Chapter 6

Toxicity Studies
Table of Contents

6.1. Introduction
6.2. Materials and Methods
   6.2.1. Preparation of the Extract
   6.2.2. Animals
   6.2.3. Acute Toxicity Studies
   6.2.4. Sub Acute Toxicity Studies
   6.2.5. Histopathological Analysis
6.3. Results
6.4. Discussion
6.1. INTRODUCTION

Toxicology is the study of how chemical substances interact with living systems and affect normal processes, and the use of this information to predict safe exposure levels. Toxicological research and testing helps us to live safely and to derive benefit from natural and synthetic substances while avoiding harm. Toxicity studies in animals are usually necessary for any pharmaceutical product intended for human use. Toxicity studies play an important role in identification and isolation of new compounds from crude extracts of medicinal mushroom. The information obtained from these studies is useful in choosing doses for repeat-dose studies, providing preliminary identification of target organs of toxicity revealing delayed toxicity. Most of these studies are conducted to assess the degree to which substances are toxic (poisonous) for humans, animals or the environment, to investigate the mechanism of toxic chemicals or to develop improved tests for specific types of chemically induced effects.

Herbal prescriptions and natural remedies are commonly employed in developing countries for the treatment of various diseases. This practice is an alternative way to compensate for some perceived deficiencies in orthodox pharmacotherapy (Sofowora 1989; Zhu 2002). Unfortunately, there is limited scientific evidence regarding safety and efficacy to back up the continued therapeutic application of these remedies. 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). For centuries, medication of herbal medicines including medicinal mushrooms has been practiced to combat a wide range of diseases and is still a significant means of medical treatment in parallel with western medicines in Eastern Asian countries (Sullivan et al, 2006). Their efficacy and non-toxicity have been indicated by numerous in vivo or in vitro studies over many years.

Mushrooms are a popular and valuable food, low in calories and high in nutritional values and some of them produce substances that have potential medical effects. Mushrooms have become attractive as functional food and sources of various drugs...
and nutraceuticals (Zheng et al, 2005). Several mushroom species such as *Ganoderma lucidum*, *Lentinus edodes*, *Schizophyllum commune*, *Grifola frondosa*, *Agaricus blazei*, *Inonotus obliquus*, *Coriolus versicolor* and *Phellinus linteus* have been reported to possess diverse medicinal effects. These include antioxidant, antiinflammatory, antitumor, antiviral, hepatoprotective, kidney tonic, immunostimulatory and anticancer effects. In folk medicine, several species of *Phellinus* are known to improve health and has been used as remedy for various diseases. *Phellinus* species has been used to treat abdominal pain, stomach problems, lymphatic tumor, and menses disorders. *Phellinus linteus* has been used since ancient times for perpetual youth and longevity. Many species of *Phellinus* spp. (e.g. *P. linteus*, *P. igniarius*, *P. gilvus*, *P. pini* and *P. hartigii*) are known and they have a variety of medicinal effects (Lee et al, 1996). Extracts obtained from *Phellinus* species have received special attention due to their potent pharmacological activities including immunostimulation, antitumor, antioxidant, and antihepatotoxicity (Kim et al 1996; Ajith and Janardhanan 2002). The immunostimulatory studies and antioxidant studies reveal that this mushroom can be used for several acute and chronic diseases induced by immunosuppression and oxidative stress.

Results of present study suggest that *P. rimosus* is a medicinally valuable mushroom and therefore, there are possibilities of developing it to a potent nutraceutical or medicine in near future. But before developing any pharmaceutical or dietary supplement it is essential to evaluate the toxicity of the compound. This chapter deals with the acute and subacute toxicity studies of PPC-Pr Complex isolated from *P. rimosus*.

### 6.2. MATERIALS AND METHODS

#### 6.2.1. Preparation of the extract

The PPC-Pr Complex of mushroom *P. rimosus* was prepared as described in section 2.2.4.
6.2.2. Animals
Male Swiss albino mice of 6 weeks old weighing 25 ± 2 g were employed for the toxic studies.

6.2.3. Acute Toxicity studies
Animals were divided into 3 groups of 6 animals each. The drug was administered intraperitoneally as a single dose as follows. The animals were observed for mortality for 72 hours.

Group I          Vehicle (distilled water).
Group II          PPC-Pr Complex  100 mg/kg body wt.
Group III         PPC-Pr Complex  50 mg/kg body wt.

6.2.4. Sub Acute Toxicity studies
Animals were divided into 3 groups of 6 animals each. The drug was administered intraperitoneally once daily for 15 days.

Group I          Vehicle (distilled water).
Group II          PPC-Pr Complex  10 mg/kg body wt.
Group III         PPC-Pr Complex  50 mg/kg body wt.

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 extract the animals were sacrificed. The blood was collected by direct heart puncture and one portion was used for studying haematological parameters such as haemoglobin, total erythrocyte count and total leukocyte count. Serum was used for determination of liver function enzymes, GOT, GPT, alkaline phosphatase and also for renal functional tests such as urea and creatinine. The methods were described in chapter 2.
<table>
<thead>
<tr>
<th>Treatments</th>
<th>Haemoglobin (g/dl)</th>
<th>Total Leukocyte count (cells/µl)</th>
<th>Total Erythrocyte count x10^6 (cells/µl)</th>
<th>Bone marrow cellularity x 10^6 (cells/µl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>14.55 ± 1.22</td>
<td>8700 ± 865</td>
<td>6.75 ± 0.54</td>
<td>15.50 ± 1.56</td>
</tr>
<tr>
<td>PPC-Pr Complex 10 mg/kg bwt</td>
<td>15.60 ± 1.46 ns</td>
<td>8467 ± 824 ns</td>
<td>5.83 ± 0.57 ns</td>
<td>14.25 ± 1.33 ns</td>
</tr>
<tr>
<td>PPC-Pr Complex 50 mg/kg bwt</td>
<td>15.96 ± 1.53 ns</td>
<td>8600 ± 832 ns</td>
<td>6.17 ± 0.62 ns</td>
<td>15.17 ± 1.41 ns</td>
</tr>
</tbody>
</table>

Table 6.1 Effect of PPC-Pr Complex on blood parameters by the subacute toxicity studies. Values are mean ± SD ns-non significant compared to normal.
<table>
<thead>
<tr>
<th>Treatments</th>
<th>Urea (mg/dl)</th>
<th>Creatinine (mg/dl)</th>
<th>ALP (IU/L)</th>
<th>GOT (IU/L)</th>
<th>GPT (IU/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>46.55 ± 4.32</td>
<td>7.68 ± 0.65</td>
<td>128.03 ± 11.87</td>
<td>149.20 ± 15.01</td>
<td>37.60 ± 3.23</td>
</tr>
<tr>
<td>PPC-Pr Complex 10 mg/kg bwt</td>
<td>48.03 ± 4.61 ns</td>
<td>6.84 ± 0.71 ns</td>
<td>116.92 ± 11.53 ns</td>
<td>139.43 ± 14.21 ns</td>
<td>33.40 ± 2.36 ns</td>
</tr>
<tr>
<td>PPC-Pr Complex 50 mg/kg bwt</td>
<td>50.89 ± 5.07 ns</td>
<td>8.52 ± 0.81 ns</td>
<td>110.96 ± 11.11 ns</td>
<td>141.69 ± 13.87 ns</td>
<td>38.90 ± 3.79 ns</td>
</tr>
</tbody>
</table>

Table 6.2 Effect of PPC-Pr Complex on the activity of liver and kidney function enzymes. Values are mean ± SD, ns-non significant compared to normal.
FIG 6.1. Effect of subacute toxicity studies of PPC-Pr Complex on liver histopathology.

A] Normal; B] PPC-Pr Complex 10mg/Kg bwt; C] PPC-Pr Complex 50mg/Kg bwt.
FIG 6.2. Effect of subacute toxicity studies of PPC-Pr Complex on kidney histopathology.
A] Normal; B] PPC-Pr Complex 10mg/Kg bwt; C] PPC-Pr Complex 50mg/Kg bwt
6.2.5. Histopathological analysis

A portion of the liver and kidney tissues were fixed in 10% neutral buffered formalin. Sections (4 µm) were taken and stained with hematoxylin-eosin and observed under oil immersion microscope (100X). Photographs were taken.

6.3. RESULTS

The animals administered with PPC-Pr Complex did not produce any external symptoms of toxicity or mortality up to the dose of 100mg/kg body weight intraperitoneally. In subacute toxicity studies, treatment with 10 mg/Kg bwt and 50 mg/Kg bwt of the extract (i.p) did not produce any statistically significant change in the hematological or biochemical parameters when compared to the normal group of animals.

Treatment of the PPC-Pr Complex for 15 days did not produce any significant changes in the animals when compared to the normal group of animals. The hemoglobin content of the administered animals was found to be almost constant for the treated groups (Table 7.1). There was no significant change in the the total leukocyte count of animals treated with both the doses of PPC-Pr Complex (Table 7.1). Similarly no significant change was observed in the R.B.C count of animals treated with PPC-Pr Complex at the end of 5th week (Table 6.1).

Treatment of the extract for 15 days did not produce any significant changes in the liver function and kidney function tests when compared to the normal group of animals. The ALP activities in the normal and PPC-Pr Complex treated (10 and 50 mg/kg bwt) group of animals were found to be 128.03 ± 11.87, 116.92 ± 11.53 and 110.96 ± 11.11 IU/L respectively. The SGPT activities in the normal and PPC-Pr Complex treated (10 and 50 mg/kg bwt) group of animals were 37.6 ± 3.23, 33.4 ± 2.36 and 38.9 ± 3.79 IU/L respectively. The SGOT activities in the normal as well as PPC-Pr Complex treated (10 and 50 mg/kg bwt) group of animals were 149.2 ± 15.01, 116.92 ± 14.21 and 110.96 ± 13.87 IU/L respectively (Table 6.2). Thus no significant change was observed in any of the liver function parameters.
The concentration of serum urea and creatinine are presented in table 7.2. The normal as well as the PPC-Pr Complex (10 and 50 mg/kg bwt) treated group of animals showed serum urea level of 46.55 ± 4.32, 48.03 ± 4.61 and 50.89 ± 5.07 mg/dl respectively. The creatinine concentration in the normal and PPC-Pr Complex treated group were found to be 7.68 ± 0.65, 6.84 ± 0.71, 8.52 ± 0.81 mg/dl respectively. Thus there was no significant change in renal function parameters indicating that this drug does not produce any renal damage (Table 6.2).

Histopathological analysis of liver and kidney did not show any pathological lesions in the organs of animals treated with PPC-Pr Complex (Fig 6.1 and Fig 6.2). So it can be concluded that PPC-Pr Complex did not produce any pathological alterations.

6.4. DISCUSSION

Recent investigations has been channeled on the development of immunotherapy to target cancer cells as well as on substances such as immunopotentiators, immunoinitiators or biological response modulators (BRM) which act to prevent carcinogenesis and induce carcinostasis (Wasser and Weis 1999). Natural polysaccharides come under this category of immunoregulators and are biodegradable, relatively inexpensive and are generally considered as safe pharmaceuticals. Less attention has been devoted to the safety of excipients obtained from natural source because of their inertia and innocuity. Polysaccharide-rich fungi and plants have been employed for centuries by cultures around the world for their dietary and medicinal benefits (Paulsen 2001; Yamada and Kiyahara 1999; Hobbs 2003; Kusaykin et al, 2008; Aloes 2003).

Animal studies reported that polysaccharides have immune system effects in the gut, spleen, bone marrow, liver, blood, thymus, lungs, and saliva. Further, controlled human studies reported evidence of immune stimulation by polysaccharides in the blood, anti-inflammatory effects in nasal lavage fluid and improved survival in cancer patients. The administration of polysaccharides can be intraperitoneal (i.p) or oral (p.o.) based on the immune responses to be enhanced.
However, i.p route was more effective than oral route for polysaccharides because of its effect on macrophages. Further, there was no loss of activity as in the case of oral administration (Sakurai et al, 1991). Some mushroom polysaccharides such as Lentinan and Schizophyllan, both large molecules, are only effective by i.v. or i.p. administration (Markova et al, 2002; Vincent and Ooi 2000). In this regard, we selected i.p route for our experiments as well as for toxicity studies.

There are several reports regarding the toxicity studies of polysaccharides. Most polysaccharide products appear to be safe, based on acute and/or chronic toxicity testing in rodents (Ramberg et al, 2010). Polysaccharides isolated from *P.linteus* was found to be non toxic by acute toxicity studies for doses upto 5g/Kg bwt for a period of 2 weeks and LD 50 was found to be greater than 5g/Kg bwt (Kim and Joo, 2008). The PPC-Pr Complex isolated from the mushroom *P.rimosus* was found to be non toxic by acute and sub acute toxicity tests.

**6.5. Conclusion**

In the present study, we checked the toxicity of isolated PPC-Pr Complex by acute and sub acute toxicity tests. Results of the study revealed that PPC-Pr Complex of *P.rimosus* did not produce any acute toxicity. The extracts up to a dose of 100mg/kg body weight, i.p was not lethal to animals and LD 50 could not be determined. No clinical signs of adverse or toxic symptoms were noticed throughout the period of sub acute toxicity study. The PPC-Pr Complex did not produce any significant hematologic toxicity as evident from the normal Hb levels and normal counts of WBC as well as RBC. Liver and kidney function parameters, which are the indicators of toxicity, were also normal in the subacute toxicity study. The study also revealed the scope of this mushroom for the production of safe and non toxic agents with therapeutic and nutraceutical properties.