Chapter V

Acute Oral Toxicity Studies
5. ACUTE ORAL TOXICITY STUDIES

5.1 Introduction

The acute toxicity study was performed to ensure the safety, to know the Therapeutic Index (TI) and for the determination of lethal dose (LD₅₀) value of drugs. In the present study the acute oral toxicity studies are conducted for hexane, ethyl acetate, methanolic extracts of *Phyllanthus amarus* aerial parts on swiss albino mice as per Organization for Economic Cooperation and Development (OECD) guideline 420.

The experimental protocol was approved by Institutional Ethical Committee (IAEC) of Regd. No. 516/PO/c/01/ CPCSEA, University College of Pharmaceutical Sciences, Andhra University, Visakhapatnam.

5.2 Material and Methods

5.2.1 Test material

Hexane (PAHE), ethylacetate (PAEA), methanolic (PAME) extracts of *P. amarus* aerial parts.

5.2.2 Animals

Healthy young adult nulliparous and non-pregnant female swiss albino mice, weighing 25-30g at the start of the experiment, were procured from Mahaveer enterprises, Hyderabad.

The animals were divided in to three groups each consisting of 6 animals. The animals were randomly selected and kept in their cages for 5 days prior to dosing to allow for acclimatization to the laboratory conditions. The animals were housed individually in clean polypropylene cages. Room temperature and humidity were maintained at 25°C (± 3°C) and 45-55% respectively with a light-dark cycle of 12hrs. The animals were fed with commercially available standard pellet chow diet (Nutravet, Hyderabad) and drinking water *ad libitum*.

5.2.3 Methodology

As per OECD guideline 420 there are two types of acute oral toxicity tests i.e., limit test and main test. The limit test is primarily used in situations where the experimenter has information indicating that the test material is likely to be nontoxic, i.e., having toxicity below regulatory limit doses. However, in those situations where
there is little or no information about its toxicity, or in which the test material is expected to be toxic, only the main test should be performed. Limit test was conducted in the present study considering the *P. amarus* as nontoxic material from the previous reports (George *et al.*, 2011).

### 5.2.4 Procedure

Prior to dosing, all the animals were fasted overnight before being weighed, dose was calculated according to the body weight and the tested sample was orally administered in a single dose of 2000mg/kg. After the sample was administered, food was withheld for next 3-4 hours.

### 5.2.5 Observations

Animals were observed continuously for toxic symptoms during the first 30 minutes after dosing and observed periodically (with special attention given during the first 4 hours) for the next 24 hours and then daily thereafter, for 14 days. Observations included changes in skin and fur, eyes and mucous membranes and behavioral pattern. Attention was given for observations of tremors, convulsions, salivation, lethargy, sleep, coma, changes in body weight and mortality.

### 5.3 Results and Discussion

Medicinal herbs and their products are widely considered to be of lower risk compared with synthetic drugs but they are not completely excluded from the possibility of having toxic or other adverse effects. There are, however, challenges unique to medicinal herbs. Often, deficiencies such as under-reporting of adverse reactions, general lack of toxicological information on herbs and the quality of the reported information present challenges when signals of safety concern arise.

#### Table 5.1. Study of acute oral toxicity of extracts and isolated compounds of *Phyllanthus amarus* administered orally to mice.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Doses (mg/kg)</th>
<th>Sex</th>
<th>D/T</th>
<th>Mortality latency</th>
<th>Toxic symptoms</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>PAHE 2000mg/kg</td>
<td>F</td>
<td>0/6</td>
<td>-</td>
<td>Nil</td>
</tr>
<tr>
<td>II</td>
<td>PAEA 2000mg/kg</td>
<td>F</td>
<td>0/6</td>
<td>-</td>
<td>Nil</td>
</tr>
<tr>
<td>III</td>
<td>PAME 2000mg/kg</td>
<td>F</td>
<td>0/6</td>
<td>-</td>
<td>Nil</td>
</tr>
</tbody>
</table>

D/T = dead/treated rats; Nil = no toxic symptoms observation; Mortality latency = time to death (in hours) after oral administration.
To determine the safety of plant products and drugs for human use, toxicological evaluation is carried out in experimental animals to predict toxicity and to provide for selecting ‘safe’ doses. The lack of adequate scientific evidence on the safety of \textit{P. amarus} is often a major issue to the acceptance and use of this medicinal plant. In this study, the plant was successfully identified as \textit{P. amarus} and therefore the results are not extrapolated beyond this species.

The absence of toxic symptoms was observed after the administration of extracts and isolated lignans (Table 5.1) and thus indicating their lethal dose (LD$_{50}$) greater than the test dose (2000 mg/kg). Previous literature on the plant extracts belonging to \textit{Phyllanthus} genus reported them as nontoxic at dose of 5g/kg body weight (George et al., 2011) which further supports the results of the present study. It was also reported that the lignans phyllanthin and hypophyllanthin were having LD$_{50}$ value of 300mg/kg for mice, 980mg/kg for rats and 3200mg/kg body weight for rabbits (www.clearsynth.com). No signs of toxicity and mortality were observed in the acute oral toxicity studies conducted on female albino mice. The LD$_{50}$ of Hexane (PAHE), ethylacetate (PAEA) and methanolic (PAME) extracts of \textit{P. amarus} aerial parts was found to be greater than the test dose (2000mg/kg).