Research Article

Immunomodulatory Potential of *Coridius nepalensis* on Cyclophosphamide Induced Mice: Most Desired Edible Insect among Ethnic People of Arunachal Pradesh, India

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**Abstract:** The traditional practice of consuming insects and exploring their medicinal usage around the world has aroused interest in the search for novel pharmaceuticals derived from natural biological resources, offering a promising avenue for drug discovery with minimal side effects and serving as a viable alternative to conventional medications. The intake of insects as food by humans is widespread among various ethnic groups of Arunachal Pradesh and other North Eastern regions of India. For centuries, *Coridius nepalensis* (Cn), a hemipteran bug, has been valued as a traditional culinary delicacy by the ethnic people of Arunachal Pradesh. Cn is rich in dietary fibres, fats, essential fatty acids, amino acids, protein, minerals and vitamins. In order to take full advantage of Cn, the current study was designed to explore the possible immunomodulatory potential of Cn on immune suppressed mice induced through cyclophosphamide (CP). The results of the present study indicate that Cn could attenuate the effect of cyclophosphamide on mice models, where Cn could restore: indices of both thymus and spleen, body weight, red blood cell (RBC) and white blood cell (WBC) count, could stimulate the response of delayed type hypersensitivity, elevate the levels of the immune factors interleukin-6 (IL-6), tumor necrosis factor-alpha (TNF-α) in addition to the proliferation of Concanavalin A induced mitogenic response for lymphocyte proliferation. Our findings suggest that Cn extract has the potential for immunomodulation, and can act as a health food supplement and a strategy to prevent the well-known immunosuppressant effects of CP. This study also reflects how sensibly the ethnic people of Arunachal had chosen this insect as one of the most preferred foods that often is little known.

**Keywords:** edible insect, *Coridius nepalensis*, Arunachal Pradesh, Hemiptera, immunomodulatory potential

1. Introduction

Insects have been widely consumed in different ethnic cultures for thousands of years as a tasty food commodity and have proven to be essential sources of nutrition [1]. Many insects are also considered to possess health-enhancing properties based on the integration of effective nutritional components. Adequate nutrition is highly recommended for the efficient functioning of the immune system across different age groups as shown by single nutrient deficiency observation in laboratory animals [2-3] and can modulate resistance to infections [4]. In traditional medicine, some species of edible insects are used to treat inflammatory diseases and a number of insects used in food systems have been
demonstrated to modulate immune responses [5-8]. To maintain a healthy state, modulation of the immune response, either through its activation or suppression, can serve as a supportive therapy to chemotherapy. Chemotherapeutic drugs like Cyclophosphamide (CP) are most widely used to treat malignant tumors but are accompanied by severe side effects, including immunosuppression [9-10]. Therefore, it is necessary to co-administer drug or food supplements with immune regulating function. Because of the various side effects of conventional drugs, the use of natural products as an alternative to conventional treatment has been rising in the past few decades [11]. As has been documented, insects can be a source of drugs in modern medicine, since compounds of insect origin can have immunological, analgesic, antibacterial, antiviral, and free radical scavenging activity [12-20]. Nevertheless, besides several intriguing and promising studies on the possible beneficial effects of insects, studies on the immunomodulatory potential of edible insects have been relatively overlooked, as highlighted by Mousavi et al. and Antonio et al. [7-8], except for scattered evidence obtained from green beetle, yellow mealworm, black ant, red velvet mites [21-25].

One of the regions in India where large numbers of insects (more than 120 species of different order) are still being appreciated as human food is Arunachal Pradesh [26-28]. In this context, members of several ethnic communities, e.g., Nyishi, Galo, Adi, Apatani, Nochte, Wancho, etc., in Arunachal Pradesh, consume a highly preferred insect locally known as Tari and scientifically identified as Coridius nepalensis (Hemiptera) [Cn] [26]. This insect is handpicked from November to February beneath the boulders alongside riverbeds. It is not consumed as a nutritional supplement or to ward off starvation. It is appreciated solely for its taste and has been regarded as a delicacy by ethnic people since time immemorial. Coridius nepalensis has high nutritive value, being rich in dietary fibres, fats with vital fatty acids, proteins with essential amino acids, minerals and vitamins [29]. So, Cn might possess health enhancing properties having immunomodulatory potential, making this insect worthy of assessment as a healthy food for humans. Therefore, the present study attempts to report the immunomodulatory activity of Cn extract on Cyclophosphamide-induced immunosuppressed mice model. This study has briefly highlighted the role of Cn, as a therapeutic agent having immunomodulatory prospects besides being nutritionally and nutraceutically potent for its utilization in the future.

2. Materials and methods

2.1 Model animal

Healthy female albino mice (22-28 g) were taken from the animal care centre of the Dept. of Zoology, Rajiv Gandhi University (RGU), Arunachal Pradesh (AP), India. They were kept in a pathogen-free laboratory at a temperature of 20-25 °C, 50% humidity, 12-h light/dark cycle, provided with food and water ad libitum and acclimatized for fifteen days before the commencement of the study. Female mice were used to study the immunomodulatory potential of Cn on CP induced mice model since female mice exhibit stronger innate and adaptive immune responses and stronger antibody responses compared to the male counterparts [30]. Moreover, many researchers have also used female mice to study immunomodulatory responses in edible insects like Tenebrio molitor larvae [23], Oxya chinensis sinuosa (grasshopper species) [24], Polyrhachis vicina (Chinese Black ant) [22].

All the experimental protocols followed with mice were approved by the Institutional Animal Care and Ethical Committee of RGU, India and the following Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) guidelines.

2.2 Coridius nepalensis [Cn] collection and extract preparation

The adults of Cn were handpicked from beneath the boulders along the Subansiri River during November to February. Live adult specimens weighing 0.530-0.645 mg were taken to the Biochemical Nutrition Laboratory, Rajiv Gandhi University, in a chilled freeze box. The insect was identified at the Zoological Survey of India, Kolkata, India. Once brought to the laboratory, the sample insects were washed thoroughly, blotted dry followed by oven-drying (40-50 °C), ground to powder, and then stored in deep freeze -20 °C under vacuum.

Powdered insect samples were extracted with water and kept for 48 hrs at room temperature in a rotatory shaker; subsequently, the extracts were filtered through Whatman filter paper No. 1 and concentrated in a rotary evaporator at 40 °C. The dried and concentrated insect extract so obtained was freeze-dried to obtain Coridius nepalensis for fur-
ther use. Prior to immunomodulatory study, an acute toxicity study was carried out as per Organisation for Economic Co-operation and Development (OECD) test guidelines 423 [31], to determine LD$_{50}$ of Cn and the effective dose for carrying out the immunological studies. The analyses of immunomodulatory activity were done based on the parameters such as measurement of immune organ (spleen and thymus) indices, body weight, hematological parameters, pro-inflammatory cytokine (TNF-α and IL-6) level, splenic-lymphocyte proliferation, hemagglutination test and delayed type hypersensitivity.

All the solvents and chemicals used in the study were of analytical grade. Enzyme-Linked Immunosorbent Assay (ELISA) kit for interleukin-6 (IL-6) and interferon (TNF-α) were obtained from Bioassay Technology Laboratory, Shanghai (China), Cyclophosphamide (CP) from Ryon Biological Technology Co. Ltd. (Shanghai, China), Concanavalin A (Con A) from Sigma-Aldrich, Roswell Park Memorial Institute Medium 1640 (RPMI 1640 medium) from Thermo Fisher Scientific. Sheep red blood cells (SRBC) were prepared from fresh blood collected from sheep (Sheep Breeding Farm at Sangti Valley, Dirang, Arunachal Pradesh), put into a sterile flask containing crystal balls and shaken to remove the fiber. The solution was rinsed three times with saline before centrifugation at 2,000 rpm for 10 min. The supernatant was discarded. The final suspension was adjusted to a concentration of $0.5 \times 10^9$ cells and $0.025 \times 10^9$ cells.

2.3 Grouping of mice and drug administration

After 15 days of acclimatization, mice were randomly divided into normal control group (NC), cyclophosphamide model control group (MC), low dose of 500 mg/kg body weight/day (CnL), medium dose of 1,000 mg/kg body weight/day (CnM) and high dose 1,500 mg/kg body weight/day (CnH). The immunomodulatory studies were run for 14 days consisting of five groups (NC, MC, CnL, CnM and CnH) of five animals each (n = 5). Apart from the control group, mice in the other four groups were exposed to immunosuppression by intra-peritoneal injection of 0.2 ml of CP (50 mg/kg/day) from day 1$^{st}$ to 4$^{th}$. From the 5$^{th}$ day, the mice in CnL, CnM and CnH groups were dosed with insect extract of 500 and 1,000 and 1,500 mg/kg/day by gavage and continued for 14 days and at the same time, mice in the NC control group and the MC model group were given 150 µl distilled water daily by gavage. After 24 hours from the last gavage, the mice were weighed and sacrificed for further measurements. The animals of each group were divided into three batches of five animals each (n = 5) with the same experimental design. Batch 1 was antigenically challenged with 0.2 ml of 10% SRBC on the 12$^{th}$ day of the experiment and used for hemagglutination and delayed-type hypersensitivity test. Batch 2 was used for studying organ indices, hematological parameters and cytokine level. Batch 3 was used for the splenic lymphocyte proliferation assay and batch 4 was assessed for Malondialdehyde (MDA), Glutathione (GSH) and Catalase (CAT) as the antioxidant markers.

2.4 Analyses

2.4.1 Measurement of body weight, organ indices

After 24 hours from the last gavage, the mice were weighed and sacrificed. The thymus and spleen were excised and rinsed in phosphate buffer saline (PBS), and the excess tissues were removed and weighed. The thymus and spleen indices were calculated according to the equation: Organ indices = organ mass (mg)/animal body mass (g).

2.4.2 Assessment of haematological parameters

White blood cell (WBC), red blood cell (RBC), lymphocyte (LYM), monocyte (MON), and granulocyte (GRA) counts were done using the MythicTM 18 Vet (ORPHEE), an Automated Hematology Analyzer (Germany) from the blood drawn by mice cardiac puncture.

2.4.3 Assessment of in-vivo oxidative stress biomarker

The in vivo studies were done through assessing the MDA, GSH and CAT activity adopting the methods of Ohkawa et al., Ellman and Aebi [32-34] respectively in the spleen of mice of all groups (NC, CP, CnL, CnM and CnH). Supernatant obtained by centrifugation at 12,000 rpm for 20 min at 4 °C from 10% tissue homogenate prepared in phosphate buffer (0.5 M, pH: 7.4) was taken for analysis.
2.4.4 Assessment of hemagglutination

Hemagglutination test was assessed following the method of Hafeez et al. [35] with minor modifications. The serum of mice was used for the determination of hemagglutination titer. Serial two-fold dilution of serum was prepared in 96-well microtiter plates. To each well, 25 µl of 1% v/v SRBC was added, and the plate was incubated at 37 °C for one hour. The plate was then observed for agglutination—the maximum dilution at which a positive hemagglutination reaction followed was considered its antibody titer.

2.4.5 Pro-inflammatory cytokine level

The tissue homogenate of mice was used for the measurement of Pro-inflammatory cytokine levels (TNF-α and IL-6) using ELISA kit.

2.4.6 Delayed type hypersensitivity (DTH) reaction

DTH sensitization was initiated by injection of SRBC suspension on the last day after measuring the volume of the hind footpad of both legs following the method of Raisuddin et al. [36]. Each mouse was injected SRBC (0.025 × 10⁹ cells) in the left paw, and an equal volume of saline was injected into the right paw under anesthesia. After five days of post-sensitization, the mice were anesthetized, and baseline measurements of footpad thickness were recorded with a caliper after 24 hours. The difference between the thickness of the left foot just before and after the challenge (mm) was taken as the measure of DTH.

2.4.7 Lymphocyte-proliferation test

After 24 hours of the last oral administration, mice spleen from different groups were isolated under aseptic conditions. After removal of fats and connective tissues, spleen lymphocyte suspension (3 × 10⁶ cells/ml) was prepared after homogenization (phosphate-buffered saline, pH 7.2) followed by filtration and centrifugation (2,000 rpm for 10 min at 4 °C). The cell viability was measured by using the trypan blue dye exclusion method. After centrifugation, 2.5 ml Roswell Park Memorial Institute Medium 1640 (RPMI 1640 media) was added to the precipitate, and 100 µl of the solution was dispensed in 96-well flat bottom culture plates and then incubated at 37 °C in an atmosphere comprising 5% CO₂/95% air and with 50 µl Concanavalin A (Con A) for 72 hours. Lymphocyte proliferation was determined using 3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide (MTT) assay. 4 hours prior to the end of the incubation, 0.7 ml RPMI 1640 culture medium and 50 µl 5 mg/ml MTT solution were added to all wells, and plates were incubated for another 4 hours under the same conditions. At the end of the incubation, the supernatant was discarded, and 100 µl of isopropanol was added to all wells and agitated thoroughly to dissolve the dark blue crystals. The optical density (OD) values were measured at the wavelength of 570 nm with a Universal Microplate Spectrophotometer. The difference in OD values between the samples incubated with and without Con A represented the proliferation ability of splenic lymphocytes as described by Tang et al. [37].

The statistical analysis was performed with Excel 2010 and GraphPad Prism Software Inc. (La Jolla, CA, USA), and were expressed as ± mean standard deviation (n = 5). The differences among the groups were evaluated by Analysis of Variance (ANOVA), followed by a Tukey’s posthoc test, and significance values were expressed as P < 0.05.

3. Results

As presented in Table 1, there were no significant differences in the initial and final body weights of the entire studied groups of mice but a decline (71%) in the model group (MC), i.e., from 23.52 g to 17.72 g, which however, recovered with an increase in doses of insect extract (Cn). Similarly, the spleen and thymus indices got reduced to about 47 to 49% in the MC mice group but were significantly increased from 1.923 (spleen), 0.76 (thymus) in CP treated model group to 3.825 (spleen), 1.45 (thymus) in group treated with respective increase in dose Cn extract, (500 and 1,000 and 1,500 mg/kg body weight) nearing to NC group i.e., 4.102, 1.56 for both spleen and thymus, respectively.
Table 1. Effects of extract of Coridius nepalensis (Cn) on body weight and organ indices in CP treated immune compromised mice

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Body weight (g) Initial</th>
<th>Final</th>
<th>Spleen index (mg/g)</th>
<th>Thymus index (mg/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control (NC)</td>
<td>Distilled water</td>
<td>24.73 ± 1.31</td>
<td>24.75 ± 1.21</td>
<td>4.102 ± 0.053</td>
<td>1.56 ± 0.032</td>
</tr>
<tr>
<td>Model control (MC)</td>
<td>CP + Distilled H₂O</td>
<td>23.52 ± 1.05</td>
<td>17.72* ± 1.32</td>
<td>1.923* ± 0.045</td>
<td>0.76* ± 0.093</td>
</tr>
<tr>
<td>CnL</td>
<td>CP + 500 mg/kgbw</td>
<td>24.02 ± 1.42</td>
<td>20.35* ± 1.23</td>
<td>3.513* ± 0.048</td>
<td>1.29* ± 0.029</td>
</tr>
<tr>
<td>CnM</td>
<td>CP + 1,000 mg/kgbw</td>
<td>23.65 ± 1.33</td>
<td>21.49* ± 1.50</td>
<td>3.612* ± 0.061</td>
<td>1.34* ± 0.037</td>
</tr>
<tr>
<td>CnH</td>
<td>CP + 1,500 mg/kgbw</td>
<td>24.22 ± 1.45</td>
<td>22.45* ± 1.25</td>
<td>3.825* ± 0.052</td>
<td>1.45* ± 0.037</td>
</tr>
</tbody>
</table>

Values are presented as Mean ± SEM (n = 5)

*p < 0.05 significant difference against normal control and model control

At the same time, there were 57 to 81% reductions in WBC, RBC, LYM, MON, and GRA in CP treated MC mice group nevertheless the respective parameters were significantly recovered from 6.30 (RBC), 1.96 (WBC), 62.94 (LYM), 1.68 (MON) and 6.20 (GRA) in the CP treated MC group to 76.78 (LYM), 2.62 (MON), 7.95 (GRA) with gradual increase in dose of insect extracts close to NC group, i.e., 76.78, 2.70 and 8.94, for LYM, MON, and GRA respectively (Table 2). Moreover, WBC and RBC count in Cn extract treated group (3.48 and 7.95) was near to the normal level (3.46, 8.24) and far off from CP treated model group (1.96 and 6.30).

Table 2. Effects of extract of Coridius nepalensis (Cn) on blood parameters in CP treated immune compromised mice

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Blood parameters</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>WBC (10⁶/µl)</td>
</tr>
<tr>
<td>Normal control (NC)</td>
<td>Distilled H₂O</td>
<td>3.46 ± 0.041</td>
</tr>
<tr>
<td>Model control (MC)</td>
<td>CP + Distilled H₂O</td>
<td>1.96* ± 0.042</td>
</tr>
<tr>
<td>CnL</td>
<td>CP + 500 mg/kgbw of insect extract</td>
<td>3.12* ± 0.025</td>
</tr>
<tr>
<td>CnM</td>
<td>CP + 1,000 mg/kgbw of insect extract</td>
<td>3.20* ± 0.033</td>
</tr>
<tr>
<td>CnH</td>
<td>CP + 1,500 mg/kgbw of insect extract</td>
<td>3.48* ± 0.045</td>
</tr>
</tbody>
</table>

Values are presented as Mean ± SEM (n = 5)

*p < 0.05 significant difference against normal control and model control

While, antioxidant enzymes (Figure 1), GSH (µmol/g tissue) and CAT (µmol/mg protein) activities as indicated in Figure 1 were significantly increased (GSH: 2.202 to 2.389 and CAT: 33.18 to 35.79) in the mice group treated with Cn extract (500, 1,000 and 1,500 mg/kgbw/day respectively) compared to CP treated MC group (GSH: 1.490 & CAT: 22.81). While MDA (µmol/g of tissue) level of cyclophosphamide (CP) treated immune-suppressed mice group was significantly higher (1.511) than that of normal control group (NC) (1.011), but the MDA level again reverted back to normal level with intensification in doses of Cn extract treated mice groups (1.162 to 1.025).
At the same time, the hemagglutination antibody titer, i.e. the relative concentration of antibody in the blood sample increased significantly (1:1,124 to 1:3,894) nearing to normal level in NC group (1:4,096) in all the Cn extract-treated mice groups compared to the CP treated MC group (1:256) as shown in Table 3.

**Table 3.** Effects of extract of *Coridius nepalensis* (Cn) on Hemagglutination antibody titer in CP treated immune compromised mice

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Hemagglutination antibody titer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control (NC)</td>
<td>Distilled H₂O</td>
<td>1:4,096</td>
</tr>
<tr>
<td>Model control (MC)</td>
<td>CP + Distilled H₂O</td>
<td>1:256*</td>
</tr>
<tr>
<td>CnL</td>
<td>CP + 500 mg/kgbw</td>
<td>1:1,124*</td>
</tr>
<tr>
<td>CnM</td>
<td>CP + 1,000 mg/kgbw</td>
<td>1:2,125*</td>
</tr>
<tr>
<td>CnH</td>
<td>CP + 1,500 mg/kgbw</td>
<td>1:3,894*</td>
</tr>
</tbody>
</table>

Values are presented as Mean ± SEM (n = 5). *p < 0.05 significant difference in normal control and model control. **p < 0.05 significant difference between Model control and Cn treated mice (evaluated by ANOVA, followed by a Tukey’s post-hoc test)
As indicated in Figure 2, the pro-inflammatory cytokine, interleukin 6 (IL-6), and tumor necrosis factor-alpha (TNF-α) levels significantly decreased to about 56% in mice tissue homogenate of the CP injected model group (106.34 & 124.09) compared to the normal control group (NC) (189.83 & 226.11). The reduced level of cytokine in the CP injected group was found to increase significantly back to normal level in mice treated with Cn extract (IL-6: 139.62 to 172.15) & (TNF-α: 173.18 to 212.45) which is about 62 to 76% for IL-6 and 58 to 72% for TNF α.

Figure 2. Effects of extract of Coridius nepalensis (Cn) on IL-6, TNF-α, Lymphocyte proliferation and Footpad edema in CP treated immune compromised mice. Values are presented as Mean ± SEM (n = 5). *p < 0.05 significant difference in normal control and model control, #p < 0.05 significant difference between Model control and Cn treated mice (evaluated by ANOVA, followed by a Tukey’s post-hoc test)

Correspondingly, the OD value of lymphocytes proliferation induced by Con A as indicated in Figure 2, was significantly increased from 0.096 in CP treated model group to 0.1219, 0.1524 and 0.1836 in group treated with 500 and 1,000 and 1,500 mg/kg body wt. respectively of Cn extract close to normal level in NC group (0.1936).

In the same way, the effect of Cn extract on DTH (Figure 2) reaction showed a significant difference in footpad volume, increasing from 0.30 mm in NC to 0.320 mm in MC group and 0.340, 0.360 and 0.375 mm in group treated with 500, 1,000, 1,500 mg/kg body wt. of Cn extract on mice.

4. Discussion

Immunomodulation is the modulation and regulation of immune responses by enhancing or suppressing their
critical function for maintaining a disease-free state. Immunomodulatory drugs are needed to treat infections, organ transplantation, cancer, rheumatoid arthritis, systemic lupus erythematosus, down syndrome, autoimmune diseases, and acquired immune deficiency syndrome (AIDS). Immunomodulators are chemical agents that modify the immune response by stimulating antibody formation and white blood cell activity, influencing innate and adaptive components of immunity.

4.1 Immunomodulation and Coridius nepalensis

4.1.1 Body weight, organ indices blood parameters

Since the spleen is the peripheral immune organ, and thymus is the central immune organ where T lymphocytes develop, proliferate, differentiate, mature, settle, and actively involved in immune responses, the respective increase in body weight and indices of the spleen as well as thymus in Coridius nepalensis (Cn) treated mice compared to CP treated model control (MC) indicates the role of Cn towards improving the respective weights impaired by CP. Similar observations also have been reported for a few insects [37]. The present result specifies the possibility of effects of CP on the differentiation and maturation of T lymphocytes by interfering with the cell cycle and proliferation of lymphocytes to cause a reduction of thymus and spleen weight, which was significantly inhibited by Cn immunosuppressed mice induced by CP thereby improving the immune status impaired by CP and enhancing the immunogenic capacity of the mice.

The leukocyte count was also significantly increased in Cn treated mice group than that of CP group showing increased immune function in mice. Leukocytes are an important part of the body’s defense system and counting the number of leukocytes is a valid method to evaluate immune function [38]. Increase in the level of WBCs enhances the immune response towards pathogens [39]. On the other hand, hemoglobin is an important part of the red blood cell (RBC) that carries oxygen to all the cells in the body. Most cancer therapies destroy cells that grow at a faster rate. Red blood cells that grow at a faster rate are often affected. A decrease in the number of leukocytes causes disorders of certain immune factors, leading to a decrease in immune function. Studies have testified that CP inhibits hematopoietic function of bone marrow to reduce the number of leukocytes [40]. The results of the present study indicated that mice immune-compromised with CP, showed decline in WBC, RBC counts while Cn could significantly inhibit the effect of CP thereby increasing the number of WBCs, RBCs in mice.

4.1.2 Oxidative stress biomarker

Since antioxidants, known to prevent or slow down the damages caused to the cell due to unstable free radicals and inability of the body to remove these free radicals result in oxidative stresses that in turn are related to various deleterious chronic diseases. Malondialdehyde (MDA) is used to assess oxidative stress and antioxidant status in patients. Similarly, glutathione (GSH), also referred to as body’s master antioxidant, helps in alleviating oxidative stress and antioxidant enzymes like catalase (CAT) also help in protecting the cells against oxidative damage by free radicals.

MDA is an end product of lipid peroxidation that reacts on cellular and tissue protein to form adducts resulting in bio-molecular damage. Lipid peroxidation is a process where oxidants such as free radicals or non-radical species attack lipids containing carbon-carbon double bonds, especially polyunsaturated fatty acids (PUFA) that involves hydrogen abstraction from a carbon and oxygen insertion resulting in lipid peroxyl radicals and hydroperoxides [41]. MDA is widely used as a biomarker for lipid peroxidation of omega-3 and omega-6 fatty acids because of its facile reaction with thiobarbituric acid (TBA) that yield intensely coloured chromogen adducts [42]. Under high lipid peroxidation rates, the extent of oxidative damage overwhelms the repair capacity and the cell undergoes apoptosis, eventually leading to molecular cell damage that facilitates the development of various pathological states and accelerated ageing. In the present study, MDA level in the liver tissue homogenate of CP treated group was significantly higher than that of the normal control group, but the MDA level again perverted back to normal level in presence of Cn extract in mice. Thus, this study indicates that the insect extract could overcome the effect of free radicals due to MDA activity in the tissue through CP treatment which might have led to restoration capacity of cells.

On the other hand, GSH plays a critical role in protecting cells from oxidative damages and the toxicity of xenobiotic electrophiles and maintaining redox homeostasis. Glutathione (GSH) is the most abundant thiol compound synthesized in the cell by the sequential addition of cysteine to glutamate followed by addition of glycine. The
sulfhydryl group of the cysteine is involved in reduction and conjugation reactions considered as the important function of GSH for the removal of peroxides. At the same time, Catalase (CAT) is an anti-oxidative enzyme present in all aerobic organisms. It is known to catalyse hydrogen peroxide ($H_2O_2$) into water and oxygen in an energy efficient manner in the cell exposed to environmental stress. It prevents the accumulation and protects the cellular organelles and tissues from the damages of peroxide that are continuously produced by numerous metabolic reactions in the animal body. The present study, GSH and CAT activity (Figure 2) was significantly increased in the mice treated with Cn extract compared to CP treated MC group is a clue that the insect extract could revert the effect of CP through enhancing GSH and CAT activity at the tissue level in immunosuppressed mice. Similar trend of MDA, GSH and CAT activity was reported by Chen et al., He et al. and Wahab et al. [43-45] while working on immunosuppressed mice taking the extract of medicinal plant as immunomodulator.

### 4.1.3 Humoral and cell mediated immunity

In the same way, hemagglutination reflects the immune response and its intensity [46]. According to Sheng et al. [47], determination of serum hemagglutination levels of SRBC-immunized animals is used to evaluate the function of humoral immunity where the antigen and antibody react, resulting in agglutination, and the maximum dilution at which agglutination is observed gives the relative concentration of antibodies. The present result advocates that, by enhancing the humoral immune function, Cn extracts might have improved hemagglutination in CP-induced immune-compromised mice. The effect might have derived from the additive action of Cn extract to macrophages, helper T cells, and B cells which are known to coordinate in immunization, activation, and antibody production [48].

Likewise, Cytokines are synthesized by stimulating immune cells and specific non-immune cells, can regulate immune response, promote hematopoietic function, participate in the development of immune cell differentiation, and mediate inflammatory reactions [49]. TNF-α naturally produced by the response of macrophages to bacterial infections or other immunogens directly causes the death of cancer cells [50]. At the same time, IL is a cell growth factor that promotes cell proliferation and differentiation, can induce the production of interferon and is involved in the process of inflammatory or autoimmune reactions [51]. It has also been reported that CP significantly reduces TNF-α levels in mice, inhibiting immune function [52]. Thus, the present results represent that Cn extract prevented the CP-induced reduction of TNF-α and IL-6 to improve the immune function of mice, i.e. Cn could raise both the levels of TNF-α and IL-6 in mice. As a result, against the effect of CP, Cn could adjust immune balance and improve low immunity in mice.

Similarly, lymphocyte proliferation test is used to evaluate the function of T lymphocyte and the ability of an animal’s immune response [53]. Lymphocyte proliferation is an important index to evaluate the function of T lymphocyte. Proliferation can be enhanced by mitogen polyclonal activators of T lymphocytes, e.g., Concanavalin A. The proliferating cells metabolize MTT by the action of the hydrolytic enzyme to produce violet formazan crystals, whose OD values reflect the proliferation of lymphocyte [54]. The current outcomes revealed that Cn extract significantly reduced the impact of splenic lymphocyte proliferation in immunosuppressed mice induced by CP, indicating that Coridius might be having promising constituents for lymphocyte proliferation. According to the literature, these health-promoting effects are attributed mainly to either direct secretion of antimicrobial peptides or indirect administration of chitin or other bioactive substances [55-56].

Delayed type hypersensitivity (DTH) is a unique type of cell-mediated immunity and a prominent feature of several chronic diseases in humans due to infectious agents. DTH recruits T cells into tissues to be activated by antigen-presenting cells to produce cytokines that mediate local inflammation. Sheep red blood cells (SRBC) are commonly employed to address humoral immune responses. SRBC acts as an antigen that could induce the activation of T cells, which would subsequently release different kinds of cytokines and attack the target cell [57]. Hence, the treatment of SRBC sensitized mice with Cn extract in the current investigation might have stimulated immunity through delayed-type hypersensitivity (DTH) in mice. The present result is similar to the findings of red velvet mite [21], flightless cockroach spp. [58], yellow mealworm [37], Chinese black ant [22] and green Mimela sp. [25]. Overall, the results of humoral and cell mediated immune system of the present investigation, are consistent with earlier studies on edible insects like black soldier fly, yellow mealworm, housefly larvae, silkworm and green Mimela sp. [7, 21, 23, 25, 37, 58], where all the edible insects showed their immunomodulatory potential. From a health perspective, it is crucial to highlight that previous studies have revealed the nutritional richness of C. nepalensis. These studies indicated its high fatty acid, high-quality protein (amino acids), 0.5 mg/100 g of vitamin C, 4.0 mg/100 g of vitamin D, vitamin E,
and 70 mg/kg of zinc content [29]. Each one of these nutritional components is known to possess immunomodulatory properties [59-61]. Thus, the present study confirms the immunomodulatory potential of *C. nepalensis*, establishing it as a valuable edible insect species. Its inclusion in the diet can enrich nutritional intake as well as offer protection against diseases to the native people of Arunachal Pradesh.

### 5. Conclusion

Above all, this preliminary study is good evidence to elucidate the protective effects of *Coridius nepalensis*, a new source of edible insects having immunomodulating potential. Thus, the present investigation provides added value to Cn from an entomoceutical point of view. Cn extract treatment potentially reduced the detrimental effect of Cyclophosphamide in mice. So, in conclusion, our study indicates that *Coridius nepalensis* could attenuate the CP induced toxicity in mice. This study also reflects how sensibly the ethnic people of Arunachal had chosen this insect as one of the most preferred foods since time immemorial, which typically is little known in a scientific context. Further studies to investigate the immune protective mechanism of *Coridius nepalensis* and protein levels for immune regulation are in progress.

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### Authors contributions

SDT, PB & NM contributed in sample collection and conducted the experiment, data collection, data analysis; JC contributed in conceptualization, fund acquisition, supervision, executed the experiment, validation, draft preparation for MS. All authors read and approved the final manuscript.

### Data availability

The datasets generated for this study are available on request to the corresponding author.

### Conflict of interest

The authors declare that they have no competing interests.

### References


