Anti-inflammatory activity of methanolic extracts of *Dillenia indica* L. leaves

Yeshwante SB, Juvekar AR, Nagmoti DM, Wankhede SS, Shah AS, Pimprikar RB, Saindane DS

*University Institute of Chemical Technology, Matunga, Mumbai 400 019, India*

**Address for correspondence:** Dr. S.B. Yeshwante; E mail: shekhar.udct@gmail.com

**ABSTRACT**

The anti-inflammatory activities of the methanol extract of *Dillenia indica* Linnaeus (Family Dilleniaceae) leaves were observed in various experimental models related to inflammation to provide some evidence for its traditional use. Anti-inflammatory activity was observed in carrageenan-induced edema and acetic acid-induced capillary permeability. The methanol extract showed significant (*P*<0.01) anti-inflammatory activity in the paw edema test and acetic acid-induced capillary permeability at 200 mg/kg and 400 mg/kg. The extract at 100 mg/kg showed significant (*P*<0.05) activity in acid-induced permeability. These findings support the folkloric use of *Dillenia indica* in diseases related to inflammatory conditions.

**Key words:** Anti-inflammatory, *Dillenia indica*, Dilleniaceae, leaves, methanolic extract

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**INTRODUCTION**

*Dillenia indica* L. is a common evergreen tree that grows widely in tropical forests in the western peninsula, Bihar, Sub Himalayan tracts, Assam, Bengal, and central and southern India from Sylhet to Sri Lanka. It has been grown in gardens for its handsome foliage and attractive flower as an ornamental plant. The plant is locally known as Karambel or Karmal in Marathi, Chalta in Hindi, and Ramphal in Nepal. The leaves, bark, and fruit of the plant are used in the indigenous system of medicine. It relieves abdominal pain and regulates the heat in the body. The fruit juice is mixed with sugar and water and used as a cooling beverage in the treatment of fever. It also tones up the nervous system and removes fatigue. The fruit juice is used as a cardiotonic. The leaves and bark are used as a laxative and astringent. Bruised bark is applied as a cataplasm for patients with arthritis. Phytchemical studies showed the presence of the lupeol group of triperpene such as betulinic acid and betulin and flavonol such as myricetin. Flavonoids such as Kaempferol, Quercetin, Isorhamnetin, Naringenin, and phenolic materials are also present. Pharmacological activity was evaluated and showed antioxidant activity in the fruit. The alcoholic extract of the *Dillenia indica* leaves is reported to possess central nervous system (CNS) depressant activity. As mentioned above, almost all traditional uses of the plant are concerned with anti-inflammatory (arthritis, cough, fever) activity. In order to prove the traditional utilization of *Dillenia indica*, this paper was intended to investigate the effect of *Dillenia indica* methanolic extract on inflammation using different animal models.
MATERIALS AND METHODS

Plant material

Leaves of *Dillenia indica* L. were collected in September 2007 from Veermata Jijabai Bhosle Udyan, Byculla, Mumbai, India. *Dillenia indica* (L.) Linnus. (Dilleniaceae) as authenticated by Dr. J.S. Yadav, Head of the Department of Botany, Pratap College, Amalner District Dhule.

Preparation of plant extract

Leaves were dried at room temperature under shade and powdered using a mixer grinder. The powder was continuously extracted using Soxhlet extractor with petroleum ether to remove oils, fats, and chlorophyll present. The powder was then extracted with methanol using Soxhlet extractor for 24 hrs at 40-50ºC. After complete extraction, methanolic extracts of *Dillenia indica* (DIM) was concentrated under a vacuum to obtain a thick extract that was then dried in a hot air oven to get a free flowing powder. The yield of the extract was found to be 17% w/w.

Animals

For the study, rats as well as mice were used. Male and female Swiss albino mice weighing 20±5 g and Wistar rats weighing 180±20 g were used. The animals were housed under 12 hr light/dark cycles in a temperature controlled room with free access to feed and water. The experimental protocol was approved by the Institutional Animal Ethics Committee under the Organisation for Economic Co-operation and Development (OECD) guidelines for use of animals.

Drug administration

After being dissolved in distilled water, all test samples (including reference drugs) were given orally to the test animals. The animals in the control group received the same experimental handling as the animals in the test groups except that the drug treatment was replaced with appropriate volumes of the dosing vehicle.

Statistical analysis

Results are presented as mean ± standard deviation (SD). Data were subjected to analysis of variance followed by Dunnett’s test. P<0.05 was considered to be statistically significant.

Carrageenan-induced rat paw edema

This method was previously described by Yin[9] and was used with some modifications. Wistar rats were divided into 5 groups (6 animals/group). The vehicle control group received distilled water. The positive control group received Indomethacin at 20 mg/kg p.o and three treatment groups received methanolic extracts of *Dillenia indica* (DIM) at 100 mg/kg, 200 mg/kg, and 400 mg/kg. The rats were injected subcutaneously with 0.1 ml of 1% carrageenan solution in normal saline (0.9% w/v NaCl) into the sub-plantar region of the left hind paw. Paw volume was measured before and 1, 2, 3, 4, and 5 hrs after injection of carrageenan using a plethysmometer. All the test substances and reference drugs were administered 60 min before injection of carrageenan. The percent increase in paw volume was calculated and compared with the vehicle control.

Acetic acid induced capillary permeability in mice

The method used by Whittle[10] was used in this study with some modifications to evaluate the effect of the extract on vascular permeability in adult albino mice of both genders. One hour after oral administration of the methanolic extract (100, 200, or 400 mg/kg); 0.1 ml of Evans Blue dye (4% in Normal saline) was intravenously administered through the tail vein. Animals in the positive control group received indomethacin (20 mg/kg) and equivalent amounts of vehicle were given to the animals in the vehicle control group. Thirty minutes later, animals received an intraperitoneal injection of 0.4 ml of acetic acid (0.5%, v/v). Treated animals were sacrificed 30 min after the injection of acetic acid and the peritoneal cavity was washed with normal saline (5 ml) into heparinized tubes and centrifuged. The dye content in the supernatant was measured at 590 nm using a spectrophotometer and the concentration of dye leaked into the peritoneal fluid was calculated using a standard curve of Evans blue dye. The percentage of inhibition was also calculated.[11]

RESULTS

Carrageenan induced paw edema

Results of anti-inflammatory activity of extract in carrageenan-induced edema are shown in Table 1. Doses of 200 and 400 mg/kg of the extract significantly inhibited the percent increase in reaction time. The inhibition was observed during the third hour after drug administration.

Acetic acid induced vascular permeability

Table 2 represents the results for the acetic acid-
Methanolic extracts of *Dillenia indica*

Table 1: Anti-inflammatory activity of *Dillenia indica* L. leaves by carrageenan-induced rat paw edema

<table>
<thead>
<tr>
<th>Groups</th>
<th>Conc. of dye (mcg/ml)</th>
<th>% inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle control</td>
<td>10.02±2.532</td>
<td>—</td>
</tr>
<tr>
<td>Indomethacin (20 mg/kg p.o.)</td>
<td>2.84±1.327**</td>
<td>71.16</td>
</tr>
<tr>
<td>100 mg/kg DIM p.o.</td>
<td>7.23±1.035*</td>
<td>22.16</td>
</tr>
<tr>
<td>200 mg/kg DIM p.o.</td>
<td>4.33±1.105**</td>
<td>52.32</td>
</tr>
<tr>
<td>400 mg/kg DIM p.o.</td>
<td>3.42±0.647**</td>
<td>61.84</td>
</tr>
</tbody>
</table>

no- 6, are expressed as mean ± SEM, * P<0.05, ** P<0.01

Table 2: Anti-inflammatory activity of *Dillenia indica* L. by acetic acid induced capillary permeability

<table>
<thead>
<tr>
<th>Groups</th>
<th>Conc. of dye (mcg/ml)</th>
<th>% inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle control</td>
<td>79.6±6.09</td>
<td>91.86±3.27</td>
</tr>
<tr>
<td>Indomethacin (20 mg/kg p.o.)</td>
<td>19.19±1.849**</td>
<td>52.32</td>
</tr>
<tr>
<td>100 mg/kg DIM p.o.</td>
<td>78.58±8.75</td>
<td>71.64±6.32</td>
</tr>
<tr>
<td>200 mg/kg DIM p.o.</td>
<td>71.47±3.34</td>
<td>79.02±8.92**</td>
</tr>
<tr>
<td>400 mg/kg DIM p.o.</td>
<td>71.94±6.85</td>
<td>51.09±6.32**</td>
</tr>
</tbody>
</table>

no- 6, are expressed as mean ± SEM, * P<0.05, ** P<0.01

induced vascular permeability model for evaluation of anti-inflammatory activity. All the doses of extract and indomethacin at a dose of 20 mg/kg (71.16%) showed a significant decrease in dye leaking in the peritoneal fluid. The effect of Indomethacin was strongest followed by 400 mg/kg (61.84%) and 200 mg/kg (52.32%).

**DISCUSSION**

**Carrageenan induced paw edema**

Carrageenan-induced paw edema as an in vivo model of inflammation has been frequently used to assess the anti-edematous effect of natural products. Carrageenan-induced paw edema is a useful model in assessing the contribution of mediators involved in vascular changes associated with acute inflammation. Edema formation in the carrageenan-induced paw edema model is a biphasic response. In the early hyperemia, 0-2 hrs after carrageenan injection, there is a release of histamine, serotonin, and bradykinin on vascular permeability. The inflammatory edema reached its maximum level at the third hour and after that it started declining. The late phase of the inflammatory response has been shown to be due to the potentiating effect of bradykinin on mediator release and prostaglandins, producing edema after mobilization of the leukocytes. The second phase is more sensitive to clinically used anti-inflammatory agents. Nitrous oxide (NO) is a potent vasodilator and is also involved in carrageenan-induced edema, which may be related to its ability to increase vascular permeability and edema through changes in local blood flow. Carrageenan induces inflammation by enhancing PGE2 release and leukocyte migration. It also enhances the expression of COX-2 in the epidermis, skeletal muscles, and inflammatory cells in air-pouch models, suggesting that prostaglandin E2 production is linked through the expression of COX-2. The DIM extract at 200 and 400 mg/kg p.o. shows inhibition after the third hour indicating an effect on the inhibition of prostaglandin release or biosynthesis. While Indomethacin shows significant activity from the first hour indicating an effect on both phases of inflammation.

**Acetic acid-induced capillary permeability**

The inflammatory response is a physiological characteristic of vascularized tissues. Histamine (released from mast cells and basophile), serotonin, and to some extent bradykinin is responsible for increased capillary permeability. It is an important step and because of this there is migration of neutrophiles and other immune cells, such as macrophages and leukocytes, to the inflamed area. Increased vascular permeability is seen in the inflammatory reaction that leads to exudation of the fluid. Exudation, which is a consequence of increased vascular permeability, is considered to be a major feature of acute inflammation. Increased vascular permeability occurs as a result of contraction and separation of endothelial cells at their boundaries to expose the basement membrane, which is freely permeable to plasma proteins and fluid. Histamine and other mediators of inflammation increase vascular permeability at various times after injury. Chemical-induced vascular permeability (such as is seen with acetic acid) causes an immediate sustained reaction that is prolonged over 24 hours and its inhibition suggests that the extract may effectively suppress the exudative phase of acute inflammation.

**CONCLUSION**

The methanolic extract of *Dillenia indica* L. (Dilleniaceae) at 200 and 400 mg/kg shows a significant anti-inflammatory activity and the possible mechanism might be inhibition of mediator release and PG biosynthesis.

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REFERENCES


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