Anti-inflammatory activity of *Jatropha gossypifolia* L. leaves in albino mice and Wistar rat

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Methanolic extract of *Jatropha gossypifolia* L. leaves showed systemic and significant anti-inflammatory activity in acute carrageenan-induced rat paw edema and chronic cotton pellet induced granuloma formation after oral treatment for 7 days in Wistar rats. Anti-inflammatory activity might be due to effects on several mediators and arachidonic granular tissue formation and leukocyte migration from vessels.

Keywords: Albino mice and rats, *Jatropha gossypifolia*, Leaves, Local and systemic anti-inflammatory activity

Introduction

*Jatropha gossypifolia* L. (Family: *Euphorbiaceae*), originated from South America, is used in folk medicine. It is locally known as *Lal bherenda*, red physic nut or bellyache bush, and grows wildly almost throughout India. It is a medium sized shrub with palmately 3-5 lobed leaves. Leaf margins, petiole and stem are covered with glandular hairs. It is used in indigenous systems of medicine for the treatment of various ailments1-4 [Carbuncles, bathing wounds, blood purifier, diarrhoea, gum infections, stomach ache, eczema, itches, fever, inflammation, skin disease (leprosy) ulcer and venereal diseases]. Based on ethnobotanical practice, leaf extract has been investigated for anti-inflammatory activity5. Root extract of *J. curcas* has also been investigated6. Aerial part was studied for anti-inflammatory activity by carrageenan induced hind paw edema. Proposed mechanism of action was through an inhibition of enzyme prostaglandin synthetase, specifically endoperoxidase7. This study presents evaluation of local and systemic anti-inflammatory activity using suitable animal models.

Experimental Section

Plant Material

Leaves of *J. gossypifolia*, collected from Pune district, Maharashtra, India, were thoroughly washed with distilled water to remove dirt and soil, and shade dried. Routine pharmacognostic studies were carried out to confirm identity of material8. Specimen of collected material was deposited in AHMA at Agharkar Herbarium of Maharashtra Association at Agharkar Research Institute (ARI), Pune, India. Leaves were coarsely powdered and subjected to successive cold solvent extraction using petroleum ether (60-80°C) and methanol. Extracts which concentrated at reduced temperature and pressure in a rotary evaporator to give following yields: pet-ether, 1.4; and methanol, 4.23%.

Leaf powder was used for topical application in paste form in acetone and its extracts after concentration were suspended in 1% carboxy methyl cellulose (CMC) in water for oral administration. TPA (12*-O*-tetradecanoylphorbol-13-acetate) was purchased from Sigma Chemical CO., St. Louis, MO. Carrageenan sodium was procured from S.D. Fine Chemical Ltd., Mumbai, India. Indomethacin was obtained from Fluka, Switzerland. All SQ grade solvents from Qualigens were used.

Experimental Animals

Swiss albino mice and Wister rat of either sex, obtained from National Institute of Virology, Pune, India, were housed in polypyrene cages at 25 ± 2°C with 10 h per14 h light/dark cycle, and were given Amrut brand balanced animal feed and water ad libitum. Optimum conditions for experiments were decided on the basis of pilot experiments carried out using three
animals per group. For further experiments, a group of at least six animals was used for individual treatment.

**TPA-induced Mouse Ear Edema**

An edema was induced on right ear by topical application of TPA (2.5 µg) in acetone (20 µg). Left ear (control) received vehicle (acetone or distilled water). Leaf powder paste in acetone (0.5 & 1 mg/ear) and indomethacin (0.5 mg/ear) was applied to right ear simultaneously with TPA. Thickness of ears was measured before and at 4th hour after induction of inflammation using a micrometer. Edema was measured as an increase in ear thickness due to TPA application (Table 1).

**Systemic Anti-Inflammatory Activity**

There was no mortality up to 2000 mg/kg oral dose of pet-ether and methanol extract. In pilot studies, only methanol extract showed activity against carrageenan-induced rat paw edema (CRPE) at 1000 mg/kg orally. Therefore, for further acute and sub-acute systemic studies, methanol extract of leaves was used by oral route up to maximum dose of 500 and 250 mg/kg, respectively.

**Carrageenan-induced Rat Paw Edema (CRPE)**

Extract of *J. gossypifolia* leaves (250 & 500 mg/kg) or indomethacin (10 mg/kg) was administered orally to different groups of rats (Table 2). Acute inflammation was induced 1/2 h after above treatment by subplantar injection of 0.1 ml freshly prepared 1% suspension of carrageenan in right hind paw in rats. Paw volume was measured initially and then at 1, 2, 3 and 4 h after carrageenan injection by using plethysmographic method.

**Cotton Pellet-induced Granuloma**

Rats were divided into three groups (n=6). After shaving fur, rats were anesthetized with ether and 10 mg of sterile cotton pellets were surgically inserted in groin region. Extracts (50 & 100 mg/kg p.o.) or indomethacin (5 mg/kg, p.o.) or vehicle was administered for 7 consecutive days from the day of cotton pellet implantation. Animals were anesthetized on 8th day and cotton pellets were removed surgically and made free from extraneous tissues. Pellets were dried at 60°C for 24 h to determine dry weight. Increment in dry weight of pellets was taken as measure of granuloma formation.

**Results**

**TPA-induced Mouse Ear Edema**

*J. gossypifolia* leaf paste (0.5 mg & 1 mg/ear) showed significant reduction in TPA-induced local inflammatory changes in mouse ear edema model. Anti-inflammatory activity of indomethacin application group was also significant (Table 1).

**Carrageenan-induced Rat Paw Edema (CRPE)**

There were dose-dependant significant reductions in CRPE at 500 and 250 mg/kg of extract and at 10 mg/kg indomethacin over a period of 4 h (Table 2).

**Cotton Pellet Implantation**

Extract treatment at 50 mg and 100 mg/kg showed significant reduction in weight of cotton pellet-induced granuloma in albino rats, so also for indomethacin treatment at 5 mg/kg for 7 days. weights of pellets (mean ±SEM mg) were as follows: control, 33.25 ± 3.20; extract (50 mg/kg, 26.9 ± 1.12; 100 mg/kg, 26.1 ± 1.46); and indomethacon (5 mg/kg), 25.7 ± 1.34.

**Discussion**

Cutaneous inflammation is produced and maintained by interaction of various inflammatory cell populations that migrate to inflammation site in response to release of soluble pro-inflammatory mediators (cytokines, prostaglandins, and leukotriene). Tumor promoting phorbol...
esters, such as TPA, when applied topically to mouse skin cause inflammation and hyperplasia. Major cellular phorbol ester receptor is a calcium and phospholipid dependent protein kinase C (PK-C), which is directly activated by TPA and most of the responses of cells to TPA appear to be mediated by PK-C, suggesting that PK-C may play a key role as a mediator of inflammation and growth in TPA-treated mouse skin\[14\]. Topical application of \textit{J. gossypifolia} leaf powder in paste form is ethnobotanical practice for treatment of inflammation. In this study, leaf paste showed significant anti-inflammatory activity against TPA-induced phlogistic response. This phorbol ester provides a skin inflammation model suitable for evaluation of topical and systemic anti-inflammatory agents. Carrageenan-induced paw inflammation has been accepted as a useful phlogistic tool for investigating systemic anti-inflammatory agent. Acute inflammation is produced when water and plasma increases in tissues during arachidonic acid metabolism via cyclo-oxygenase and lipoxygenase enzyme pathways\[15\]. It has two phases: first phase (begins immediately after injection and lasts for 1 h) is characterized by release of histamine and serotonin; and second phase (begins after 1 h and lasts 3 h) is characterized by bradykinin release via prostaglandins mediator pathways\[16\]. Extract showed dose-dependent inhibitory activity in CRPE inflammation over a period of 4 h. This indicates action against release of histamine, serotonin and kinins in early phase, while later phases are suspected to be arachidonate metabolites producing an edema dependent on mobilization of neutrophils\[17,18\]. Moreover, TPA-induced local inflammation and carrageenan-induced paw edema are more effectively controlled with arachidonate cyclo-oxygenase inhibitors than arachidonate lipo-oxygenase inhibitors\[19-22\]. In order to assess its efficacy against proliferative phase of inflammation, in which tissue degeneration and fibrosis occur, cotton pellet granuloma test was employed. During repair process of inflammation, there is proliferation of macrophages, neutrophils, fibroblasts and multiplication of small blood vessels, which are basic sources of forming a highly vascularised reddish mass, termed granulation tissue\[23,24\]. In case of cotton pellet-induced granuloma, there was significant reduction in granular tissue formation. This result is in confirmation with anti-proliferative activity of extract. Thus, in the light of results of TPA-induced phlogistic model, CRPE and cotton pellet induced granuloma \textit{J. gossypifolia} leaf powder for local and systemic application in inflammatory conditions can be explained.

Acknowledgement

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References

5. \textit{The Useful Plants of India} (NISCAIR, CSIR, New Delhi) 1992, 302-303.

Table 2— Acute anti-inflammatory activity of \textit{Jatropha gossypifolia} leaf methanol extract in carragennan-induced rat paw edema

<table>
<thead>
<tr>
<th>Treatment</th>
<th>1 h</th>
<th>2 h</th>
<th>3 h</th>
<th>4 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>36.67 ± 4.9</td>
<td>52.42 ± 6.5</td>
<td>61.78 ± 5.2</td>
<td>70.13 ± 3.9</td>
</tr>
<tr>
<td>Extract 250 mg/kg</td>
<td>24.24 ± 5.9</td>
<td>47.80 ± 8.4</td>
<td>57.83 ± 10.2</td>
<td>67.6 ± 10.6</td>
</tr>
<tr>
<td>Extract 500 mg/kg</td>
<td>18.08 ± 2.4*</td>
<td>35.024 ± 2.8***</td>
<td>43.18 ± 3.0*</td>
<td>49.19 ± 3.1**</td>
</tr>
<tr>
<td>Extract 1000 mg/kg</td>
<td>19.89 ± 2.2*</td>
<td>40.69 ± 3.5</td>
<td>41.26 ± 2.4***</td>
<td>46.19 ± 3.7**</td>
</tr>
<tr>
<td>Indomethacin 10 mg/kg</td>
<td>7.36 ±2.7**</td>
<td>12.26± 3.7</td>
<td>20.36±3.7**</td>
<td>28.32 ± 4.5**</td>
</tr>
</tbody>
</table>

Data represent mean ± SEM (n=6)

*Significant as compared to control P< 0.01; ** P< 0.001; ***P<0.05