Methodology

Authentication of the plant from BSI (Botanical survey of India).

Plan to collect Achyranthes aspera L leaves from Avsari Forest Park (Pune), Maharashtra, India. Herbaria of the plant will be sent for authentication from BSI (Botanical Survey of India), Pune, India.

Method – 1

Acute toxicity study

Plan to collect Achyranthes aspera L leaves from Avsari Forest Park (Pune), Maharashtra, India. After collection of the required quantity of Achyranthes aspera Leaves, it will be carefully segregated, cleaned and dried in shade to constant weight. The plant material will be planned to keep in preset oven for eight days at 45°C. The dried plant material free of moisture will be made into powder and sieved through a BSS Mesh No. 85 sieve and then planned to store in an airtight container. The study protocol will be use for the study is given in table No. I

<table>
<thead>
<tr>
<th>Name of the study</th>
<th>Acute toxicity study</th>
</tr>
</thead>
<tbody>
<tr>
<td>Test material</td>
<td>Achyranthes aspera L leaves powder as slurry.</td>
</tr>
<tr>
<td>Animal model</td>
<td>Albino Swiss Mice</td>
</tr>
<tr>
<td>Route of administration</td>
<td>Raj Biotech (INDIA) Ltd., Pune</td>
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<tr>
<td>Sex</td>
<td>Male and Female</td>
</tr>
<tr>
<td>Weight range of animals</td>
<td>Between 35 to 45 g</td>
</tr>
<tr>
<td>No. of dose groups</td>
<td>Four groups each</td>
</tr>
<tr>
<td>Animals per group</td>
<td>Two (1 male and 1 female)</td>
</tr>
<tr>
<td>Route of administration</td>
<td>Intragastric</td>
</tr>
<tr>
<td></td>
<td>administration with the</td>
</tr>
</tbody>
</table>
Assessment of the behavior of animals will be check by general observations of each animal on a daily basis from the stage of dosing to the end of the study. Any changes or an abnormality is an indication of toxicity.

The above methodology (method-1) is taken from the past experiments done by Shirish S. Pingale on Acute toxicity (37, 38).

Method – 2

Hepatoprotection study

Liver disorders constitute a major health problem in India. There is dearth of effective modern drugs for the treatment of liver disorder, like Jaundice. Many herbal preparations have been marketed for the same. The current investigation on Achyranthes Aspera L as to the
hepatoprotective activity was undertaken as an extension of Dr. Shirish S. Pingale earlier work on Argemone Mexicana, centella asiatica, ricinus communis and tonospora cordifolia.

Thirty Wistar rats of either male or female sex will be planned from Raj Biotech (INDIA) Ltd., Pune, India. The animals will be planned to keep in the polyurethane cage houses. The cages will be provided with rice husk bedding and cleaned daily. The animals will be provided with drinking water and adlibitum feed. The animals will be given measured volume (250 ml) of drinking water and weighed amount (200g) of food during the experiment. All animals plan to use for the study in the weight range of 130-150g. The animals will be randomly divided into five groups of six (3 male and 3 female) animals each. The male and female rats will be housed in separate cages.

After an acclimatization period of fourteen days the rat cages will be randomly assigned the following treatments;

Group I – Normal (Vehicle) control,
Group II - toxicant (CCl4) control,
Group III - toxicant (CCl4) recovery,
Group IV - CCl4 + Silymarin treated,
Group V - Plant (Achyranthes Aspera L) control.

The animals from Group I plan to give an intra peritoneal (i.p.) injection of 0.5ml of liquid paraffin and those from Group II, III, IV and V will receive an i.p. injection of 0.75ml/Kg of CCl4 in 0.5ml liquid paraffin per animal on the first day of the study. The animals from Group I, II and III will receive an oral dose of 2ml of distilled water (D/W) once daily. The animals from Group IV will receive an oral dose of 0.0075g/Kg of Silymarin suspension in 2ml of distilled water per animal. The animals from Group V will receive an oral dose of 0.65g/Kg of sieved leaves powder of Achyranthes Aspera suspension in 2ml of distilled water per animal. The animals from Group I, II, IV and V will be sacrificed on the fourth day (72 hours after dosing) and those from Group III will be sacrificed on seventh day of the study.

The above methodology (method-2) is taken from the past experiments done by Shirish S. Pingale on Hepatoprotection (30,31,32,33,34,35,36,39,40,41,42).
Work plan for 2 years (For every six months)

➢ First six (1-6) months work plan is as follows
   a) Introduction and selection of research topic.
   b) Review and study on medicinal plants.
   c) Review and literature study.

➢ Second six (6-12) months work plan is as follows
   a) Plant material Sample collection and sent for Authentification from BSI (Botanical Survey of India).
   b) Grinding and Extraction of plant Leaves by different methods.
   c) Plan of Experiments on Phytochemical analysis and proximate analysis.

➢ Third six (12-18) months work plan is as follows
   a) Preparation for Acute toxicity study Experiments.
   b) Writing paper on acute toxicity study.
   c) Writing paper on Review of selected medicinal plant.

➢ Fourth six (18-24) months work plan is as follows.
   a) Preparation for Hepatoprotection study Experiments.
   b) Writing paper on Hepatoprotection study.
   c) Writing summary of research work.
   d) Final Thesis writing.