ANTI-INFLAMMATORY ACTIVITY OF SIDA CORDIFOLIA LINN SEEDS EXTRACT

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Summary
The seed oil obtained from sida cordiofolia seeds was found to possess anti-inflammatory activity against carrageenan induced paw edema in albino rats. The acute toxicity studies up to a dose level of 2000 mg/kg body weight of the rats showed the signs of toxicity like tremors and convulsions. After determination of acute toxicity study up to a therapeutic dose of 400 mg/kg body weight of petroleum ether extract of seeds of sida cordiofolia was found to possess anti-inflammatory activity against carrageenan induced paw edema in albino rats. The inflammation in carrageenan induced rat paw edema was reduced to 1.10 (p< 0.05) at 30 minute, 1.00 (p< 0.01) at 60 minute, 0.650 (p< 0.01) at 180 minute and 0.375 (p< 0.01). After comparing with control group and standard drug diclofenac sodium the value of seed extract was significant.

Keywords: Sida cordifolia seed Linn, Anti-inflammatory effect, Carrageenan induced paw edema, Diclofenac sodium etc.

Introduction
Inflammation is fundamental patho-physiological response of the host involving a series of events to eliminate noxious stimulus, such as radiant, chemical, Physical infection and immune provocation. Cornelius celsius, a roman writer in 1st century A.D. described the four cardinal signs of inflammation: Rubor, Tumor, Calor and Dolor (redness, swelling, heat, and pain respectively).¹ In 1973, the Scottish surgeon, John Hunter wrote the inflammation is not a disease but a non-specific response that had a “Salutary” effect on its host.¹ Depending upon the cause and duration of response, inflammation can be classified as acute and chronic.²
In India *Sida cordifolia* L. (Family: Malvaceae) is popularly known as “Country mallow” and “Bala” found along roadsides throughout the tropical and subtropical plains of India and Sri Lanka. It is reported to possess analgesic, anti-inflammatory, anticancer, diuretic, laxative, hypoglycemic and hepatoprotective activities. Further, studies showed that aqueous fraction of hydroalcoholic extract of leaves induces vasorelaxation, hypotension and bradycardia. Furthermore, it is reported to possess antioxidant property. The leaves of *sida cordifolia* were used as antioxidant in myocardial injury.

Several indigenous drugs have been described in ayurveda for the management of inflammatory disease. The extracts of different parts of the plants such as root, stem, bark, flower and seeds have found medicinal properties, and relatively these properties are less in seeds but then some example where seed oil are effective medicine such as *Hydrocarpus anthelmintica* is effective against leprosy, *Colchicum luteum* as anti-rheumatic, *Chemopodium ambiosioids* is used as anthelmintic. There is no report revealed on anti-inflammatory studies of the seed oil of *Sida cordifolia*. In the present study anti-inflammatory activity of seed oil if *sida cordifolia* Linn have been investigated against carrageenan induced paw edema in albino rats with refers to standard drug Diclofenac sodium.

**Methods**

**Plant materials and chemicals**

Seeds of *Sida cordifolia* were collected from Chennai and identified by Botanist, Department of Botany, RLS College, Belgaum, Karnataka, India.

Albino wistar rats (150-200 gm) and albino mice(20-30gm) was purchased from Sri Venkateshwara Traders; enterprises, No.4304, 13th main, 1st cross subramanya nagar, Bangalore; 560021. Regestration No. CPC237 used for the study. The experimental protocol was approved by the institutional animal ethics committee (IAEC) and care of laboratory animals was taken as per CPCSEA guidelines (Reg. No. 0436).The animal were housed in polypropylene cages in the adequately ventilated room. The rats were fed standard feed and water ad libitum throughout the course of the study. Lightning was natural sequence being 12 hours light and 12 hours dark.

**Preparation of petroleum ether extract**

The collected seeds were air dried under the shade in laboratory for 7-12 days. After complete drying, powdered and extracted thoroughly with light petroleum ether (40° -60° c) in a soxhlet extractor for 24-48 hours. The petroleum ether extracts were dried over anhydrous sodium sulphate and solvents was removed in vacuum at 40° c by using rotary evaporator (rotavapour bucchi, Switzerland) to recover oil. The seed oil was filtered through whattman filter paper No. 1 to remove any foreign particles and store properly.
Acute toxicity studies

The albino mice were divided into three groups of six each. The test seed oil of *Sida cordifolia* was administered orally to different groups in increasing dose levels of 50 mg/kg body weight and continued up to 2000 mg/kg and observed continuously for 1 hour and then frequently for 24 hours and thereafter daily for 14 days. During this period the animals were observed for gross behavioral morphological profiles.\textsuperscript{13,14}

Anti-inflammatory studies

Carrageenan-induced hind paw edema model was used for the assessment of anti-inflammatory effect.\textsuperscript{15} Rats of either sex ranging from 150–200 gm were randomly selected and fasted for 18 hours before the experiment, but had free access to water. The animals were divided into three groups of 6 rats each. Standard drug Diclofenac sodium 40 mg/kg body weight, seed extract 200 mg/kg body weight and control saline 0.2 ml and dose were given prior to 60 min. Then acute inflammation was induced by injecting carrageenan (0.1 ml of 1\% suspension in 0.9\% saline) in sub-plantar region of the left hind paw of the rat and the paw volume was measured at 0, 30, 60, 180, & 300 mins with the help of Plethysmometer (UGO Basile, Italy). The difference between one reading and one of the subsequent readings provides the actual edema volume at that time. The mean paw volume at different time was calculated and compared with the control and standard. The percentage inhibition was calculated by using the formula.\textsuperscript{16}

\begin{equation}
\text{Percentage inhibition} = \frac{\text{Final Paw Volume (ml)} - \text{Initial Paw Volume (ml)}}{\text{Initial Paw Volume (ml)}}
\end{equation}

Statistical analysis

The data were calculated per group as Mean ± SEM. The significance was calculated using ANOVA followed by Dunette test. The minimum level of significance was set at $p < 0.05$.

Results

The acute toxicity study of the seed oil of *Sida cordifolia* up to a dose level of 2000 mg/Kg body weight showed no mortality. The petroleum ether extract of seeds of *sida cordifolia* were evaluated for anti-inflammatory activity activity. As illustrated in Table 1, The Petroleum ether extract of *Sida cordifolia* displayed significant anti-inflammatory activity at a dose of 400 mg/Kg body weight. The inflammation was reduced to 1.10 ($P < 0.05$) at 30 minutes from 0.350 at 0 minutes, 1.00 ($P < 0.01$) at 60 minutes, 0.650 ($P < 0.01$) at 180 minutes and 0.375 ($P < 0.01$) at 300 minutes, against carrageenan induced rat paw edema. After comparing with control group the value of seed extract was significant. While Diclofenac sodium as reference drug showed inhibition by 52.6\% inhibition at 0.68 ($P < 0.01$) at 30 minutes, 0.55 ($P < 0.01$) at 60 minutes, 0.35 ($P < 0.01$) at 90 minutes and 0.30 ($P < 0.01$) at 180 minutes. The study therefore revealed that the seed oil of Sida cordifolia is anti-inflammatory and more detailed study is needed to conclusively show its therapeutic potential has anti-inflammatory agent.
Table 1: Anti-inflammatory activity in carrageenan induced paw edema.

<table>
<thead>
<tr>
<th>S.N</th>
<th>Group</th>
<th>Dose (Mg/Kg)</th>
<th>Difference in Paw Edema Volume (Mean ± SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>0 min</td>
</tr>
<tr>
<td>01</td>
<td>Control</td>
<td>Saline</td>
<td>0.38 ±0.05</td>
</tr>
<tr>
<td>02</td>
<td>Standard</td>
<td>40mg/kg</td>
<td>0.35 ±0.03</td>
</tr>
<tr>
<td>03</td>
<td>Test Extract</td>
<td>400mg/kg</td>
<td>0.35 ±0.03</td>
</tr>
</tbody>
</table>

Note:  P < 0.05 – Slightly significant *;  P < 0.01 – Significant **;  P < 0.001 – Highly significant *** (One way followed by Dunettes test).

Discussion

The petroleum ether extract of seeds of *Sida Cordiofolio* were studied for acute toxicity test to establish effective dose. These toxicity tests were performed in albino mice, test substances administrated orally in the form of suspension by triturating with water and 0.5% gum acacia CMC.

Acute inflammatory response, characterized by erythema, edema and pain, is a complex phenomenon involving various chemical mediators such as histamine, 5-HT, various chemotactic factors, bradykinin, leukotrienes, and prostaglandins. The calcium is responsible for the release of these mediators. The calcium is also involve in the action of these mediators on vascular endothelium to induce vasodilation and enhance permeability, resulting in characteristic feature of inflammation. Many mediator appears to be involve in the carrageenan induced inflammation including histamine, 5-HT, kinins and prostaglandins.

The early phase (1-2 hours) of the carrageenan model in mainly by Histamine, serotonin, and increased synthesis of prostaglandins in the damaged tissue surrounding. The late phase is sustained mediated by prostaglandin release and mediated by bradykinin, leukotrienes.

In the present study, we observed that the seed extract of *sida cordifolio* significantly reduced carrageenan induced paw edema at 0, 30, 60, 180, 300 minutes. The observation suggested that the test compound may act in both early and late phase of carrageenan induced acute inflammation.

Conclusion

In present study the seed extract of *sida cordifolia* was used for pharmacological screening. Acute toxicity study was carried out according to OECD guidelines in albino mice (20- 30 gm). The acute toxicity study of petroleum ether extract showed the signs of toxicity like tremors and convulsion at 2000 mg/kg body weight. Therefore dose of petroleum ether extract is taken 400 mg/kg body weight as therapeutic dose. The pharmacological screening of *sida cordifolia* seed oil shown significant anti-inflammatory, activity in albino rats.
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References