CHAPTER 8: Toxicity studies of aqueous-ethanol extract of *G. lucidum*, protein bound polysaccharides and total triterpenes
TABLE OF CONTENTS

8.1 INTRODUCTION

8.2 MATERIALS AND METHODS

8.2.1 Preparation of the extract, isolation of protein bound polysaccharides and triterpenes from *G. lucidum*

8.2.2 Animals

8.2.3 Acute Toxicity studies

8.2.4 Sub Acute Toxicity studies

8.3 RESULTS

8.4 DISCUSSION
8.1. INTRODUCTION

Clinical toxicology can be defined as the study of the clinically significant changes caused by xenobiotics and or therapeutic exposure, which are adverse in nature for the patient. Fundamentally toxicology has two goals, identification of the tissues that are susceptible to the toxic effects of the xenobiotics and determination of the level of acute and chronic exposures that these tissues can tolerate without clinical significance (Parchment, 1998). Acute toxicity is defined as the adverse changes occurring immediately or at a short time following administration of a single exposure of substance (Walum, 1998).

Herbal medicine is one of the most commonly used complementary and alternative therapies in the world (Bent and Ko, 2004). The beneficial effects of several commonly used botanicals are documented (Ernst, 2002), but herbal safety data are much less available than any other drug trials (Cohen et al., 2000). Although there is limited data to indicate whether these herbs are safe, many people nevertheless consume various dosages of herbal supplements everyday (Arab, 2000). Thus, there is a need for absolute proof of safety of herbs to be adopted.

*Ganoderma lucidum* (*G. lucidum*), also known as Reishi or Ling-zhi (literally, “spiritual mushroom”), has been in use in traditional Chinese medicine (TCM) for over 4000 years for the general promotion of health and longevity (Sliva, 2003). Contrary to TCM’s holistic approach, researchers in Japan, Taiwan, China, the United States, Canada, and Poland have been studying *G. lucidum* for over 30 years in an attempt to isolate specific parts of the mushroom depending on what type of disorder one is studying. Generally Ganoderma species are described as beneficial to all viscera and non-toxic (Liu, 1999). Recently, a detailed study recently had done by Wicks et al. (2007) on evaluating the safety and tolerance of oral intake of *G. lucidum* extract in healthy human volunteers showed that using the recommended dosage for current Chinese consumers, the extract was well-tolerated and no significant subjective or objective side effects were observed during 10 consecutive days of administration. It appears that additional Phase I trial with the herb dose-escalating design is needed. Previous data obtained from many cancer patients with over a month of extract administration in China suggested that *G. lucidum* has beneficial effects in cancer patients (Ren et al., 2004a; Ren et al., 2004b).
But till now there are no detailed toxicity evaluation of the triterpenes and protein bound polysaccharides from the *G. lucidum*. Thus, this chapter deals with the acute and sub acute toxicity studies of aqueous ethanol extract, protein bound polysaccharide and total triterpenes of *G. lucidum*.

8.2 MATERIALS AND METHODS

8.2.1 Preparation of the extract, isolation of protein bound polysaccharides and triterpenes from *G. lucidum*

Aqueous-ethanol (70%) extract of *G. lucidum* was prepared as described in section 2.2.1. The protein bound polysaccharides were isolated from the water extract of *G. lucidum* as described in the section 2.2.2, and the total triterpenes were isolated from the ethanol extract of *G. lucidum* as described in the section 2.2.3.

8.2.2 Animals

Male Swiss Albino mice weighing 25 ± 5 g (8-12 months age) were used for the acute toxicity studies. Male wistar rats weighing 200 ± 20 g (10-15 months age) were employed for the subacute toxicity studies.

8.2.3 Acute Toxicity studies

Acute Toxicity studies were performed according to the acute oral toxicity- fixed dose procedure recommended by OECD (Organization for economic cooperation and development)-420. The animals were divided into 8 groups of 6 animals each. The drug was administered orally as a single dose as follows. The animals were observed for mortality for 72 hours.

Group I - distilled water
Group II - olive oil control (1 ml olive oil)
Group III - extract of *G. lucidum* 2500 mg/kg b.w (p.o) suspended in 1 ml d.w
Group IV - extract of *G. lucidum* 1000 mg/kg b.w (p.o) suspended in 1 ml d.w
Group V - protein bound polysaccharide 500 mg/kg b.w (p.o) suspended in 1 ml d.w
Group VI - protein bound polysaccharide 250 mg/kg b.w (p.o) suspended in 1 ml d.w
Group VII - total triterpene fraction 50 mg/kg b.w (p.o) suspended in 1 ml olive oil
Group VIII - total triterpene fraction 25 mg/kg b.w (p.o) suspended in 1 ml olive oil
The extract and the total polysaccharide were suspended in distilled water and the total triterpene fraction was suspended in olive oil and employed for the experiment.

### 8.2.4 Sub Acute Toxicity studies

Animals were divided into 8 groups of 6 animals each. The drug was administered orally once daily for 30 days.

- **Group I** - distilled water
- **Group II** - Oil control (1 ml olive oil)
- **Group III** - extract of *G. lucidum* 50 mg/kg b.w (p.o) suspended in 1 ml d.w
- **Group IV** - extract of *G. lucidum* 250 mg/kg b.w (p.o) suspended in 1 ml d.w
- **Group V** - protein bound polysaccharide 25 mg/kg b.w (p.o) suspended in 1 ml d.w
- **Group VI** - protein bound polysaccharide 50 mg/kg b.w (p.o) suspended in 1 ml d.w
- **Group VII** - total triterpene fraction 5 mg/kg b.w (p.o) suspended in 1 ml olive oil
- **Group VIII** - total triterpene fraction 2.5 mg/kg b.w (p.o) suspended in 1 ml olive oil

The changes in body weights were recorded weekly with simultaneous observation of toxic manifestation and mortality. Twenty-four hours after the last dose of the drug administrations animals were sacrificed. The blood was taken out by heart puncture. The changes in the haematological parameters such as haemoglobin (section 2.2.21), total erythrocytes (section 2.2.22), total leukocyte count (section 2.2.23) were determined. Serum was used for determination of liver function enzymes, GOT (section 2.2.14), GPT (section 2.2.15) and alkaline phosphatase (section 2.2.16) and also for renal function test such as urea (section 2.2.17) and creatinine (section 2.2.18). The liver and kidney samples were dissected out and were kept in formalin for the histopathological analysis of any possible toxicity.

### 8.3 RESULTS

The animals administered with aqueous ethanol extract of *G. lucidum* up to the dose of 2500 mg/kg body weight, polysaccharide up to the dose of 500 mg/kg body weight and the total triterpene fraction up to the dose of 50 mg/kg body weight orally did not produce any symptoms of toxicity or mortality. In subacute toxicity studies, treatment with the different concentration of the extract (50 and 250 mg/kg), protein bound
polysaccharides (25 and 50 mg/kg) and total triterpene fraction (2.5 and 5 mg/kg) did not produce any statistically significant change in the hematological or biochemical parameters compared to the normal group of animals. The histopathological examination of the liver and kidney tissues of the treated animals also supported the non toxic nature of the extract, protein bound polysaccharides and total triterpene fraction. The administration of olive oil to the rats showed no significant changes in haematological parameters, biochemical assays of liver and kidney markers and histopathology.

Treatment with the olive oil, extract, protein bound polysaccharides and total triterpene fraction for 30 days did not produce any significant changes in the liver function and kidney function tests compared to the normal group of animals. The ALP activity in the total triterpene fraction at 5 mg/kg treated group had slight increase than that of the normal group. The ALP activity in the total triterpene fraction at 5 mg/kg treated group was 168.05 ± 44.36. Other groups had no increase in the mean value of the ALP activities (Table 8.1). The SGPT and SGOT activities in the olive oil, extract, protein bound polysaccharides and total triterpene fraction treated groups of animals showed non-significant changes than that of normal group. Further, the mean values were also almost same as that of normal group (Table 8.1).

The levels of serum urea and creatinine are presented in table 8.2. The olive oil, extract, protein bound polysaccharides and total triterpene fraction for 30 days did not produce any significant changes in the kidney function tests. There was slight increase in the mean values of the levels of urea and creatinine in the extract, protein bound polysaccharides and total triterpene fraction and the highest values were observed for total triterpene fraction at the concentration 5 mg/kg and the values were 66.24 ± 12.45 mg/dl and 0.55 ± 0.088 mg/dl for urea and creatinine respectively.

The haemoglobin contents of the olive oil, extract, protein bound polysaccharides and total triterpene fraction administered animals were also found to be almost constant throughout the period of study and there was no significant changes in these from the normal group of animals (Table 8.3). In the total leukocyte count of animals treated with the extract, protein bound polysaccharides and total triterpene fraction were slightly increased in the mean value than that of normal group of animals and the highest value was observed in the case of total triterpene fraction at the concentration 5 mg/kg and it was 8950.25 ± 1650.50 (cells/µl). Similarly, in the
Table 8.1. Effect of extract, protein bound polysaccharides and total triterpenes from the *G. lucidum* and olive oil on the activity of liver function enzymes

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatments (mg/kg)</th>
<th>ALP (IU/L)</th>
<th>SGPT (IU/L)</th>
<th>SGOT (IU/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>Vehicle</td>
<td>158.35 ± 55.15</td>
<td>55.56 ± 14.25</td>
<td>72.25 ± 17.25</td>
</tr>
<tr>
<td>Oil control</td>
<td>Olive oil</td>
<td>156.44 ± 47.22(^{ns})</td>
<td>53.55 ± 13.55(^{ns})</td>
<td>74.22 ± 14.22(^{ns})</td>
</tr>
<tr>
<td>Extract of <em>G. lucidum</em></td>
<td>50</td>
<td>152.45 ± 60.05(^{ns})</td>
<td>48.25 ± 10.25(^{ns})</td>
<td>69.25 ± 11.25(^{ns})</td>
</tr>
<tr>
<td>Extract of <em>G. lucidum</em></td>
<td>250</td>
<td>149.15 ± 69.20(^{ns})</td>
<td>47.25 ± 13.33(^{ns})</td>
<td>68.27 ± 11.22(^{ns})</td>
</tr>
<tr>
<td>Protein bound polysaccharides</td>
<td>25</td>
<td>150.45 ± 29.35(^{ns})</td>
<td>49.25 ± 5.25(^{ns})</td>
<td>71.25 ± 10.24(^{ns})</td>
</tr>
<tr>
<td>Protein bound polysaccharides</td>
<td>50</td>
<td>155.05 ± 43.33(^{ns})</td>
<td>50.25 ± 18.2(^{ns})</td>
<td>67.23 ± 14.44(^{ns})</td>
</tr>
<tr>
<td>Total triterpene fraction</td>
<td>2.5</td>
<td>145.15 ± 32.85(^{ns})</td>
<td>49.77 ± 11.25(^{ns})</td>
<td>74.25 ± 10.24(^{ns})</td>
</tr>
<tr>
<td>Total triterpene fraction</td>
<td>5</td>
<td>168.05 ± 44.36(^{ns})</td>
<td>51.25 ± 17.14(^{ns})</td>
<td>75.26 ± 19.11(^{ns})</td>
</tr>
</tbody>
</table>

Values are the mean ± SD; n = 6. \(^{ns}\)p>0.05 non-significantly different from normal (Bonferroni test)
Table 8.2. Effect of extract, total protein bound polysaccharides and total triterpenes from the *G. lucidum* and olive oil on the serum urea and creatinine concentration

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatments</th>
<th>Urea (mg/dl)</th>
<th>Creatinine (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>Vehicle</td>
<td>55.20 ± 10.25</td>
<td>0.46 ± 0.099</td>
</tr>
<tr>
<td>Oil control</td>
<td>Olive oil</td>
<td>54.21 ± 14.55&lt;sub&gt;ns&lt;/sub&gt;</td>
<td>0.42 ± 0.044&lt;sub&gt;ns&lt;/sub&gt;</td>
</tr>
<tr>
<td>Extract of <em>G. lucidum</em></td>
<td>50</td>
<td>59.15 ± 13.29&lt;sub&gt;ns&lt;/sub&gt;</td>
<td>0.48 ± 0.077&lt;sub&gt;ns&lt;/sub&gt;</td>
</tr>
<tr>
<td>Extract of <em>G. lucidum</em></td>
<td>250</td>
<td>60.35 ± 8.15&lt;sub&gt;ns&lt;/sub&gt;</td>
<td>0.48 ± 0.055&lt;sub&gt;ns&lt;/sub&gt;</td>
</tr>
<tr>
<td>Protein bound polysaccharides</td>
<td>25</td>
<td>56.65 ± 19.05&lt;sub&gt;ns&lt;/sub&gt;</td>
<td>0.51 ± 0.044&lt;sub&gt;ns&lt;/sub&gt;</td>
</tr>
<tr>
<td>Protein bound polysaccharides</td>
<td>50</td>
<td>59.29 ± 20.65&lt;sub&gt;ns&lt;/sub&gt;</td>
<td>0.49 ± 0.088&lt;sub&gt;ns&lt;/sub&gt;</td>
</tr>
<tr>
<td>Total triterpene fraction</td>
<td>2.5</td>
<td>63.36 ± 14.20&lt;sub&gt;ns&lt;/sub&gt;</td>
<td>0.53 ± 0.078&lt;sub&gt;ns&lt;/sub&gt;</td>
</tr>
<tr>
<td>Total triterpene fraction</td>
<td>5</td>
<td>66.24 ± 12.45&lt;sub&gt;ns&lt;/sub&gt;</td>
<td>0.55 ± 0.088&lt;sub&gt;ns&lt;/sub&gt;</td>
</tr>
</tbody>
</table>

Values are the mean ± SD; *n* = 6. <sup>ns</sup>*p* > 0.05 non-significantly different from normal (Bonferroni test)
Table 8.3. Effect of extract, protein bound polysaccharides and total triterpenes from the *G. lucidum* and olive oil on total hemoglobin concentration and total WBC and RBC counts

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatments (mg/kg)</th>
<th>Hb (g/dl)</th>
<th>WBC (cells/µl)</th>
<th>RBC (10^6 cells/µl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>Vehicle</td>
<td>16.89 ± 2.55</td>
<td>8250.50 ± 1550.25</td>
<td>80.22 ± 9.99</td>
</tr>
<tr>
<td>Oil control</td>
<td>Olive oil</td>
<td>15.99 ± 5.58^ns</td>
<td>8130.25 ± 1450.50^ns</td>
<td>81.25 ± 12.54^ns</td>
</tr>
<tr>
<td>Extract of <em>G. lucidum</em></td>
<td>50</td>
<td>17.01 ± 5.22^ns</td>
<td>8120.25 ± 1025.50^ns</td>
<td>86.25 ± 7.01^ns</td>
</tr>
<tr>
<td>Extract of <em>G. lucidum</em></td>
<td>250</td>
<td>17.22 ± 3.33^ns</td>
<td>8450.25 ± 1440.25^ns</td>
<td>87.22 ± 8.88^ns</td>
</tr>
<tr>
<td>Protein bound polysaccharides</td>
<td>25</td>
<td>16.99 ± 5.28^ns</td>
<td>8550.50 ± 1750.25^ns</td>
<td>82.25 ± 7.11^ns</td>
</tr>
<tr>
<td>Protein bound polysaccharides</td>
<td>50</td>
<td>17.22 ± 4.14^ns</td>
<td>8650.30 ± 1325.25^ns</td>
<td>86.66 ± 9.22^ns</td>
</tr>
<tr>
<td>Total triterpene fraction</td>
<td>2.5</td>
<td>17.05 ± 3.31^ns</td>
<td>8425.50 ± 1250.20^ns</td>
<td>84.44 ± 7.62^ns</td>
</tr>
<tr>
<td>Total triterpene fraction</td>
<td>5</td>
<td>17.14 ± 5.00^ns</td>
<td>8950.25 ± 1650.50^ns</td>
<td>91.22 ± 10.25^ns</td>
</tr>
</tbody>
</table>

Values are the mean ± SD; n = 6. ^ns_p>0.05 non-significantly different from normal (Bonferroni test)
Fig. 8.1 Effect of aqueous ethanol extract, the protein bound polysaccharides and total triterpenes from the *G. lucidum* and olive oil on the body weight of animals before and after the treatments

Values are the mean ± SD; n = 6. ns p >0.05 non significantly different from before (Bonferroni test)
Fig. 8.2. Effect aqueous ethanol extract, the protein bound polysaccharides and total triterpenes from the *G. lucidum* and olive oil on the liver histopathology.

(A) Group I – normal control liver (B) Group II – Olive oil group (C) Group III – *G. lucidum* (50 mg/kg) group (D) Group IV – *G. lucidum* (250 mg/kg) group, (E) Group V – protein bound polysaccharides (25 mg/kg) group, (F) Group V – protein bound polysaccharides (50 mg/kg) group, (G) total triterpenes (2.5 mg/kg) group and (H) total triterpenes (5 mg/kg) group. Magnification X20.
Fig. 8.3 Effect of aqueous ethanol extract, protein bound polysaccharides and total triterpenes from the *G. lucidum* and olive oil on the kidney histopathology

(A) Group I – normal control kidney (B) Group II – Olive oil group (C) Group III – *G. lucidum* (50 mg/kg) group (D) Group IV – *G. lucidum* (250 mg/kg) group, (E) Group V - protein bound polysaccharides (25 mg/kg) group, (F) Group V - protein bound polysaccharides (50 mg/kg) group, (G) - total triterpenes (2.5 mg/kg) group and (H) - total triterpenes (5 mg/kg) group. Magnification X20.
case of the total erythrocyte count and haemoglobin contents also there were slightly increase in their mean value than that of normal group of animals and the highest values were observed in the case of total triterpene fraction at the concentration 5 mg/kg. The total erythrocyte count was 91.22 ± 10.25 cells/µl and the haemoglobin content was 17.14 ± 5.00 cells/µl in the case of total triterpene fraction at the concentration 5 mg/kg.

Treatment with the olive oil, extract, protein bound polysaccharides and total triterpene fraction did not produce any significant changes as compared to the normal group of animals. Body weight of the animals remained almost constant throughout the period of study. In the group of animals treated with the extract at 250 mg/kg body weight and protein bound polysaccharides at 50 mg/kg showed a slight increase in the average body weight was observed at the end of the experiment (Fig. 8.1).

The histopathological observation also supported the above non toxic results of the olive oil, extract, protein bound polysaccharides and total triterpene fraction. The histopathology of liver and kidney samples form the treated groups and normal group had been represented as figure 8.2 and figure 8.3.

8.4 DISCUSSION

Determining the toxicological profile of a substance or preparation is required by regulations for the use and marketing of a product and is also an essential prerequisite for guaranteeing public health. Every drug before being declared as a potential medicine for any disease has to be tested for its chemical, bio-physiological and pathological characters. This process ensures that the compound that becomes a medicine is safe for consumption and can be commercially marketed.

Results of the study reveal that the olive oil and the aqueous ethanol extract, protein bound polysaccharides and total triterpenes from G. lucidum did not produce any acute toxicity. The extracts up to a dose of 2500 mg/kg body weight, protein bound polysaccharides extracts up to a dose of 500 mg/kg body weight and total triterpenes up to a dose of 50 mg/kg body weight were not lethal to animals and thus LD$_{50}$ of any of these could not be determined.

The hematotoxicology studies were performed to determine the adverse effects of toxicants on mature blood cell in the hematopoetic tissue (Parchment, 1998). Blood is an index of physiological and pathological status in animals and the parameters usually measured are haemoglobin, packed cell volume, white blood cell
count, platelets count (Schalms et al., 1975). The values of these parameters can be changed by the ingestion of some toxic drugs (Ajagbonna et al., 1999).

The olive oil and the aqueous ethanol extract, protein bound polysaccharides and total triterpenes from *G. lucidum* did not produce any significant haematologic toxicity as evident from the normal counts of WBC and RBC. After 30 days of treatment only a slight non significant increase in the WBC and RBC count was observed which does not indicate any toxicity.

Serum transaminases (GOT and GPT) and ALP activities are good indices of liver damage. In general with liver disease, the activities of serum SGPT and SGOT rise and fall at the same time (Haweroft, 1987). A mild elevation of SGPT activity has been shown to be associated with liver injury or myocardial infarction. The higher the activity of SGPT has been observed in larger infarction size (Feldman and Zinkl, 2000). After 30 of treatment with the olive oil and the aqueous ethanol extract, protein bound polysaccharides and total triterpenes from *G. lucidum*, a slight increase in both SGPT and SGOT were observed compared to normal group of animals. However, the observed changes were statistically non significant and not sufficient to support any toxicity of the extract.

The urea and creatinine are markers of kidney function (Jesse, 1982). But there were no significant changes in the level of urea and creatinine by the administration of the olive oil and the aqueous ethanol extract, protein bound polysaccharides and total triterpenes from *G. lucidum*. A slight increase in creatinine level was observed by an unknown reason, which is not sufficient to prove toxicity. Further, the histological examination of the liver and kidney revealed no abnormality in the architecture of the organs and this supports the biochemical and haematologic parameters assessed in the current study.

Hence the toxicity studies indicates that the olive oil and the aqueous ethanol extract, protein bound polysaccharides and total triterpenes from *G. lucidum*, did not produce any toxic symptoms in the animals at the tested doses. The findings suggests the scope of this mushroom and its major constituents such as protein bound polysaccharides and total triterpenes for the production of safe and non toxic nutriceuticals or food supplements with therapeutic and nutriceutic properties.