Anti-inflammatory Activity of the Aqueous Leaf Extract of *Ixora coccinea*  

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**Abstract**

The aim of this study was to investigate the anti-inflammatory potential of an aqueous leaf extract (ALE) of *Ixora coccinea* (Rubiaceae) in rats after oral administration (500, 1000 and 1500 mg/kg). This was done using the carrageenan-induced paw edema (acute inflammatory model) and cotton pellet granuloma tests (chronic inflammatory model). In the former test, ALE significantly impaired both early and late phases of the inflammatory response and also the edema maintained between the two phases. In the latter test, it significantly suppressed granuloma formation (only highest dose tested). Collectively, these data show promising anti-inflammatory activity against both acute and chronic inflammation. ALE showed strong antihistamine and antioxidant activities that can account for its anti-inflammatory potential. In addition, inhibition of prostaglandins and bradykinins may play a role.

**Keywords:** Antihistamine, anti-inflammatory activity, antioxidant, *Ixora coccinea*.

**Introduction**

The search for new pharmacologically active agents obtained by screening natural sources such as microbial fermentations and plant extracts has led to the discovery of many clinically useful drugs that play a major role in the treatment of human diseases. The ethnomedicinal uses as well as certain biological activities exhibited by *Ixora coccinea* indicate it to be a rich source of phytomedicine, *Ixora coccinea* Linn. (Rubiaceae) (Sinhala: ratmal; Tamil: vedchi) is a shrub (Jayaweera, 1982) with small, obovate to oval-oblong, rounded to subcordate base leaves on branched hard heavy twigs (Jayaweera, 1982). It is very common everywhere in the low country of Sri Lanka. The wide distribution in Sri Lanka has led to the extensive use of this species in the traditional system of medicine. A decoction of the roots is given for dysentery and as a sedative for hiccoughs, nausea, loss of appetite, fever, and gonorrhea. The flowers and bark are used on reddened eyes and eruptions in children (Jayaweera, 1982). Further, a decoction of the flowers is given for hemoptysis, catarrhal bronchitis, and dysmenorrhea. The leaves of *I. coccinea* are used in the treatment of dermatological disorders in the traditional system of medicine in Sri Lanka (Jayaweera, 1982). A preliminary report (Reena et al., 1993) on the anti-inflammatory effect of ethanol extract of the leaves of *I. coccinea* prompted us to study this effect in detail. We report here our investigation of the anti-inflammatory activity using the rat carrageenan-induced paw edema technique (acute inflammatory model) and cotton pellet test (chronic inflammatory model).

**Materials and Methods**

**Collection of the herb and preparation of aqueous leaf extract (ALE)**

Fresh leaves of *I. coccinea* were collected from Keleniya and Mirigama in the Gampaha district of Sri Lanka in August 2001 and were identified and authenticated by Professor B.A. Abeywickrama of the Botany Department of the University of Colombo. A voucher specimen (wdr/sad 1003) was deposited at the museum of the Department of Zoology. The leaves were washed under running water, air-dried, and cut into small pieces. The pieces (234 g) were macerated with water and were then...
refluxed with 3 l of water for 2 days in a round-bottom flask fitted to a Leibig condenser. The brownish red solution was filtered and freeze-dried (12 g, yield 4.3%) and stored airtight at room temperature (30–32°C). The freeze-dried powder was dissolved in distilled water (DW) to obtain the required dosages in 1 ml solution (500, 750, 1000, or 1500 mg/kg).

Experimental animals

Healthy adult cross-breed albino male rats (200–250 g) were used in study. The animals were kept in plastic cages (six per cage) under standardized animal house conditions (temperature, 28–31°C; photoperiod, approximately 12 h natural light per day; relative humidity, 50–55%) with continuous access to pelleted food (Master Feed Ltd., Colombo, Sri Lanka) and tap water. Except at the time of experimental procedures, the animals were handled only during cage cleaning. All efforts were made to minimize animal suffering and to reduce the number of animals used.

Anti-inflammatory activity

Carrageenan-induced paw edema

Forty-four male rats were selected and randomly divided into five groups. The rats in groups 1, 2, and 3 were orally treated with 500, 1000, and 1500 mg/kg ALE, respectively. The rats of group 4, which served as the control, were treated with 1 ml of DW. The rats in the fifth group were treated with 5 mg/kg indomethacin (Dharmasiri et al., 2002) (State Pharmaceutical Corporation, Colombo, Sri Lanka), the reference drug (Laurence & Bennett, 1992). After 1 h, 0.05 ml of 1% carrageenan (Sigma Chemical Company, St. Louis, MO, USA) suspension was injected subcutaneously into the planter surface of the left hindpaw as described by Winter et al. (1962). The volume of the injected paw of animals used.

Cotton pellet granuloma

Twelve rats were randomly assigned into two groups (n = 6/group). Autoclaved cotton pellets (10 mg) were implanted subcutaneously, one on each side above the scapula region, under ether anesthesia using aseptic precautions (Dhawn & Srimal, 2000). Either 1500 mg/kg of ALE or 1 ml DW was administered orally for 7 consecutive days starting from the day of injection. On day 8, the animals were killed and the pellets along with granuloma removed and dried in an oven at 60°C until a constant weight was obtained.

Antihistamine effect

Fur on left lateral side of the back of 18 rats was shaved. Twenty-four hours later, these rats were randomly assigned into three equal groups. Group 1 was treated with 1500 mg/kg of ALE orally. The other groups were treated, respectively, with 0.67 mg/kg of chlorpheniramine and 1 ml of water (Dharmasiri et al., 2002). After 1 h, these rats were subcutaneously injected with 50 μl of 200 μg/ml histamine dihydrochloride (Fluka, Buchs, Switzerland) into the skin where the fur had been shaved, and 2 min later the area of the wheal formed was measured (Spector, 1956).

Evaluation of antioxidant activity

Antioxidant activity was assessed using thiobarbituric acid reactive substances assay as described by Dorman et al. (1995). Vials containing the reagents were treated in triplicate with the ALE so that the final concentrations of the extract in the various vials was 0.25, 2.5, 12.5, 18.8, 25, 50, or 125 μg/ml. Butylated hydroxy toluene (BHT) (100 μg/ml) was used as the positive reference and DW was used as the control. The vials were mixed well and incubated at 95°C for 60 min and then allowed to cool. Butanol (5 ml) was added, mixed well, and centrifuged at 1500 × g for 5 min. The absorbance of the butanol layer was measured at 532 nm, and the antioxidant index was calculated as follows: Antioxidant index = (1 – T/C) × 100 (where T = absorbance of test and C = absorbance of control).

Evaluation of diuretic activity

This was done as described previously by us (Ediriweera & Ratnasooriya, 2002). Briefly, 12 rats were deprived of water but not food for 18 h. Their urinary bladders were emptied by gentle compression of the pelvic area and by pull of their tails. Each of these rats was then orally treated with 15 ml of isotonic saline (NaCl, 0.9% w/v) to impose a uniform water load. After 45 min, these rats were randomly assigned into two groups (n = 6/group) and treated orally in the following manner. Group 1, 2 ml of DW; group 2, 1500 mg/kg of ALE. Each of these rats was individually placed in metabolic cages and cumulative urine output was determined at hourly intervals for 5 h.

Phytochemical analysis

Phytochemical screening of the aqueous extract was carried out. The ALE was subject to column chromatography (30 cm length and 3.7 cm diameter) on reverse-phase C-18 silica gel (Fluka Chemie G, Buchs, Switzerland). The column was eluted with water, mixtures of methanol and water, methanol, mixtures of methanol and ethyl acetate, ethyl acetate, mixtures of ethyl acetate and dichloromethane, dichloromethane, mixtures of dichloromethane and hexane, and
The ALE caused a significant (p < 0.05) and marked inhibition (by 36.1%) of granuloma weight as compared to control (control vs. treatment: 29.2 ± 9.6 vs. 18.6 ± 7.1 mg).

**Antihistamine effect**

The ALE induced a significant (p < 0.05) and profound impairment (by 42.6%) of the area of wheal formed by the subcutaneous injection of histamine. This antihistamine effect was comparable to that produced by chlorpheniramine.

**Antioxidant activity**

As shown in Table 2, the ALE had promising (compared to BHT control) and dose-dependent (r² = 0.9, p < 0.05) antioxidant activity.

**Diuretic activity**

The ALE did not induce a significant (p > 0.05) increase in cumulative urine output (control vs. treatment 4.9 ± 0.8 vs 4.2 ± 1.1 ml).

**Phytochemical analysis**

Phytochemical screening of the ALE showed the presence of quaternary base alkaloids, flavonoids, tannins/polyphenols, steroids, and/or terpenoids and saponins. Thin-layer chromatography of the methylene chloride, methylene chloride/hexane, and hexane column, fractions showed the presence of phenols (Rf 0.76, 0.12, 0.08), flavonoids (Rf 0.83, 0.7), steroids (Rf 0.09), and triterpenoid glycosides (Rf 0.64, 0.17, 0.73) and a quaternary base (Rf 0.15) on spraying with characteristic reagents.

**Discussion**

This study examined the anti-inflammatory activity of ALE of *Ixora coccinea* in rats using the carrageenan-induced paw edema test (acute inflammatory model) and cotton-pellet test (chronic inflammatory model) after oral

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**Table 1.** Effect of oral administration of *Ixora coccinea* aqueous leaf extract on the carrageenan-induced paw edema in rats (mean ± SEM).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>First hour</th>
<th>Second hour</th>
<th>Third hour</th>
<th>Fourth hour</th>
<th>Fifth hour</th>
</tr>
</thead>
<tbody>
<tr>
<td>500 mg/kg (n = 6)</td>
<td>18.4 ± 2.53*</td>
<td>26.62 ± 5.96*</td>
<td>30.63 ± 6.32**</td>
<td>32.63 ± 6.42**</td>
<td>31.80 ± 6.34**</td>
</tr>
<tr>
<td>1000 mg/kg (n = 10)</td>
<td>15.21 ± 2.60**</td>
<td>27.92 ± 4.94*</td>
<td>35.15 ± 7.67*</td>
<td>31.01 ± 5.93**</td>
<td>30.39 ± 5.14**</td>
</tr>
<tr>
<td>1500 mg/kg (n = 8)</td>
<td>20.63 ± 3.24*</td>
<td>28.12 ± 1.60**</td>
<td>33.38 ± 2.85**</td>
<td>26.50 ± 5.32**</td>
<td>23.50 ± 3.0**</td>
</tr>
<tr>
<td>Control (n = 11)</td>
<td>28.75 ± 3.29</td>
<td>48.83 ± 4.49</td>
<td>57.71 ± 6.85</td>
<td>58.12 ± 4.48</td>
<td>57.46 ± 4.4</td>
</tr>
<tr>
<td>Indomethacin 5 mg/kg (n = 9)</td>
<td>5.23 ± 1.17**</td>
<td>11.16 ± 2.34**</td>
<td>9.09 ± 1.55**</td>
<td>8.69 ± 2.13**</td>
<td>5.58 ± 1.19**</td>
</tr>
</tbody>
</table>

*Values are significant at p ≤ 0.05.
**p < 0.001.

finally with hexane. The fractions with similar thin-layer chromatography (TLC) spots were combined after inspecting under UV light. The combined fractions were subject to TLC (Aldrich silica gel precoated on plastic plates and Fluka Chemie G reverse-phase C-18 precoated glass plates). The mobile phases were 60% dichloromethane in hexane, 10% methanol in dichloromethane for normal phase chromatography, and methanol and 50% methanol in water for reverse-phase chromatography. The TLC plates were sprayed with color reagents specific for various classes of compounds (Stahl, 1965). (AICl₃ test; for flavonoids; diazotized para-nitroaniline: for phenols; para-toluene sulphonic acid: for flavonoids and steroids; SbCl₃ in acetic acid: for flavonoids and steroids; Libermann Burchardt spray; for triterpenoid glycosides; and Dragendorff’s reagent and iodoplatinate reagent: for alkaloids.)

**Statistical analysis**

The data are expressed as the mean ± SEM. Statistical analysis was performed using Mann-Whitney U-test. Significant values were set at p ≤ 0.05. Linear regression analysis was performed to assess dose-dependencies.

**Results**

**Carrageenan-induced paw edema**

The results obtained are summarized in Table 1. As shown, all the doses of ALE tested caused a significant (p < 0.05 to 0.001) and marked reduction in paw edema (28–59%) compared to control at each time point measured. Overall, this anti-inflammatory effect seemed dose-related. Indomethacin also impaired the edema formation, but this anti-inflammatory effect was much stronger (77–90%).

**Cotton pellet granuloma**

The ALE caused a significant (p < 0.05) and marked inhibition (by 36.1%) of granuloma weight as compared to control (control vs. treatment: 29.2 ± 9.6 vs. 18.6 ± 7.1 mg).
administration. The results show that the ALE has promising anti-inflammatory activity both against acute (exudative phase) and chronic (proliferative phase) inflammation.

The carrageenan-induced paw edema test is widely accepted as a sensitive phlogistic tool for investigating potential anti-inflammatory agents, particularly the non-steroidal type (Vineger et al., 1969). In this test, development of edema (inflammatory response) is a biphasic event with a maintenance phase in between (Vineger et al., 1969). In this study, ALE simultaneously inhibited all these phases. The initial phase (1–2 h) is primarily mediated by histamine and serotonin (Vineger et al., 1969), but platelet activating factor and arachidonic acid metabolites also play a role (Boughton-Smith et al., 1993). The ALE had strong antihistamine activity that could impair microvascular leakage induced by carrageenan (Kuriyama et al., 2000) and thereby inhibits the initial phase of the edema test. Histamine stimulates vessel endothelial cells to increase vascular permeability (Kuriyama et al., 2000). Further, the ALE contained triterpenoid glycosides. Many kinds of triterpenoids from angiosperms are known to impair histamine release from mast cells and exert anti-inflammatory effects (Janaki et al., 1999). Such a mode of action is possible in this study as well. The edema maintained between the first and second phase (2–3 h) is due to kinin-like substances, especially bradykinin (Vineger et al., 1969). Curtailment of this maintenance phase indicates that the ALE had inhibited bradykinin release and/or its vascular permeability promoting action. The ability of the ALE of *Ixora coccinea* to inhibit the late phase of the formalin test of nociception (Ratnasooriya et al., 2004) provides support to this notion. However, new research is needed to clarify this point. On the other hand, the delayed phase of the carrageenan test (3–6 h) has been linked to release of prostaglandins, arachidonate metabolites, neutrophil migration, release of oxygen free radicals, proteolytic enzymes, as well as other neutrophil-derived mediators (Boughton-Smith et al., 1993; Bouriche et al., 2003). The ALE contained flavonoids and tannins. Flavonoids (Carlo et al., 1999; Kim et al., 1996) and tannins (Muruganadan & Raviprakash, 2001) impair cyclooxygenase/lipoxygenase activities that would reduce the levels of prostaglandins and other arachidonic acid metabolites. Such a mechanism may account for impairment of the late phase. In addition, the ALE showed marked and dose-dependent antioxidant activity. Carrageenan paw edema is sensitive to antioxidants (Boughton-Smith et al., 1993). Thus, it may be inferred that this antioxidant is one of the mechanisms by which ALE mediates impairment in the late phase of the anti-inflammatory response. The antioxidant activity of the ALE may be attributed to its flavonoids and phenols (Carlo et al., 1999; Kim et al., 1996). On the other hand, the ALE had failed to inhibit heat-induced hemolysis of rat erythrocytes in vitro (Ratnasooriya et al., 2004). This indicates that ALE cannot stabilize lysosomal membranes to inhibit the release of proteolytic enzymes: lysosomes play a major role in the inflammatory reaction (Hess & Millonig, 1972; Thabrew et al., 2003), and there is a close similarity between erythrocyte and lysosomal membrane system (Hess & Millonig, 1972).

In addition to these specific mechanisms, several other nonspecific mechanisms may account for the simultaneous and more or less equal impairment of early and late phases of the carrageenan-induced paw edema induced by ALE. Diuresis is one such mechanism (Barrachina et al., 1995). However, this mode of action is unlikely as ALE failed to increase urinary output. Opioid agonists can induce acute anti-inflammatory actions (Ahmadiani et al., 1998), but this mechanism is also unlikely to be operative, as ALE has no opioid-mimetic activity (Ratnasooriya et al., 2004). Because the primary effect of carrageenan as an inflammatory agent is the activation of phospholipase A2 (PLA2) (Chungag et al., 2003), its inhibition can impair both phases of the inflammatory response in equal intensities as evident in this study. Such an action may result from flavonoids present in the ALE. Flavonoids are powerful inhibitors of PLA2 (Carlo et al., 1999; Kim et al., 1996). Alternatively, such a response may result from ALE-induced release of glucocorticoids (Spector, 1969) and/or having a glucocorticoid-mimetic activity, as it contained steroidal constituents. Further, triterpenoids of the lupane class that have structural similarity to steroidal compounds inhibit cortisol inactivation (Manez et al., 1997). The impairment of granuloma formation of the cotton pellet test provides indirect evidence in favor of this glucocorticoid-related mechanism (Manez et al., 1997).

The cotton pellet test is considered a model for studies on chronic inflammation (Dhawn & Srimal, 2000), and inflammatory granuloma is considered as a typical feature of established chronic inflammatory reaction (Spector, 1969). The fact that the ALE was effective in suppressing granuloma formation in this model indicates that it may be effective in chronic inflammatory conditions. This inhibitory effect was seen when ALE

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**Table 2. In vitro antioxidant activity of aqueous leaf extract of *Ixora coccinea*.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Concentration (mg/ml)</th>
<th>Antioxidant index</th>
</tr>
</thead>
<tbody>
<tr>
<td>Standard (BHT)</td>
<td>0.1</td>
<td>91.20 ± 5.68</td>
</tr>
<tr>
<td>Extract</td>
<td>0.10</td>
<td>10.81 ± 3.65</td>
</tr>
<tr>
<td></td>
<td>0.50</td>
<td>14.12 ± 2.29</td>
</tr>
<tr>
<td></td>
<td>0.75</td>
<td>17.45 ± 3.78</td>
</tr>
<tr>
<td></td>
<td>1.00</td>
<td>42.68 ± 7.43</td>
</tr>
<tr>
<td></td>
<td>2.00</td>
<td>45.47 ± 5.23</td>
</tr>
<tr>
<td></td>
<td>5.00</td>
<td>74.95 ± 8.32</td>
</tr>
</tbody>
</table>

BHT, botylated hydroxyl toluene.
Ixora coccinea and inflammation

was administered after the onset of inflammation, which is claimed to reflect a genuine anti-inflammatory action (Duwiejua et al., 1994) and also shows the potential to be used as curative in different inflammatory conditions.

Our previous study has shown that this ALE does not induce unacceptable side effects including gastric lesions and is well tolerated even after subchronic administration (Ratnasooriya et al., 2004). Further, it has strong antiinflammatory activity (Ratnasooriya et al., 2004). The presence of both inflammatory and antinociceptive activities in a single drug, which is devoid of major side effects, is extremely valuable, as inflammation is generally accompanied with pain (Anonymous, 2000), and currently available both steroidal and nonsteroidal allopathic anti-inflammatory drugs are associated with unacceptable and often severe side effects (Anonymous, 2000).

In conclusion, this study scientifically demonstrates, for the first time, promising anti-inflammatory activity in I. coccinea leaves. This is an important finding, both globally and locally, because inflammation is a common medical condition for which available drug therapies are poor (Anonymous, 2000). About 3.5 to 4 billion people in the world rely on plants as sources of drugs (Farnsworth, 1988), and, in Sri Lanka, 35% of the population is dependent on traditional medicine for their primary health care needs (Mahindapala, 2000). Leaves of I. coccinea are noncommercial and abundantly available throughout the year.

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References


