IMPACT OF GANODERMA LUCIDIIUM ON CARBON TETRA CHLORIDE (CCl₄) INDUCED HAEMATOLOGICAL PROFILES OF MICE

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ABSTRACT

Aim: To investigate the impact of Ganoderma lucidium on carbon tetrachloride (CCl₄) induced haematological profiles of swiss albino mice. Methods: Thirty mice were used in the study and were grouped into three with 10 mice in each group. Group A: untreated mice kept as control and served with equal volume of distilled water by gavage method. Group B: mice treated with 2.8 ml/kg b. w. of (CCl₄) twice a week for 5 weeks to make anemic & immunosuppressant model. Group C: mice treated with Ganoderma lucidium followed by CCl₄ by gavage method @ 75 mg/kg b.w. (body weight) for eight weeks. After making several trials of dose of Ganoderma lucidium, oral dose of 75 mg/kg b.w. (body weight) was used for amelioration. The treated and control group of mice were sacrificed on targeted day for haematological study. Haematological parameters such as RBC count, Total WBC count, PCV, Hb (%), and platelets count were determined.

Results: There was significant statistical difference (p < 0.001) was observed in the PCV of CCl₄ treated group with compare to control. A significant increase however was seen in the G. lucidium treated group of PCV. There was also significant statistical difference (p < 0.001) were observed in the red blood cells (RBC), haemoglobin, white blood cells (WBC) & platelets (PLT) with compare to control. A marked significant statistical increase (p < 0.001) was observed in the RBC, WBC, & Hb during the period of the study in the G. lucidium treated group except platelets count. There was no significant statistical difference (p > 0.05) in the platelets count was seen in all groups during the period of the study. Conclusion: Oral administration of G. lucidium significantly reduces CCl₄-induced haematological alterations.
in mice, probably by exerting a protective effect by its free-radical scavenging and immunomodulator ability.

**Key words**: *Ganoderma lucidium*, CCl₄, swiss albino mice, haematological profiles.

**INTRODUCTION**

*Ganoderma lucidum*, an oriental fungus has a long history of use for promoting health and longevity in China, Japan, and other Asian countries. In China, *G. lucidum* is called lingzhi, whereas in Japan the name for the Ganodermataceae family is reishi. Ganoderma has a unique double walled basidiospore with a shining skin. Some of the active compounds identified in the cell wall of the mushrooms include protein bound polysaccharides or long chain glucose[4,8,15]. Most mushrooms are composed of around 90% water by weight. The remaining 10% consists of 10–40% protein, 2–8% fat, 3–28% carbohydrate, 3–32% fiber, 8–10% ash, and some vitamins and minerals, with potassium, calcium, phosphorus, magnesium, selenium, iron, zinc, and copper accounting for most of the mineral content [1]. These compounds along with probably others have been found useful in the treatment of malignancies such as leukemia as well as immunodeficiency states. Similarly extracts of *Ganoderma lucidum* specifically has been found useful in the treatment of viral, bacterial as well as some parasitic infections and infestations[6,7,10].

This study was therefore set up to ascertain the impact of *Ganoderma lucidum* on carbon tetrachloride (CCl₄) induced haematological profiles of swiss albino mice. It has been found that metabolism of CCl₄ involves the production of free radicals through its activation by drug-metabolizing enzymes located in the endoplasmic reticulum[11] and causes hematological alterations.

**MATERIALS & METHODS**

**Animals** : In the present investigation, experiments were performed on 10-12 weeks old healthy Swiss albino mice, *Mus musculus*. For the optimal growth and development, the mice were kept in ideal condition under a well regulated light and dark (12h:12h) schedule at 23±1°C in the animal house, Mahavir cancer Institute & Research centre, patna, India (CPCSEA Regd. No. 1129/bc/07/CPCSEA, dated 13/02/2008) and the experiment was duly approved by the IAEC. Animals were given food and water *ad libitum*. 
Carbon tetrachloride: 2.8 ml/kg b. w. of (CCl4) was used to make anemic and immunosuppressant model of mice & it was procured from Sigma-Aldrich Chemicals Pvt Limited, BANGALORE (INDIA).

Ganoderma lucidium: G. lucidum, was obtained from the Daxen Agritech India Pvt. Ltd.

Methodology

Study Design: Thirty mice were used in the study and were grouped into three with 10 mice in each group. Group A: untreated mice kept as control and served with equal volume of distilled water by gavage method. Group B: mice treated with 2.8 ml/kg b. w. of (CCl4) twice a week for 5 weeks to make anemic & immunosuppressant model. Group C: mice treated with Ganoderma lucidum followed by CCl4 by gavage method @ 75 mg/kg b.w. (body weight) for eight weeks.

After making several trials of dose of Ganoderma lucidum, oral dose of 75 mg/kg b.w. (body weight) was used for amelioration. The treated and control group of mice were sacrificed on targeted day for haematological study.

Collection of Blood: The blood from the control and treated mice have been taken out as a sample to test and collect the data. Blood samples were obtained from mice by orbital sinus puncture. Mice were anaesthetized for this purpose. Collection of blood from orbital sinus with a Hematocrit tube is one of the most effective methods, which causes least stress to the animal. The blood was collected in EDTA vaccutainer tube for hematological study.

Haemoglobin estimation - A 1: 250 dilution of blood was made by adding 0.02 mL of blood to 5 mL of Drabkins solution in a test tube. This was mixed and allowed to stand for 5 minutes, for complete conversion. The test was read colorimetrically at a wavelength of 540 nm.

White blood cell count (WBC) - A 1:20 dilution of blood was made by adding 0.02 mL of blood to 0.38 mL of Turks solution in a 75 mm 10 mm plastic tube. After tightly corks the tube the suspension was well mixed by rotation. The improved Neubauer counting chamber was loaded with the diluted blood by means of pasteur pipette. The loaded counting chamber was allowed for two minutes for cells to settle, after which the preparation was viewed under the microscope 10 mm objective. The cells were counted in the 4 large corner squares of the
counting chamber. The calculation of total white blood cells was made using the formula N x 2.5 x 20.

**Red blood cell count (RBC)** - A 1:200 dilution of blood was made in formol citrate solution by diluting 200 mL of blood into 4 mL of diluents in a plastic tube. A clean dry improved Neubauer counting chamber with cover slip already in position was loaded with diluted blood using pasteur pipette. The chamber was left undisturbed for 2 minutes for the cells to settle. The cells were counted under the microscope using 40 mm objective. Cells were counted in 80 small squares in the central ruled area of the counting chamber. The calculation of Red blood cells was made using the formula Nx50 x 200.

**Statistical analysis**

Data were analyzed with statistical software (Graphpad Prism 5) and values were expressed as Mean ± SEM. And differences between the groups were statistically analyzed by one-way analysis of variance (ANOVA) using the Dunnett’s test.

**RESULTS**

**Analysis of Haematological Parameters**

The results for the haematological parameters are presented in Table. A days dependent study of *Ganoderma lucidium* was done against CCL₄ induced mice. Effect of *G. lucidium* showed increased value of haematological parameters in Group III & IV.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control I</th>
<th>CCL₄ Treated II</th>
<th><em>G. lucidium</em> 6 wks Treated III</th>
<th><em>G. lucidium</em> 8 wks Treated IV</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCV (%)</td>
<td>40.97 ± 0.45</td>
<td>35.30 ± 0.17</td>
<td>36.20 ± 0.26</td>
<td>38.32 ± 0.28</td>
</tr>
<tr>
<td>RBC (10⁶/umm)</td>
<td>5.14 ± 0.08</td>
<td>3.38 ± 0.05</td>
<td>3.91 ± 0.05</td>
<td>4.5 ± 0.04</td>
</tr>
<tr>
<td>Hb (g/dl)</td>
<td>12.64 ± 0.08</td>
<td>7.29 ± 0.14</td>
<td>8.57 ± 0.18</td>
<td>9.96 ± 0.23</td>
</tr>
<tr>
<td>WBC (10⁹/umm)</td>
<td>10.39 ± 0.40</td>
<td>4.81 ± 0.32</td>
<td>7.61 ± 0.25</td>
<td>9.95 ± 0.23</td>
</tr>
<tr>
<td>PLT (10³/cumm)</td>
<td>583.8 ± 21.48</td>
<td>531.6 ± 9.70</td>
<td>550.4 ± 4.71</td>
<td>544.5 ± 8.77</td>
</tr>
</tbody>
</table>

Values are expressed as Mean ± SEM, one way ANOVA followed by Dunnet’s Test, Treated groups are compared with control group. PCV = Packed Cell Volume, RBC = Red Blood cells, WBC = White Blood Cells, Hb = Haemoglobin, PLT = Platelets.

There was significant statistical difference (p < 0.001) was observed in the PCV of CCL₄ treated group with compare to control. A significant increase however was seen in the *G. lucidium* treated group of PCV. There was also significant statistical difference (p < 0.001) were observed in the red blood cells (RBC), haemolobin, white blood cells (WBC) &
platelets (PLT) with compare to control. A marked significant statistical increase (p < 0.001) was observed in the RBC, WBC, & Hb during the period of the study in the *G. lucidium* treated group except platelets count. There was no significant statistical difference (p>0.05) in the platelets count was seen in all groups during the period of the study.

**DISCUSSION**

The present study was therefore set up to ascertain the haematological impact of *Ganoderma lucidum* on swiss albino mice induced by carbon tetrachloride (CCl4) in mice. 2.8 ml/kg b. w. of (CCl4) was used to make anemic model of mice. It has been found that metabolism of CCl4 involves the production of free radicals through its activation by drug-metabolizing enzymes located in the endoplasmic reticulum[11] and causes hematological alterations. *G. lucidum* has been reported to have a number of pharmacological effects including immunomodulating, antiatherosclerotic, anti-inflammatory, analgesic, chemopreventive, antitumor, radioprotective, sleeppromoting, antibacterial, antiviral (including anti-HIV), hypolipidemic, antifibrotic, hepatoprotective, diabetic, antioxidative and radical-scavenging, anti-aging, hypoglycemic, and anti-ulcer properties.[2,3,5,9,12,13] A compound from *G. lucidum* suppressed the growth of K562 leukemic cells in a dose- and time-dependent manner and induced their differentiation into more mature erythrocytic cells.[14]. In the present investigation similar results were obtained in production of RBC and haemoglobin level. A marked significant statistical increase (p < 0.001) was observed in the RBC, PCV & Hb during the period of the study in the *G. lucidium* treated group.

The major immunomodulating effects of active substances derived from *G. lucidum* include mitogenecity and activation of immune effector cells such as T lymphocytes, macrophages, and NK cells leading to the production of cytokines including ILs, TNF-a, and IFNs. Other effects, such as inhibition of mast cells, activation of B lymphocytes, and the complement system have also been reported.[16]. Similarly in my investigation a significant increase was observed in the production of total WBC count during the period of the study in the *G. lucidium* treated group except platelets count. There was no significant statistical difference in the platelets count was seen in all groups during the period of the study.

**CONCLUSION**

The present study was aimed to evaluate haematological impact of *G. lucidium* in mice. The mice were subjected to anemic as well as immunosuppressant through induction of CCl4. *G. lucidium* @ 75 mg/kg b.w. (body weight) for eight weeks showed significant increase in the
production RBC, PCV, Hb and total WBC count however platelets count was decreased during the whole period of the study. Thus it can be concluded from this study that G. lucidium might be helpful to combat anemia and immunosuppressant conditions.

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REFERENCES


