Chapter 6: Toxicity studies of Phellinus rimosus
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6.1. 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 the adverse changes occurring immediately or at a short time following administration of a single exposure of substance (Walum, 1998).

Mushrooms have recently become attractive as a source for the development of many drugs. Many pharmaceutical substances with potent and unique properties have been isolated from mushrooms. It is generally believed that many mushrooms are safe because of their long history of usage. However, there exists an insidious fear of mushroom poisoning which can even approach phobic levels. Before developing any pharmaceutical or dietary supplement, it is essential to evaluate the toxicity of the compound. This chapter deals with the acute and sub acute toxicity studies of aqueous-ethanol extract of *P. rimosus*.

6.2. MATERIALS AND METHODS

6.2.1. Preparation of the extract.

Aqueous-ethanol (70%) extract of *P. rimosus* was prepared as described in section 2.2.1.

6.2.2. Animals

Male Swiss albino mice of 6 weeks old weighing 25 ± 2 g were employed for the toxicity studies.

6.2.3. Acute toxicity study

Animals were divided into 3 groups of 6 animals each. The drug was administered orally as single dose as follows (Walum, 1998).

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td>Vehicle (distilled water).</td>
</tr>
<tr>
<td>Group II</td>
<td>Aqueous-ethanol (70%) extract of <em>P. rimosus</em> 500 mg/kg body wt</td>
</tr>
</tbody>
</table>
Group III  Aqueous-ethanol (70%) extract of *P. rimosus* 2500 mg/kg body wt

The animals were observed for toxic symptoms and mortality for 72 hours.

**6.2.4. Sub acute toxicity study**

Animals were divided into 3 groups of 6 animals each. The drug was administered orally once daily for 30 days (Parchment, 1998).

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td>Vehicle (distilled water).</td>
</tr>
<tr>
<td>Group II</td>
<td>Aqueous-ethanol (70%) extract of <em>P. rimosus</em> 50 mg/kg body wt</td>
</tr>
<tr>
<td>Group III</td>
<td>Aqueous-ethanol (70%) extract of <em>P. rimosus</em> 250 mg/kg body wt</td>
</tr>
</tbody>
</table>

The body weights of animals were recorded weekly with simultaneous observation of toxic manifestation and mortality. Twenty-four hours after the last dose of the drug administration 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 2.2.26), and total leukocyte count (section 2.2.27) were determined. Serum was used for determination of liver function enzymes, SGOT (section 2.2.16), SGPT (section 2.2.17) and alkaline phosphatase (section 2.2.18) and also for renal function test such as urea (section 2.2.19) and creatinine (section 2.2.20). The liver and kidney samples were dissected out and were kept in formalin for the histopathological analysis (Section 2.2.28).

**6.3. RESULTS**

The animals administered with aqueous ethanol extract of *P. rimosus* up to the dose of 2500 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) did not produce any statistically significant change in the hematological or biochemical parameters compared to the normal group of animals.

Treatment with extract for 30 days did not produce any significant changes in the liver function (SGOT, SGPT and ALP) and kidney function tests (Urea and Creatinine)
compared to the normal group of animals. The SGOT and SGPT activities in the normal and the extract treated (250 mg/kg body wt) were 106.51±14.58, 36.49 ± 3.62, and 111.73 ± 11.67, 37.2 ± 2.49 respectively. The ALP level in the normal and the extract treated (250 mg/kg body weight) was 52.3 ± 5.85 and 55.17 ± 7.45 (Table 6.1). The SGOT, SGPT and ALP levels in the extract treated groups of animals showed non-significant changes than that of normal group.

The concentration of serum urea and creatinine are presented in Table 6.2. The normal animals showed serum urea level 56.01 ± 9.07 and creatinine level 0.55 ± 0.03 mg/dl, while the extract (250 mg/kg body wt) treated group showed 59.40 ± 5.99 mg/dl and 0.58 ± 0.05 mg/dl respectively (Table 6.2).

The haemoglobin contents of the extract were also found to be almost constant throughout the period of study and there were no significant changes in these from the normal group of animals (Table 6.3). The leukocyte count in the normal and the extract treated groups were (250 mg/kg body wt) 10300 ± 1267.40 (cells/µl) and 10500 ± 1110.27 (cells/µl) respectively. Total leukocyte count of animals treated with the higher dose of extract (250 mg/kg body wt) was slightly increased in the mean value than that of normal group of animals. Similarly the total erythrocyte count was also slightly increased in their mean value than that of normal group of animals. The total erythrocyte count of normal and extract treated groups (250 mg/kg) were 85.22 ± 10.27 and 91.27 ± 7.25 cells/µl.

Treatment with the extract 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 (Fig. 6.1).

The histopathological observation also supported the above non toxic results of the extract. The histopathology of liver and kidney samples form the treated groups and normal group had been represented as figure 6.2 and figure 6.3.
Table 6.1: Effect of *P. rimosus* 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>52.3 ± 5.85</td>
<td>36.49 ± 3.62</td>
<td>106.51 ± 14.58</td>
</tr>
<tr>
<td><em>P. rimosus</em></td>
<td>50</td>
<td>52.612 ± 6.27</td>
<td>ns</td>
<td>34.96 ± 7.54</td>
</tr>
<tr>
<td><em>P. rimosus</em></td>
<td>250</td>
<td>55.17 ± 7.45</td>
<td>ns</td>
<td>37.2 ± 2.49</td>
</tr>
</tbody>
</table>

Values are the mean ± SD; n = 6.

ns P>0.05 non-significantly different from normal (Bonferroni test)

Table 6.2: Effect of *P. rimosus* on serum urea and creatinine levels.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatments (mg/kg)</th>
<th>Urea (mg/dl)</th>
<th>Creatinine (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>Vehicle</td>
<td>56.01 ± 9.07</td>
<td>0.55 ± 0.03</td>
</tr>
<tr>
<td><em>P. rimosus</em></td>
<td>50</td>
<td>57.83 ± 9.71</td>
<td>0.53 ± 0.02</td>
</tr>
<tr>
<td><em>P. rimosus</em></td>
<td>250</td>
<td>59.40 ± 5.99</td>
<td>0.58 ± 0.05</td>
</tr>
</tbody>
</table>

Values are the mean ± SD; n = 6.

ns P>0.05 non-significantly different from normal (Bonferroni test)
Table 6.3: Effect *P. rimosus* on total haemoglobin concentration, 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>15.94 ± 2.81</td>
<td>10300.00 ±1267.40</td>
<td>85.22 ± 10.27</td>
</tr>
<tr>
<td><em>P. rimosus</em></td>
<td>50</td>
<td>15.69 ± 0.53 ns</td>
<td>10090.00 ± 1321.2 ns</td>
<td>89.21 ± 9.22 ns</td>
</tr>
<tr>
<td><em>P. rimosus</em></td>
<td>250</td>
<td>16.28 ± 1.47 ns</td>
<td>10500.00 ±1110.27 ns</td>
<td>91.27 ± 7.25 ns</td>
</tr>
</tbody>
</table>

Values are the mean ± SD; n = 6.

ns P>0.05 non-significantly different from normal (Bonferroni test)
Fig. 6.1: Effect of aqueous ethanol extract of *P. rimosus* on the body weight of animals before and after the treatments.

Values are mean ± SD; n=6. $^\text{ns}$ P>0.05 non significantly different from before (Bonferroni test).
Fig. 6.2: Effect of sub acute toxicity studies of *P. rimosus* on liver histopathology

Normal

![Histopathology images](image)

*P. rimosus* (50 mg/kg)  *P. rimosus* (250 mg/kg)
Fig. 6.3: Effect of sub acute toxicity studies of *P. rimosus* on kidney histopathology

Normal

*P. rimosus* (50 mg/kg)  *P. rimosus* (250 mg/kg)
6.4. 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 aqueous ethanol extract of *P. rimosus* did not produce any acute toxicity. The extracts up to a dose of 2500 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 hematopoitic 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 aqueous ethanol extract of *P. rimosus* 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 where as, the higher activity of SGOT has been observed in larger infarction size (Feldman and Zinkl, 2000). After 30 days treatment with the extract, a slight increase in both SGPT and SGOT were observed compared to normal group of animals. 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 extract. 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.

Body weight is an important factor to monitor the health of an animal. Loss in body weight is frequently the first indicator of the onset of an adverse effect. All the animals from treated groups did not show any decrease in body weights as compared with the first day values.

Hence, the toxicity studies indicate that the aqueous ethanol extract of *P. rimosus* did not produce any toxicity in the animals at the tested doses. The findings suggest the scope of this mushroom and its major constituents for the production of safe and non-toxic nutriceuticals or food supplements.