>
</tr>
<tr>
<td>Eosinophils (%)</td>
<td>2.5000±1.2910</td>
<td>2.4000±0.8944</td>
<td>2.5000±1.2910</td>
</tr>
<tr>
<td>Neutrophils (%)</td>
<td>22.333±0.5773</td>
<td>20.800±1.3038</td>
<td>17.333±0.5773*</td>
</tr>
<tr>
<td>Basophils (%)</td>
<td>1.0000±0.8165</td>
<td>1.2000±0.8367</td>
<td>1.0000±0.8367</td>
</tr>
</tbody>
</table>

$P<0.05$; **$P<0.001$ significant value
parenchymal liver cells damage resulting in the release of these enzymes into the blood circulation. In the present study there was no increase in SGOT and SGPT indicating no toxicity of LCM of C. sinensis to the liver. Moreover, normal values of glucose, triglycerides and cholesterol in the blood of rats treated with LCM of C. sinensis are the indication of normal function of liver. Increased creatinine level in the blood is a sign of abnormal kidney function or indication of nephrons damage in kidney. In this study, creatinine and urea levels were found within the normal range and no significance difference was recorded as compared to control group. The glucose level in blood of test animals was slightly decreased but cholesterol and triglycerides levels were not changed. The lower value of glucose in test group may be as a result of increased glucose metabolism in normal rats. Thus the results of hematobiochemical study suggest that LCM of C. sinensis is non toxic and safe for animals in sub-acute treatment. Gross pathological examination of animals as a whole and internal organ did not reveal any abnormalities, presence of lesions or changes in the colour of the internal organs and related organ weight were not found to have significant difference as compared to control. Histopathological study of the vital organs also confirmed the non-toxic effect of LCM of C. sinensis.

Conclusion

In the view of above findings, it can be concluded that powder of LCM of C. sinensis given to animals
by oral route did not show any signs of toxicity as observed by no adverse changes in organ weight, body weight, food intake, serological and hematological parameters. Histopathology of tissue samples also supported the non toxic effect of *C. sinensis* at above dose rates. Hence powdered LCM of *C. sinensis* (cultured at DIBER Field Station, Pithoragarh) is safe and non toxic at given doses. This powder can be used in product development for improving human health and quality of life. However, for long term use, chronic and organ specific toxicological studies are still required.

**Acknowledgement**

Thanks are due to Director and Dr. S C Pant, Defence Research & Development Establishment, Gwalior for facilities for histopathological study, and to Mr. M.C. Arya and Dr. H.K. Pandey for support and suggestions during the study.

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