4. ACUTE TOXICITY STUDIES

4.1 INTRODUCTION

Acute toxicity studies are tests in which single doses of the drug are used in each animal on one occasion only for the determination of LD$_{50}$ or median lethal dose, i.e. the dose which will kill 50% of the animals of a particular species.$^{[220]}$

LD$_{50}$ values of the drugs were determined following Organization for Economic Co-operation and Development (OECD) Guidelines$^{[221]}$ for acute oral toxicity-Up-and-Down Procedure, using AOT425 Software.$^{[222]}$ The Up-and-Down testing method was first suggested by Dixon and Mood.$^{[223-225]}$ Later Bruce (1985) proposed to use this procedure for determining acute toxicity of chemicals.

OECD adopted the revised AOT 425 on 17$^{th}$ December 2001 based on the recommendations by expert meetings. The advantages of this test procedure are$^{[221]}$:

i) Number of animals required to study acute toxicity are minimized.

ii) A common international agreement has been reached on harmonized LD$_{50}$ cut-off values for the classification of chemical substances.

iii) Testing in one sex only (usually females) is considered sufficient.

iv) Estimation of confidence intervals is included in order for a point estimate to be meaningful.
In this test a single ordered dose progression is to be followed and animals are to be dosed one at a time at a minimum of 48h intervals and the dose progression by a factor of 3.2 as recommended by the guidelines (3.2 is the default factor corresponding to one half log units). Dosing continues depending on the outcomes of all the animals up to (48h). Survival after 48h is marked as ‘O’ and death as ‘X’. Animals found to be in moribund condition should be killed humanely. Dosing is stopped when one of the following three criteria is satisfied.

i) Three consecutive animals survive at the upper bound.

\[ O \ O \ O \]

ii) 5 reversals occur in any six consecutive animals tested.

\[ O \ X \ O \ X \ O \ X \]
\[ \downarrow \downarrow \downarrow \downarrow \downarrow \]
\[ 1 \ 2 \ 3 \ 4 \ 5 \text{ reversals} \]

iii) At least four animals have followed the first reversal and the specified likelihood-ratios exceed the critical value.

When the stopping criteria are attained the AOT425 software automatically calculates the LD$_{50}$ with Confidence Interval (CI).

**3.2 MATERIALS AND METHODS**

**Animals:**

220-250g weighing, 8-12 weeks old, nulliparous, non-pregnant female Wistar rats$^{[226]}$ (procured from M/s Sainath animals registered with CPCSEA) were taken and maintained at temperature 22$^\circ$C ± 3$^\circ$C, 50-60% relative humidity, 12:12h time cycle of light and dark and were housed individually with standard pellet diet (Amrut Lab Animal Feed)
and water ad libitum. Rats were fasted overnight with water ad libitum prior to the test.

**Drugs:**

Panchagavya Ghrutham (PG), Hab-e-Jund (HJ), Kushmanda Lehyam (KL), Itrifal Kishneezi (IK), extracts of Cynodon dactylon (CD) and Barleria cristata (BC) were taken. Except PG all the drugs were given as suspensions in 1% gum acacia (as the drugs formed thick suspensions) and PG was given in sesame oil (as it was neither suspending nor emulsifying in gum acacia). The drugs were administered such that the final volume was not more than 1ml/kg body weight.

**Dose:**

The dose progression followed in this study was 175, 550, 1750, 5000mg/kg as per the OECD guidelines. Drugs were administered as single doses by gavages using gastric tubes to overnight fasted animals. Food was given after 4h of administration of the drugs.

**4.3 RESULTS**

The formulations showed relatively higher LD$_{50}$ values compared to the methanolic extracts of crude drugs. PG showed highest LD$_{50}$ of all the drugs and least by CD. The rats at higher doses of CD died of convulsions. Among the formulations, HJ showed lower LD$_{50}$ values. The results are shown in table 4.1
4.4 DISCUSSION

PG was relatively safer than the other drugs, followed by IK, HJ and KL. This could be explained by the higher therapeutic doses in humans and the formulations and large quantities of safer substances like clarified butter, honey, candied sugar, etc. Convulsions with higher doses of CD supports its use in folklore medicine for diabetes. Higher doses of PG, HJ and KL showed sedation where as IK and BC showed anxiety, writhing, palpitation and tremors.

Table 4.1 LD$_{50}$ Values of Drugs

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Drugs</th>
<th>Estimated LD$_{50}$ * (mg/kg)</th>
<th>Approximate 95% CI (mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Panchagavya Ghrutham</td>
<td>1909</td>
<td>1750-5000</td>
</tr>
<tr>
<td>2.</td>
<td>Hab-e-Jund</td>
<td>1750</td>
<td>468.6-2660</td>
</tr>
<tr>
<td>3.</td>
<td>Cynodon dactylon</td>
<td>802.3</td>
<td>550-1750</td>
</tr>
<tr>
<td>4.</td>
<td>Kushmanda Lehyam</td>
<td>1750</td>
<td>288.6-2910</td>
</tr>
<tr>
<td>5.</td>
<td>Itrifal Kishneezi</td>
<td>1750</td>
<td>775.6-4280</td>
</tr>
<tr>
<td>6.</td>
<td>Barleria cristata</td>
<td>1030</td>
<td>550-1750</td>
</tr>
</tbody>
</table>

*Based on assumed sigma of 0.5  CI-Confidence Interval

4.5 CONCLUSIONS

From the LD$_{50}$ studies, the doses for further studies were chosen in logarithmic progression of 30,100, 300 mg/kg body weight as suggested by Turner.\textsuperscript{[227]}