Chapter 3

Plan of Work
Literature has reports on the pharmacological actions of *Lawsonia inermis* Linn., where its anti-inflammatory potentials and hemotoxicity are revealed. Experiments were designed to evaluate the anti-inflammatory and hemotoxic effects of the extracts of Lawsonia against acute inflammation.

### 3.1 Objectives of Investigation

1. To prepare the aqueous, alcoholic and chloroform extracts of the leaves of *Lawsonia inermis* Linn.
2. To induce inflammation in right hind paw in rats by subplantar injection of Carrageenin and other phlogistic agents; histamine, bradykinin, serotonin, prostaglandin E₂ and hyalluronidase. Assessment of the edema during the following six hours by measuring the paw volume. Blood samples for estimation of biochemical markers of inflammation. Finally animals are to be sacrificed for histopathological studies.
3. To study the effect of the pretreatment of aqueous, alcoholic and chloroform extracts of leaves of *Lawsonia inermis* Linn., against Carrageenin, histamine, bradykinin, serotonin, prostaglandin E₂ and hyalluronidase induced inflammation in rats.
4. To compare the activity of the extracts with that of respective standard drugs, in the different phlogistic agents induced inflammations.
5. To study various biochemical and histopathological changes produced by the extracts of *Lawsonia inermis*.
6. To study the analgesic effect of the extracts of *Lawsonia inermis*.
7. To study the toxic effects of the extracts of *Lawsonia inermis* by biochemical, hematological and histopathological evaluation.
8. To carry out the phytochemical standardization the extracts of *Lawsonia inermis* Linn.
3.2 Design of Research work

Accordingly investigations on the extracts were planned as per the following details:
The following experiments were designed to study the pharmacological actions of the three extracts of *Lawsonia inermis*, viz.; Aqueous, Alcoholic and Chloroform.

3.2.1 Preparation of the extracts

Experiment 1

- Collection of leaves of Lawsonia from the source.
- Authentication of the leaves
- Drying of the leaves
- Extraction in Soxhlet apparatus.

3.2.2 Design of Animal Study

The studies were performed by grouping the rats with 6 rats in each. Each group was kept in the laboratory animal house for a week for proper acclimatization, before starting the experiment.

In each group rats were given below mentioned treatment orally for 3 days and on 3rd day respective phologistic agent was injected s. c. in the subplanter region. Development of paw edema / inflammation was assessed plethysmographically for 6 hours. After 6 hours blood samples were withdrawn for biochemical estimations. Liver was removed and preserved in 10% Formaline for histopathological assessment.
3.2.2.1 Carrageenin induced inflammation

Experiment 2

Pretreatment with test drugs was done for 3 days prior to the administration of carrageenin (0.1 ml of 1% suspension) into the sub-planter site of right hind paw in each rat.

Table 2: Animal grouping and treatment for Carrageenin induced inflammation

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment (For 3 days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group N</td>
<td>Normal control Normal diet. (Only for basal Biochemical values)</td>
</tr>
<tr>
<td>Group I</td>
<td>Toxic control Normal saline (1 ml/100g p.o.)</td>
</tr>
<tr>
<td>Group II</td>
<td>Stand drug Phenylbutazone (100 mg/kg p.o.)</td>
</tr>
<tr>
<td>Group III</td>
<td>Test drug 1 Aqueous extract (0.75 g/kg p.o.)</td>
</tr>
<tr>
<td>Group IV</td>
<td>Test drug 1 Aqueous extract (1.5 g/kg p.o.)</td>
</tr>
<tr>
<td>Group V</td>
<td>Test drug 2 Alcoholic extract (0.75 g/kg p.o.)</td>
</tr>
<tr>
<td>Group VI</td>
<td>Test drug 2 Alcoholic extract (1.5 g/kg p.o.)</td>
</tr>
<tr>
<td>Group VII</td>
<td>Test drug 3 Chloroform extract (0.75 g/kg p.o.)</td>
</tr>
<tr>
<td>Group VIII</td>
<td>Test drug 3 Chloroform extract (1.5 g/kg p.o.)</td>
</tr>
</tbody>
</table>

Assessment of Anti-inflammatory Activity

1. Physical method - Paw edema by plethysmometer (Transducer, UGO Basil 7140)
2. Biochemical method (Diagnostic estimations)
   - In Serum
     - i) Serum Glutamyl oxaloacetate Transaminase / SGOT / AST
     - ii) Serum Glutamyl pyruvate Transaminase / SGPT / ALT
     - iii) Serum γ- Glutamyl Transpeptidase / GGTP
3. Histopathological study (10% formal saline buffer fixed liver)
   - Hematoxylin and Eosin staining
3.2.2.2 Other phologistic agents induced inflammation

Histamine ($10^{-3}$ g/ml) [Chlorpheniramine], Serotonin ($10^{-3}$ g/ml) [Cyproheptadine], PG $E_2$ ($10^{-6}$ g/ml) [Indomethacin], Bradykinin ($2 \times 10^{-5}$ g/ml) [Aspirin], Hyalluronidase (2400 IU/ml) [Indomethacin].

Experiment 3 (A to E)

Pretreatment with test drugs was done for 3 days prior to the administration of Phologistic agent (0.1ml) into the sub-planter region of right hind paw.

Table 3: Animal grouping and treatment for Phologistic agents induced inflammation

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>N=6</td>
<td></td>
</tr>
<tr>
<td>Group N</td>
<td>Normal control. Normal diet. (Only for basal Biochemical values)</td>
</tr>
<tr>
<td>Group I</td>
<td>Toxic control</td>
</tr>
<tr>
<td>Group II</td>
<td>Stand drug</td>
</tr>
<tr>
<td>Group III</td>
<td>Test drug 1</td>
</tr>
<tr>
<td>Group IV</td>
<td>Test drug 2</td>
</tr>
<tr>
<td>Group V</td>
<td>Test drug 3</td>
</tr>
</tbody>
</table>

Assessment of Anti-inflammatory Activity

1. Physical method - Paw edema by plethysmometer (Transducer, UGO Basil 7140)
2. Biochemical method (Diagnostic estimations)
   In Serum i) Serum Glutamyl oxaloacetate Transaminase / SGOT / AST.
   ii) Serum Glutamyl pyruvate Transaminase / SGPT / ALT.
3.2.2.3 Analgesic activity

Experiment No 4 (A & B)

On the 3\textsuperscript{rd} day animals were subjected to painful stimuli 1 hr after the final dose.

Table 4: Animal grouping and treatment for analgesic activity

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>N=6</td>
<td>(For 3 days)</td>
</tr>
<tr>
<td>Group I</td>
<td>Toxic control Normal saline (1 ml/100g p.o.)</td>
</tr>
<tr>
<td>Group II</td>
<td>Standard drug Standard drug- Aspirin (100mg /kg p.o.)</td>
</tr>
<tr>
<td>Group III</td>
<td>Test drug 1 Aqueous extract (1.5 g/kg p.o.)</td>
</tr>
<tr>
<td>Group IV</td>
<td>Test drug 2 Alcoholic extract (1.5 g/kg p.o.)</td>
</tr>
<tr>
<td>Group V</td>
<td>Test drug 3 Chloroform extract (1.5 g/kg p.o.)</td>
</tr>
</tbody>
</table>

Pain stimuli

Tail Immersion in hot water at 55±1°C
Eddy's Hot plate

Assessment of Analgesia

Tail immersion method - Time of tail withdrawal
Eddy's hot plate method - Jumping or paw licking
3.2.2.4 Toxicity evaluation of the extracts of *Lawsonia inermis* Linn.

Experiment No 5

Table 5: Animal grouping and treatment for toxicological activity

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td>Normal control</td>
</tr>
<tr>
<td>Group II</td>
<td>Test drug 1 Aqueous extract (2 g/kg p.o.)</td>
</tr>
<tr>
<td>Group III</td>
<td>Test drug 2 Alcoholic extract (2 g/kg p.o.)</td>
</tr>
<tr>
<td>Group IV</td>
<td>Test drug 3 Chloroform extract (2 g/kg p.o.)</td>
</tr>
</tbody>
</table>

General behaviour

Body weight

Ulcerogogenic study

Biochemical Studies

- In Serum
  - i) Serum Glutamyl oxaloacetate Transaminase / SGOT / AST
  - ii) Serum Glutamyl pyruvate Transaminase / SGPT / ALT

Hematological estimations

- i. Clotting time
- ii. Hemoglobin content
- iii. RBC count
- iv. Total WBC count
- v. Platelet count

Histopathological study

10% formal saline fixed hematoxylin and eosin stained sections of

- i. Kidney
- ii. Liver
3.2.3 Standardization

Experiment 6

- Preliminary phytochemical screening
- Qualitative identification of flavonols
- Quantification of the identified active constituents