CHAPTER IV

Toxicity studies of the formulation: biochemical parameters
TOXICITY STUDIES OF THE FORMULATION: BIOCHEMICAL PARAMETERS

Introduction

In order to assess the safety of a drug, various toxicity studies are carried out in animals like mice, rats, guinea pigs, dogs and monkeys under varying conditions of drug administration. The basic objective of the preclinical toxicological studies is a definition of an initial dose for humans and projection of the qualitative and quantitative toxic events that are likely to be encountered during the initial clinical trial.222-267 Extensive efforts were directed to determine the doses of new drugs that killed 10% (LD10), 50% (LD50) and 90% (LD90) of the animals receiving the agent on varied schedules.268-269

Toxicological studies usually involve two phases. The first phase - acute toxicity studies are used as dose range finding studies. In these, drugs are tested for the effects of single doses in different groups of animals. The main objective of an acute toxicity study is not only to establish a figure for LD50 with precision, but also to learn something about the way in which the drug acts as a poison.

Second phase - the chronic toxicity studies are carried out in different animal species with different doses and usually lasts for a period of 90 days to one year. Chronic toxicity studies of shorter duration (14-21 days) are called sub acute toxicity studies. The major objective of the second stage or more detailed studies
is the final prediction of the safe starting dose definition of the safe specific organ toxicity likely to be encountered in the patients.\textsuperscript{270}

Generally the toxicity of indigenous drugs has largely been neglected as it is argued that these drugs are used in traditional clinical practices. However, it is suggested that all natural products and active principles must be subjected to the same stringent toxicity studies as in the case of synthetic drugs.\textsuperscript{271}

Eventhough all the plants selected for the formulation are individually analysed earlier during different analytical studies,\textsuperscript{97, 154, 162, 272-275} it was found to be important to evaluate the toxic effects of this formulation of selected plants, essential to select a safe dose. Some of the selected plants have been used against cyclophosphamid-induced toxicity \textsuperscript{162} and against carbon tetrachloride induced liver damage.\textsuperscript{165} Therefore an effort is made to determine the acute and sub acute toxicity studies of the herbal formulation, ‘Caps HT2’ and their tolerance in laboratory animals, the rats.

**Materials and methods**

**Animals**

The male rats (200-250g) are caged in uniform hygienic conditions and fed with standard pellet diet (Lipton Laboratories, Bangalore) and water *ad libitum*. Acute and sub acute toxicity studies were conducted in rats for the detection of the toxic effect of the formulation.\textsuperscript{222}
**Acute toxicity study**

Single doses of the formulation (2.5, 5 and 10gm/kg body wt.) were administered into three groups of rats (2: 12a) by oral intubations and were observed for pathological symptoms for a period of 72 hrs. The body weights of the animals were taken initially and after the experimental period. Animals were fasted overnight and sacrificed using ether anaesthesia and the serum GPT, GOT, ALP, LDH, GGT, creatinine and urea were estimated (2: 12c - 2: 12i) and compared with the normal groups for determining the normal functioning of the vital organs, heart, liver and kidney.

**Sub acute toxicity study**

1/10th of the dose formulations of acute study were administered (250 and 500 mg/kg body weight) in rats for a period of 21 days by oral intubations (2: 12b). The initial and final body weights of the animals were recorded. After the experimental period of 21 days the rats were fasted overnight and sacrificed using ether anaesthesia. The serum biochemical parameters, the ALP, GPT, GOT, LDH, GGT, creatinine and urea were estimated (2:12c - 2: 12i) for assessing the functions of heart, liver and kidney and compared with the normal group.

**Results**

During this study, the formulation did not produce any external symptoms or mortality. The acute toxicity study revealed clearly that the single doses of formulation administered have no relation with LD50, as LD50 was not
observed even up to 10,000mg/kg dose. The weight (239±11.7) change was not observed in any of the treated rats after the period of 72 hrs. The results of the acute toxicity studies are shown in the table (4: 1).

In the sub acute toxicity study the weight changes (11-13%) noticed in the experimental groups were not significant between the normal and treated groups. The animal behaviour, food and water intake were normal during the experimental period. The results of the sub acute toxicity studies are shown in the table (4: 2).

**Effect on heart function**

No significant change in the activity of the marker enzymes GOT and LDH noticed in the serum of rats (Table 4: 2) indicating the effectiveness of the formulation without any toxic effects on heart.

**Effect on liver function**

The GOT, GPT and ALP activity of the serum also exhibited no variation among the experimental groups. We have not observed any toxic effects at the dosages of acute as well as sub acute toxicity studies. The serum GGT study also revealed no significant change between the formulation treated and normal rats.

**Effect on kidney function**

The urea and creatinine levels did not show any variation in all the experimental groups, indicating that the formulation had no toxic effects in the kidney.
Table 4: Serum biochemical parameters on acute toxicity study of the formulation in rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>Urea (mg/dl)</th>
<th>ALP (KA Unit)</th>
<th>Creatinine (mg/dl)</th>
<th>GGT (IU/l)</th>
<th>LDH (IU/l)</th>
<th>SGOT (IU/l)</th>
<th>SGPT (IU/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>41.5±4.0</td>
<td>23.8±1.3</td>
<td>0.84±0.07</td>
<td>2.6±0.2</td>
<td>569.7±47.7</td>
<td>171.1±12.5</td>
<td>49.9±4.7</td>
</tr>
<tr>
<td>Formulation (2.5g /kg)</td>
<td>45.3±1.9</td>
<td>24.0±2.5</td>
<td>1.13±0.09</td>
<td>3.3±0.3</td>
<td>570.2±43.2</td>
<td>190.7±15.4</td>
<td>51.0±4.8</td>
</tr>
<tr>
<td>Formulation (5g /kg)</td>
<td>38.4±2.01</td>
<td>23.8±2.3</td>
<td>0.91±0.08</td>
<td>4.0±0.4</td>
<td>590.3±52.4</td>
<td>186.0±11.6</td>
<td>48.3±4.2</td>
</tr>
<tr>
<td>Formulation (10g /kg)</td>
<td>36.3±2.9</td>
<td>24.1±2.3</td>
<td>1.18±0.11</td>
<td>4.2±0.32</td>
<td>589.8±47.5</td>
<td>187.5±10.3</td>
<td>47.5±3.21</td>
</tr>
</tbody>
</table>

The values are mean ± SD of 6 animals/group
Table 4: Serum biochemical parameters on sub acute toxicity study of the formulation in rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>Urea (mg/dl)</th>
<th>ALP (KA Unit)</th>
<th>Creatinine (mg/dl)</th>
<th>GGT (IU/l)</th>
<th>LDH (IU/l)</th>
<th>SGOT (IU/l)</th>
<th>SGPT (IU/l)</th>
<th>% Weight increase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>40.8±4.1</td>
<td>23.6±2.0</td>
<td>0.86±0.05</td>
<td>2.5±0.3</td>
<td>568.5±45.9</td>
<td>168.3±11.8</td>
<td>51.0±5.0</td>
<td>11.5±0.9</td>
</tr>
<tr>
<td>Formulation (250mg/kg)</td>
<td>42.3±1.9</td>
<td>22.9±1.9</td>
<td>0.90±0.07</td>
<td>3.03±0.4</td>
<td>554.5±45.7</td>
<td>150.0±13.4</td>
<td>54.3±4.8</td>
<td>11.3±1.1</td>
</tr>
<tr>
<td>Formulation (500mg/kg)</td>
<td>39.3±2.1</td>
<td>23.0±2.1</td>
<td>0.87±0.07</td>
<td>2.9±0.3</td>
<td>588.0±52.2</td>
<td>153.0±10.6</td>
<td>53.8±5.2</td>
<td>12.6±1.0</td>
</tr>
</tbody>
</table>

The values are mean ± SD of 6 animals/group.
Discussion

Changes in body weight have been used to assess the course of the disease and the response to therapy of the drugs.\textsuperscript{276} The weight increase observed was normal for the period of 21 days, indicating non-toxicity of formulation. The formulation was non-toxic and did not induce any significant alterations by its administration as a single dose up to a level of 10,000mg/kg body weight. No significant alterations are observed in haematological screening indicating no toxic effect.

Transaminases (GOT and GPT) and alkaline phosphatases (ALP) are good indices of liver damage,\textsuperscript{277} and the formulation effected no damage to liver, which could be inferred from normal activity of the enzymes.

In the present study, the alterations observed in the levels of urea and creatinine are not considerable, the slight variations observed of the acute toxicity study was not sufficient to regard as renal failure. Raised urea and non-protein nitrogen level in blood have been observed with impaired renal function and chronic renal failure.\textsuperscript{278}

Gama-Glutamyl transferase\textsuperscript{,279} catalyses the transfer of gama-glutamyl group from a gama-glutamyl peptide to an amino acid or another peptide. It has been found in several tissues, with the highest activity noticed in the brush border of the proximal tubules in the kidney and in the epithelium of the intrahepatic bile ducts. The formulation treated rats exhibited normal enzymatic activity corresponding to the activity of normal rats.
LDH is a hydrogen transfer enzyme that catalyses the oxidation of L-lactate to pyruvate with the mediation of NAD⁺ as hydrogen acceptor. The serum enzyme activity changes following myocardial infarction, and the values may be moderately increased in myocarditis and cardiac failure. Elevation of LDH activity is observed also in liver diseases and especially high (10 times as normal) in toxic hepatitis. In the present study LDH activity exhibited only slight (insignificant) variations between the normal and formulation administered groups indicating that there is no toxic effect for this formulation.

The present study showed that formulation, ‘Caps HT2’ does not induce any toxic manipulation on the biochemical parameters investigated. From this we can infer and hypothesize that the formulation is non-toxic and can be used as a therapeutic agent for treating the diseases effectively.