STUDIES ON ANTI-INFLAMMATORY ACTIVITY
• Introduction

• Pharmacological screening
  ➢ Ethanolic extract of the leaves of *Ricinus communis*
  ➢ Ethanolic extract of the seeds of *Ricinus communis*
ANTI-INFLAMMATORY ACTIVITY

- **Introduction**

  Inflammation is the body’s response to noxious or injurious stimuli, characterized by warmth, redness of the skin, pain, swelling and loss of function. The response to only stimuli (chemical, physical and microbial) is quite similar. Inflammation is a part of host defense mechanism. There are several tissue factors that are known to be involved in the inflammation reactions such as release of histamine, bradykinin and prostaglandins.

  Essentially the inflammations are of two types: acute and chronic. The classical signs of acute inflammation are warmth, redness, pain, swelling and loss of function. Chronic inflammation is also characterized by long lasting pain, redness and swelling and is caused by the persistence of an irritant, which may be biological, physical or chemical in nature.

  ➢ **Experimental methods for testing anti-inflammatory activity**:

    Acute inflammatory condition is produced in the animals by adopting the following methods.

    a) Carrageenan – induced pedal inflammation
    b) Egg – white induced pedal inflammation
    c) Dextrin – induced pedal inflammation

    Chronic inflammatory condition is produced in the animals by adapting the following methods.

    a) Formaldehyde – induced pedal inflammation
    b) Implantation of cotton pellets
    c) Granular pouch
    d) Tuberculin sensitivity
    e) Freund’s adjuvant.

  Instruments used to measure the paw oedema are

    a) Plethysmograph
    b) Zeithins apparatus
PHARMACOLOGICAL SCREENING –
ANTI - INFLAMMATORY ACTIVITY

• Effect of the ethanolic extract of the leaves of *Ricinus communis*.

  ➢ Preparation of the extract

  The preparation of ethanolic extract of the leaves of *Ricinus communis*
  has already been discussed by the author under the chapter –III (chemical
  investigations). The extract was used to evaluate anti-inflammatory activity.

  ➢ Selection of Method

  Anti-inflammatory activity was evaluated by carrageenan – induced rat
  hind paw oedema\textsuperscript{34} using plethysmograph apparatus to measure the paw volume.

  ➢ Selection of Materials

  All the materials used for this experiment were of analytical grade.
  Carrageenan was procured from (Aman Scientific Products, Vijayawada),
  Tween 80 (S.D. Fine Chemicals Pvt. Ltd., Bombay) and Saline (Core Health Care,
  Bombay) were purchased from the local supplier. Diclofenac sodium sample was
  obtained as gift sample from M/S Jagsonpal Ltd., New Delhi.

  ➢ Preparation of Tween 80 (1%)

  Stock suspension of Tween 80 was prepared by triturating 1 g of
  Tween 80 in distilled water and made up to 100 ml. It was used for
  suspending the test compound and standard drug.

  ➢ Preparation of carrageenan suspension

  1% suspension of carageenan was prepared by sprinkling 100 mg of
  carrageenan powder in 10 ml of saline (0.9% w/v NaCl) solution and set aside
  and soaked for 1 h. A homogenous suspension was then obtained by thorough
  mixing with magnetic stirrer.
Selection of animals

Wistar albino rats of either sex weighing between 150–200 g obtained from Kshema Breeders, Deralakatte, Mangalore, India were used for the study. They were housed in polypropylene cages in an adequately ventilated room. The rats were allowed standard rat feed pellets supplied by Hindustan Lever Co., Bombay and water ad libitum throughout the course of the study.

Acute toxicity studies

The animals were stabilized for one week. They were maintained in standard condition at room temperature; 60±5% relative humidity and 12 h light-dark cycle. They had been given standard pellet diet supplied by Hindustan Lever Co., Bombay and water ad libitum throughout the course of the study. The animals were divided into 6 groups having 6 animals each. The test extract was administered orally as 1% solution of Tween 80 to the different groups in increasing dose levels of 10, 40, 100, 400, 1000 and 4000 mg/kg body weight.

The animals were then observed continuously for 3 hours for general behavior, neurological and autonomic profiles and then for every 30 minutes for next 3 hours and finally leading to death after 24 hours.

Selection of dose

From preliminary toxicity studies it was observed that none of the treated animals died and thus it was concluded that the compound was non toxic up to 4000 mg/kg body weight. Hence a dose of 100, 200 and 400 mg/kg body weight were chosen for the study.

Anti-inflammatory activity

Albino rats of either sex weighing between 150–200 g were divided into 5 groups having 6 animals each.
Group I

Animals served as control and received vehicle orally (Tween 80, 3 ml of 1% solution).

Group II

Animals were administered with standard drug diclofenac sodium at a dose of 100 mg/kg body weight, p.o. as a suspension in Tween 80 (3 ml of 1% solution).

Group III

Animals were treated with ethanolic extract of the leaves of *Ricinus communis* obtained by the author at a dose of 100 mg/kg body weight, p.o. as a suspension in Tween 80 (3 ml of 1% solution).

Group IV

Animals were treated with ethanolic extract of the leaves of *Ricinus communis* obtained by the author at a dose of 200 mg/kg body weight, p.o. as a suspension in Tween 80 (3 ml of 1% solution).

Group V

Animals were treated with ethanolic extract of the leaves of *Ricinus communis* obtained by the author at a dose of 400 mg/kg body weight, p.o. as a suspension in Tween 80 (3 ml of 1% solution).
A mark was made on both the hind paws just below the tibio-tarsal junction so that every time the paw could be dipped in the mercury column of plethysmograph up to the mark to ensure constant paw volume. After 30 min. of above treatment, an inflammatory oedema was induced in the left hind paw by injecting 0.1 ml carrageenan (1%) in the planter tissue of the paw of all the animals. The right paw served as a reference to non-inflammed paw for comparison. The initial paw volume was measured plethysmographically within 30 sec. of the injection. The relative increase in the paw volume was measured in control, standard and treated groups, 4h after carrageenan injection. The percent increase in paw volume over the initial reading was also calculated. This increase in the paw volume in animals treated with standard drug and the different doses of ethanolic extracts of the leaves of *Ricinus communis* were compared with the increase in paw volume of untreated control animals after 4h. The percentage inhibition of oedema volume was calculated using the formula,

\[
\% \text{ Inhibition} = \left\{ 1 - \frac{V_t}{V_c} \right\} \times 100
\]

Where \( V_t \) and \( V_c \) are the relative changes in the oedema volume of the test and control respectively. The results are summarised in the Table –8.
RESULTS AND DISCUSSION

In carrageenan induced rat hind paw method; statistically it was found that ethanolic extract of the leaves of *Ricinus communis*, at a dose of 400 mg/kg body weight significantly reduced the oedema volume, which was comparable to standard drug diclofenac sodium. The ethanolic extract of the leaves of *Ricinus communis* also showed dose dependent anti-inflammatory activity.

In carrageenan induced rat hind paw oedema the standard anti-inflammatory drug (Diclofenac sodium 100 mg /kg, p.o.) as well as the test drug of ethanolic extract of the leaves of *Ricinus communis* obtained by the author in doses of (100,200 and 400 mg /kg, p.o.) exhibited a significant reduction respectively (p < 0.01 and p < 0.001) in the volume of paw oedema in rats as compared to the control rats. All the drugs showed maximum inhibition of carrageenan induced rat paw oedema at the end of 4h. Diclofenac sodium (100 mg /kg) showed maximum of 73.3% inhibition of oedema. Ethanolic extract of the leaves of *Ricinus communis* (100,200 and 400 mg /kg) produced 13.3%, 26.6% and 66.6% inhibition of oedema (Table 8).
### Table 8

**Anti-inflammatory activity of the ethanolic extract of the leaves of *Ricinus communis***

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg/kg,)</th>
<th>Oedema Volume (ml)</th>
<th>Inhibition (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (Tween 80,1%)</td>
<td>–</td>
<td>0.15± 0.004</td>
<td>–</td>
</tr>
<tr>
<td><em>Ricinus communis</em></td>
<td>100</td>
<td>0.13± 0.003**</td>
<td>13.3</td>
</tr>
<tr>
<td></td>
<td>200</td>
<td>0.11±0.003*</td>
<td>26.6</td>
</tr>
<tr>
<td></td>
<td>400</td>
<td>0.05±0.007*</td>
<td>66.6</td>
</tr>
<tr>
<td>Diclofenac sodium</td>
<td>100</td>
<td>0.04±0.004*</td>
<td>73.3</td>
</tr>
</tbody>
</table>

*p < 0.001 compared to control, **p<0.01 compared to control

Values are mean ±SEM, of six animals in each group.

Data was analysed by unpaired ‘t’ test.

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Fig 8: Percentage inhibition of carrageenan induced paw oedema in rats

![Percentage inhibition of carrageenan induced paw oedema in rats](chart.png)
Effect of the ethanolic extract of the seeds of *Ricinus communis*.

Anti-inflammatory activity was evaluated by carrageenan induced rat hind paw oedema\(^3^4\) using plethysmograph apparatus to measure the paw volume. The preparation of the ethanolic extract of the seeds of *Ricinus communis* has already been discussed by the author under chapter – IV (Studies on hepatoprotective activity). The extract was used to evaluate anti-inflammatory activity. The detailed procedures followed in this study were described under various profiles Viz; selection of materials, preparation of Tween-80 (1%), preparation of carrageenan suspension, acute toxicity studies, selection of animals and selection of dose were narrated in detailed under the evaluation of anti-inflammatory effect of the ethanolic extract of the seeds of *Ricinus communis* and hence results and discussions are presented here under.
RESULTS AND DISCUSSION

In carrageenan induced rat hind paw method; statistically it was found that ethanolic extract of the seeds of *Ricinus communis*, at a dose of 400 mg/kg body weight significantly reduced the oedema volume, which was comparable to diclofenac sodium. The ethanolic extract of the seeds of *Ricinus communis* also showed similar results of dose dependent anti-inflammatory activity.

In carrageenan induced rat hind paw oedema the standard anti-inflammatory drug (Diclofenac sodium 100 mg/kg, p.o.) as well as ethanolic extract of the seeds of *Ricinus communis* obtained by the author in doses of (100, 200 and 400 mg/kg, p.o.) exhibited a significant reduction respectively (p < 0.01 and p < 0.001) in the volume of paw oedema in rats as compared to the control rats. All the drugs showed maximum inhibition of carrageenan induced rat paw oedema at the end of 4 hours. Diclofenac sodium (100 mg/kg) showed maximum of 70.58% inhibition of oedema. Ethanolic extract of the seeds of *Ricinus communis* (100, 200 and 400 mg/kg) produced 17.64%, 29.41% and 64.70% inhibition of oedema (Table 9).

It can be concluded that it will be safe and harmless to use ethanolic extract of leaves / seeds of *Ricinus communis*, since they are able to show the efficacy on par with diclofenac sodium in anti-inflammatory activity.
Table –9

Anti-inflammatory activity of ethanolic extract of Ricinus communis seeds

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg/kg, p.o.)</th>
<th>Oedema Volume (ml)</th>
<th>Inhibition (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (Tween 80,1%)</td>
<td>……………………</td>
<td>0.17±0.007</td>
<td>……………………</td>
</tr>
<tr>
<td><em>Ricinus communis</em></td>
<td>100</td>
<td>0.14±0.004***</td>
<td>17.64</td>
</tr>
<tr>
<td></td>
<td>200</td>
<td>0.12±0.006*</td>
<td>29.41</td>
</tr>
<tr>
<td></td>
<td>400</td>
<td>0.06±0.004*</td>
<td>64.70</td>
</tr>
<tr>
<td><em>Diclofenac sodium</em></td>
<td>100</td>
<td>0.05±0.005*</td>
<td>70.58</td>
</tr>
</tbody>
</table>

*p < 0.001 compared to control, **p<0.01 compared to control
Values are mean ±SEM, of six animals in each group.
Data was analysed by unpaired ‘t’ test.

Fig 9: Percentage inhibition of carrageenan induced paw oedema in rats