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Anti-inflammatory Activity of Decoctions of Leaves and Stems of *Anisomeles indica* at Preflowering and Flowering Stages

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Abstract

A decoction of leaves and stems of *Anisomeles indica* (Lamiaceae) is claimed to possess anti-inflammatory activity in Sri Lankan traditional medicine. The plants at both preflowering and flowering stages are used as an anti-inflammatory agent. However, the anti-inflammatory activity of the plant has not been scientifically evaluated thus far. The aims of this study were to evaluate scientifically the anti-inflammatory activity of decoctions of leaves and stems of *A. indica* at preflowering and flowering stages and possible toxic effects of the decoctions. Three doses of the freeze-dried decoction of a preflowering plant (E1) (125, 250 and 500 mg/kg) and one dose of the decoction of a plant at flowering stage (E2) (500 mg/kg) were orally administered to rats. The anti-inflammatory activity was evaluated using the carrageenan-induced paw edema, formaldehyde-induced paw edema and adjuvant-induced paw edema models in rats. E1 demonstrated a significant ($P < 0.01$) and dose-dependent anti-inflammatory effect in all three models, while E2 did not demonstrate significant anti-inflammatory activity. E1 demonstrated a significant ($P < 0.01$) and dose-dependent antihistamine activity and free radical scavenging activities in addition to the previously reported membrane stabilising and cyclooxygenase-I inhibitory activities. However, E1 failed to impair significantly the *in vitro* activity of lipoxygenase. A 30-day treatment with 500 mg/kg of E1 was not liver toxic or renotoxic, and it did not have a significant effect on body weights. It was concluded that the anti-inflammatory activity of E1 is contributed by cyclooxygenase-1 inhibition, plasma membrane stabilisation, antihistamine and free radical scavenging activities, but not by the inhibition of lipoxygenase. These observations prove scientifically the anti-inflammatory activity of *A. indica*, mentioned in the Sri Lankan traditional medicine, while revealing a loss of the activity after flowering.

Keywords: *Anisomeles indica* Kuntze, anti-inflammatory activity, antihistamine activity, free radical scavenging activity, Sri Lanka.

Introduction

Anisomeles indica Kuntze (Lamiaceae), Yakwanassa in Sinhala and Peyameratti in Tamil, is a large perennial herb which grows commonly as a weed in waste places, roadsides, forest clearings and shrub jungles in Sri Lanka, India, Malaya China, and the Philippines (Dassanayake & Fosberge, 1981; Jayaweera 1981). A decoction made of leaves and stems of this plant (both at preflowering and flowering stages) is used in Sri Lankan traditional medicines (Deshiya Chikitsa System of Medicine) as an anti-inflammatory agent. However, a scientific validation of the anti-inflammatory activity remains to be carried out.

The main aim of this study was to assess the anti-inflammatory potentials of decoctions made from leaves and stems of preflowering and flowering plants of *A. indica*, by using rats. The other aim was to investigate possible toxic effects of the decoction.

Materials and methods

Collection of plants and preparation of plant extracts

Fresh *A. indica* plants at the preflowering and flowering stages were collected separately from a field area around Colombo, Sri Lanka, between September and November 1998, and was authenticated by Professor R.N. de Fonseka, Department of Botany, University of Colombo, Sri Lanka. The two categories of plants were cut into small pieces and

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boiled separately in distilled water (DW), (200 g of plant material in 1000 ml of water) under reflux conditions, for 3 h. After 3 h, the boiled extracts were filtered through cotton wool and each filtrate was further reduced to 100 ml by boiling under reduced pressure. The concentrated extracts were then freeze-dried. The freeze-dried extracts were dark brown solid masses (yield: 5% w/w), which were made into small pieces using a glass rod. The masses obtained from the preflowering and the flowering plants were designated E1 and E2, respectively, and they were reconstituted in DW to obtain desired concentrations in 1 ml solution during the treatment.

Animals

Cross-bred albino rats from a colony maintained at the Department of Zoology, University of Colombo, were used: males and females weighing 150–200 g. The rats were housed under standardised animal house conditions (temperature; 28–31°C, photoperiod: approximately, 12 h natural light and relative humidity: 55–60%). All animals had free access to pelleted food (Vet House Ltd., Colombo, Sri Lanka) and water at all times.

Effect on carrageenan-induced paw edema

Seventy-eight male rats were selected and randomly divided into seven groups. The rats in groups 1, 2 and 3 (n = 12/group) were orally treated with 500, 250, and 125 mg/kg of E1, respectively. The rats of group 4 and 5 (n = 12/group) were respectively treated with 500 mg/kg E2 and 1 ml of DW, and group 5 served as the control. The rats in the sixth group (n = 12) were treated with 4 mg/kg indomethacin (State Pharmaceutical Corporation, Colombo, Sri Lanka) (Laurence & Bennett, 1992) in 1 ml 1% methylcellulose (Griffin and George Ltd, London, UK) and the seventh group (n = 6) of rats were treated with 1 ml 1% methyl cellulose. After 1 h, 0.05 ml of 1% carrageenan (Sigma Chemical Company, St. Louis, MO, USA) suspension was injected subcutaneously into the plantar surface of the left hind paw (Winter et al., 1962); of all these rats were under mild ether anaesthesia using a 25G needle and 1 ml syringe (Hindustan Syringes & Medical Devices Ltd, Faridabad, India). The volume of the injected paw of these rats was measured 1 h prior to the injection of carrageenan and 1, 2, 3 and 4 h after the injection using the water displacement method described by Ratnasooriya and Dharmasiri (1999).

Using these volumes, the increase in paw volume from their pre-injection volumes was calculated. The areas under the curves of anti-inflammatory activity vs. time plots were calculated.

Effect on formaldehyde-induced paw edema

Sixty male rats were randomly assigned into five equal groups (n = 12/group). The rats in groups 1, 2 and 3 were orally treated with 500, 250 and 125 mg/kg/day of E1, respectively, while those in the other two groups were treated with 500 mg/kg/day of E2 or 1 ml/day of DW (control group), respectively, for 7 consecutive days. On day 1 and 3 of the treatment, all these rats were injected with 0.1 ml of 2% formaldehyde in normal saline into the plantar surface of the left hind paw (Singh et al., 1989) under mild ether anaesthesia. The paw volume of these rats were measured using the water displacement method described previously. Paw volumes were measured prior to the injection of formaldehyde and at 4 h after the injection on day 1 and again once a day, every day after 1 h of treatment upto the 7th day of treatment, as by the 7th day of experiment paw edema had almost disappeared. On day 3 of the treatment, the paw volume was measured before the injection of formaldehyde.

The increase in paw volume was calculated. The areas under the curves of anti-inflammatory activity versus time plots were calculated.

Effect on adjuvant-induced paw edema

Fifty-four male rats were randomly assigned into six groups (n = 9/group). For 21 consecutive days, the rats in groups 1, 2 and 3 were orally treated with 500, 250, 125 mg/kg/day E1, respectively, and group 4, the control group, was treated with 1 ml/day DW. Groups 5 and 6 were treated, respectively, with 500 mg/kg/day E2 and 4 mg/kg/day indomethacin for the same period. One hour after the treatment of plant extracts, DW or indomethacin on day 1, all the rats were subcutaneously injected with 0.05 ml of Fruend's complete adjuvant (Sigma Chemical Company, St. Louis, MO, USA) into the plantar surface of the left hind paw (Singh et al., 1989). The volumes of the injected paws of all rats were measured prior to the injection, at 18 h after the injection, and on every other day upto day 14 of treatment, and finally on the day 21 of treatment, using the water displacement method as previously mentioned. The amount of increase in paw volume was calculated. The areas under the curves of anti-inflammatory activity versus time plots were calculated.

Effect on *in vitro* lipoxygenase activity (low ethanol method)

To investigate whether the anti-inflammatory activity of E1 could be attributed to an inhibition of leukotriene mediated effects, the lipoxygenase inhibitory effect of E1 was tested *in vitro* using the assay kit purchased from Sigma Chemical Company, St. Louis, MO, USA. E1 was mixed with the recommended amounts of reagents so that its final concentration in the reaction vessel would be 150 µg/ml. (This concentration of E1 was used because it was highly active in other *in vitro* assays) (Dharmasiri, 2001). After mixing the reagents, the absorbance of the mixture was measured at 234 nm at 1 min intervals for 3 min against reagent blank, using a spectrophotometer (V500, Jasco Corporation, Tokyo, Japan). The change of absorbance/minute was calculated.

The temperature of the reaction mixture was maintained at 25°C.

Antihistamine effect

The experiment was carried out as described by Spector (1956). Seventy-two male rats were selected and their fur on the posterior lateral side was completely shaved under ether anaesthesia. Twenty-four hours later, these rats were randomly assigned into seven equal groups (n = 12/group). The rats in groups 1–3 were orally treated with 125, 250, and 500 mg/kg of E1, respectively. The other three groups of rats were, respectively, treated with 0.67 mg/kg of chlorpheniramine (State Pharmaceutical Corporation, Colombo, Sri Lanka) (Laurence & Bennett, 1992) in 1% methylcellulose, 1 ml DW and 1 ml of 1% methylcellulose (control). After 1 h, these rats were lightly anaesthetised with ether and subcutaneously injected with 0.05 ml of 200 µg/ml histamine dihydrochloride (Fluka, Buchs, Switzerland) and the area of the wheal formed was calculated.

Free radical scavenging activity

2,2-Diphenyl-1-picrylhydrazyl (DPPH) (Sigma Chemical Company, St. Louis, MO, USA) was dissolved in absolute methanol (20 mg/l) (Joyeux et al., 1995). Aliquots (1.5 ml) of the solutions were pipetted into Eppendorf tubes. These tubes were treated with E1 (in triplicate) so that the final concentrations of E1 in the tubes were 20, 40, 60, 80 and 100 µg/ml. A triplet of tubes containing 1.5 ml of the DPPH solution with 25 µl of DW served as the control. After a 5 min incubation at room temperature (28–31°C) the absorbance of the solutions in the tubes were measured at 517 nm against a methanol blank. The percentage scavenging activity (decolorisation) with respect to the control was calculated.

Toxic effects

Twelve male rats were randomly divided into two equal groups. They were orally treated either with 500 mg/kg of E1 or 1 ml of DW for 30 consecutive days. During this period, the rats were observed every day for overt signs of acute toxicity (salivation, ataxia, diarrhoea) or stress (fur erection and exopthalmia). On day 1 of post-treatment, the rats were weighed (MP 6000, Chyo Balance Corporation, Tokyo, Japan). Blood was collected from the tail of these rat under mild ether anaesthesia using aseptic precautions. Blood was allowed to clot, sera were separated out and the serum activities of aspartic transaminase (AST), alanine transaminase (ALT) and the serum concentration of total protein and albumin were determined using a Randox assay kit (Randox Laboratories Ltd, Co. Antrim, UK). The serum creatinine concentration was determined according to Merck (1974).

Statistical analyses

Statistical analysis was performed using Student's *t*-test, one way ANOVA followed by Tukey's Family Error Rate test, and linear regression analysis. $P \leq 0.05$ was considered as significant.

Results

Carrageenan-induced paw edema

Treatment with 250 and 500 mg/kg of E1 resulted in significant reduction in the paw edema from 1–4 h of post-injection of carrageenan, as compared with controls. However, E2 did not significantly reduce the paw edema (Table 1). There was a significant suppression of inflammation among the treatment groups with E1 when the increase in paw volume was compared with each other using one way ANOVA. The areas under the curves for 125, 250, 500 mg/kg of E1 and indomethacin were 1735.5, 5323.5, 2913.0 and 4028.5 mm^2, respectively. The dose-response relationship showed a bell shape demonstrating the highest suppression of inflammation with the dose of 250 mg/kg as calculated from the areas under the curves. The anti-inflammatory activity of 250 mg/kg of E1 was higher by 32% than that of

Table 1. Effect of the oral treatment with preflowering (E1) and flowering (E2) plant decoctions of *A. indica* on the carrageenan-induced paw edema in rats (means ± SEM, n = 12).

	Increase in paw volume (ml)			
	1 h	2 h	3 h	4 h
Control	0.30 ± 0.02	0.54 ± 0.03	0.59 ± 0.03	0.56 ± 0.03
125 mg/kg E1	0.21 ± 0.03	0.39 ± 0.04*	0.43 ± 0.06*	0.47 ± 0.07
250 mg/kg E1	0.14 ± 0.03**	0.18 ± 0.02**	0.25 ± 0.03**	0.17 ± 0.04**
500 mg/kg E1	0.17 ± 0.02**	0.36 ± 0.04**	0.38 ± 0.02**	0.33 ± 0.03**
500 mg/kg E2	0.32 ± 0.07	0.58 ± 0.07	0.66 ± 0.07	0.60 ± 0.08
4 mg/kg indomethacin	0.14 ± 0.03**	0.28 ± 0.03**	0.27 ± 0.05**	0.17 ± 0.03**

As compared with controls: * $P < 0.05$, ** $P < 0.01$ (Student's *t*-test).

indomethacin. The EC_{50} for the anti-inflammatory effect was 223.43 ± 15.13 mg/kg of E1.

Formaldehyde-induced paw edema

Treatment with E1 significantly reduced the formaldehyde-induced increase in paw volume while E2 was unable to reduce significantly the increase in paw volume as compared with control (Table 2). The areas under the curves for 125, 250 and 500 mg/kg of E1 were 310.5, 423.0, 1105.0 mm^2, respectively. The suppression of inflammation by E1 was dose-dependent ($r^2 = 0.98$, $P < 0.05$) as calculated using the logarithmic values of areas under the curves. The EC_{50} of E1 for the suppression of inflammation showed a significant ($r^2 = 0.28$, $P < 0.01$) decrease with time.

Adjuvant-induced paw edema

Treatment with E1 caused a significant reduction in the adjuvant-induced increase in paw volume compared to the control while E2 had no significant effect (Table 3). The areas under the curves for 125, 250, 500 mg/kg of E1 and indomethacin were 284, 790, 2410 and 2241 mm^2, respectively. E1 showed a ($r^2 = 0.97$, $P < 0.05$) dose-dependent suppression of inflammation as calculated using the areas under the curves. The EC_{50} of E1 with time demonstrated a significant ($r^2 = 0.64$, $P < 0.01$) reduction with time. However, there was no significant difference between the EC_{50} of E1 in the formaldehyde model and the adjuvant model (EC_{50}; formaldehyde model vs. adjuvant model: 464.4 ± 63.9 vs. 454.4 ± 31.1 mg/kg).

The suppression of inflammation at 18 h was higher in 500 mg/kg of E1 than that of 4 mg/kg of indomethacin. Therefore, the anti-inflammatory activity of 500 mg/kg of E1 against the adjuvant-induced inflammation at 18 h is higher by 24% than that of 4 mg/kg of indomethacin.

Lipoxygenase activity *in vitro*

E1 did not significantly reduce the change of absorbance per minute at 243 nm *in vitro* with respect to the control (change of absorbance; control vs. treatment: 0.169 ± 0.008 vs. 0.169 ± 0.011).

Antihistamine effect

E1 significantly reduced the area of the wheal as seen with 0.67 mg/kg of chlorpheniramine. The area of the wheal formed: control versus 125 vs. 250 E1 versus 500 mg/kg of E1 versus 0.67 mg/kg of chlorphenaramine: 132.9 ± 6.1 versus 116.5 ± 7.6 versus 99.3 ± 10.5 versus 75.8 ± 6.6 versus 84.3 ± 9.5 mm^2. It was dose-dependent ($r^2 = 0.99$, $P < 0.05$) as calculated from the logarithmic values of the areas versus concentrations of E1. The antihistamine activity of E1 was 16% higher than that of 0.67 mg/kg of chlorpheniramine. The EC_{50} of E1 was 568.19 mg/kg.

Free radical scavenging effect

E1 showed a significantly dose-dependent ($r^2 = 0.97$, $P < 0.01$) free radical scavenging effect as indicated by the % decolourisation of DPPH in methanol (Fig. 1). The EC_{50} of E1 for the decolourisation of DPPH were 46.64 µg/ml.

Toxic effect

There was no overt signs of acute toxicity or stress observed in the treated rats. The body weights were not significantly altered (control vs. E1: 252.7 ± 4.9 vs. 254.9 ± 7.2 g). The serum activities of AST and ALT and the serum concentration of albumin were also not significantly changed (AST; control vs. E1: 48.7 ± 6.4 vs. 62.7 ± 7.0 u/l, ALT; control vs. treatment: 17.0 ± 2.9 vs. 22.8 ± 4.4 u/l, total protein; control vs. treatment: 7.4 ± 0.3 vs. 7.1 ± 0.1, albumin; control vs. E1: 3.5 ± 0.1 vs. 3.3 ± 0.1 g/dl). However, the 30 day treatment with E1 significantly ($P < 0.01$) reduced the serum concentration of creatinine (control vs. E1: 2.9 ± 0.1 vs. 2.0 ± 0.1 mg/dl).

Discussion

The results show that E1 in contrast to E2 has a dose-dependent anti-inflammatory activity when evaluated in both acute (carrageenan-induced paw edema) and chronic (formaldehyde-induced paw edema and adjuvant-induced paw edema) inflammatory models. E1 did not show overt signs of general toxicity, hepatotoxicity (in terms of unaltered serum activities of AST and ALT and concentration serum proteins) or renotoxicity (as indicated by serum creatinine level). This is an important finding, because the World Health Organisation has estimated that about 80% of the global population rely chiefly on traditional drugs, derived chiefly from plant extracts for their primary health care (WHO Symposium, 2001).

In the carrageenan-induced paw edema test, E1 inhibited in the early and in the late phases of an acute inflammation as well. The inhibition of the early phase can be attributed to the antihistamine effect and the previously reported COX-I inhibitory effect (Dharmasiri et al., in press) of E1. Both prostaglandins synthesised via COX-I pathway, and histamine, have been shown to be powerful mediators of the early exudative phase of inflammation in the carrageenan model (Antonio & Brito, 1998; Tsai & Lin, 1999). The inhibition of the late phase indicates that E1 may have COX-II inhibitory activity as well: COX-II is induced at the late phase of inflammation in the macrophages infiltrated into the area of inflammation (Tsai & Lin, 1999). Opioid receptor agonists can also induce acute anti-inflammatory activity (Ahmadiani et al., 1998). Since E1 had no agonist activity on opioid receptors (Dharmasiri et al., in press), its anti-inflammatory activity can not be caused by opioid receptor mediation.

Bradikinin and serotonin are also important mediators in acute inflammation (Guyton & Hall, 1996; Kuby, 1997).

Table 2. Effect of oral administration with preflowering (E1) and flowering (E2) plant decoctions of *A. indica* on formaldehyde-induced paw edema in rats (means ± SEM, n = 12).

	Increase in paw volume (ml)							
	4th hour	day 1	day 2	day 3	day 4	day 5	day 6	day 7
Control	0.49 ± 0.05	0.52 ± 0.05	0.37 ± 0.04	0.36 ± 0.04	0.46 ± 0.06	0.45 ± 0.05	0.30 ± 0.04	0.08 ± 0.02
125 mg/kg E1	0.32 ± 0.01**	0.30 ± 0.03**	0.24 ± 0.02*	0.41 ± 0.04	0.33 ± 0.07*	0.26 ± 0.07**	0.23 ± 0.08*	0.12 ± 0.06
250 mg/kg E1	0.38 ± 0.01	0.25 ± 0.02**	0.18 ± 0.02**	0.34 ± 0.04*	0.30 ± 0.03*	0.21 ± 0.03**	0.11 ± 0.02**	0.03 ± 0.01*
500 mg/kg E1	0.26 ± 0.02**	0.22 ± 0.04**	0.15 ± 0.04**	0.20 ± 0.03**	0.16 ± 0.03**	0.16 ± 0.03**	0.11 ± 0.02**	0.07 ± 0.02
500 mg/kg E2	0.51 ± 0.04	0.53 ± 0.05	0.41 ± 0.05	0.64 ± 0.04	0.58 ± 0.04	0.46 ± 0.03	0.26 ± 0.04	0.11 ± 0.03

As compared to controls: * P < 0.05, ** P < 0.01 (Student's *t*-test).

Table 3. Effect of oral administration with preflowering (E1) and flowering (E2) plant decoctions of *A. indica* for 21 consecutive days on the adjuvant-induced paw edema in rats (means ± SEM, n = 9).

	Increase in paw volume (ml)								
	18 h	day 2	day 4	day 6	day 8	day 10	day 12	day 14	day 21
Control	0.83 ± 0.06	0.88 ± 0.06	0.68 ± 0.06	0.56 ± 0.04	0.47 ± 0.05	0.50 ± 0.03	0.51 ± 0.03	0.49 ± 0.04	0.29 ± 0.04
125 mg/kg E1	0.75 ± 0.07*	0.69 ± 0.07	0.59 ± 0.06	0.56 ± 0.03	0.53 ± 0.04	0.46 ± 0.02	0.41 ± 0.02**	0.33 ± 0.03**	0.15 ± 0.03**
250 mg/kg E1	0.65 ± 0.07*	0.68 ± 0.05*	0.53 ± 0.06*	0.45 ± 0.08	0.42 ± 0.05	0.37 ± 0.05*	0.37 ± 0.06*	0.30 ± 0.06**	0.12 ± 0.03**
500 mg/kg E1	0.39 ± 0.08**	0.45 ± 0.09**	0.33 ± 0.06**	0.23 ± 0.05**	0.19 ± 0.04**	0.27 ± 0.06**	0.25 ± 0.06**	0.18 ± 0.06**	0.07 ± 0.02**
500 mg/kg E2	0.81 ± 0.05	0.74 ± 0.05	0.69 ± 0.07	0.56 ± 0.07	0.54 ± 0.07	0.55 ± 0.06	0.48 ± 0.04	0.47 ± 0.04	0.28 ± 0.05
4 mg/kg Indomethacin	0.45 ± 0.07**	0.46 ± 0.06**	0.32 ± 0.05**	0.27 ± 0.06**	0.20 ± 0.06**	0.16 ± 0.04**	0.12 ± 0.05**	0.08 ± 0.03**	0.15 ± 0.03**

As compared with controls: * P < 0.05, ** P < 0.01 (Student's *t*-test).

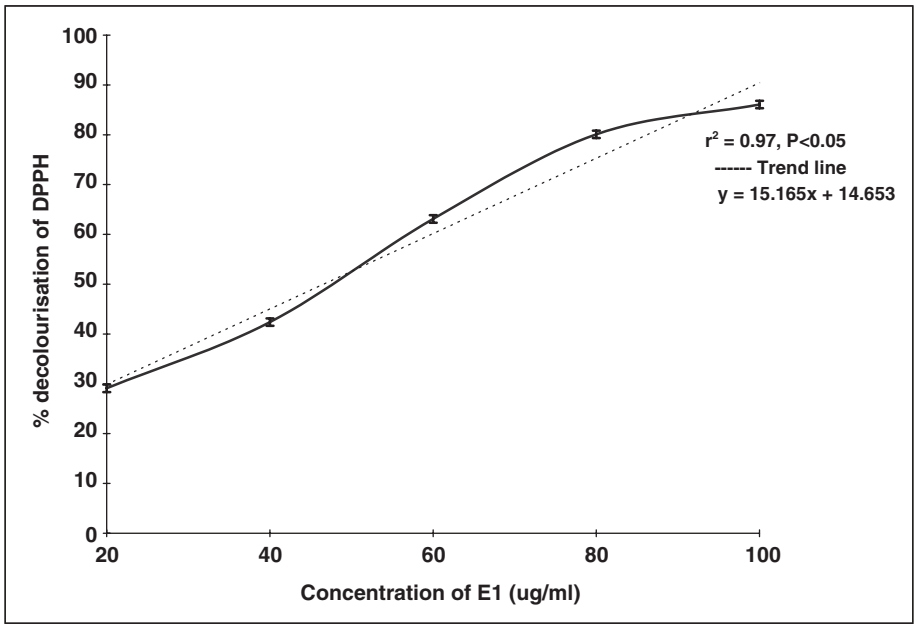

Figure 1. Variation of the free radical scavenging effect of the extract of preflowering *A. indica* as indicated by the % decolorisation of DPPH (means ± SEM).

However, in this study, we did not investigate the effects of E1 on their synthesis, release and activity.

The anti-inflammatory activity of E1, seen against chronic models of inflammation, is likely to be due to the potent membrane stabilising effect (Dharmasiri et al., in press) and free radical scavenging activity of E1 in addition to its PG synthesis inhibition and antihistamine activities. The membrane stabilising effect is considered as a biochemical index of anti-inflammatory activity of a drug (Perez et al., 1995). Reactive oxygen free radicals produced by the neutrophils, activated macrophages and lymphocytes that infiltrate into an inflammatory site contribute to the tissue damage, leading to an aggravation of the chronic inflammation (Hanna & Poste, 1991). E1 can scavenge radicals. This could be another mechanism involved in its anti-inflammatory activity of E1. Prostaglandins produced via COX-II pathway, cytokines produced by activated macrophages and immunological factors such as complement and lymphocytes are also important mediators of chronic inflammation (Rang et al., 1995). The effect of E1 on the action of these factors cannot be excluded. However, the anti-inflammatory effect caused by the inhibition of the leukotrienes via the inhibition of 5-lipoxygenase can be excluded, as E1 failed to inhibit lipoxygenase *in vitro*.

The EC_{50} value of E1 was reduced with time in the chronic inflammatory models, indicating an increase of the efficacy of the extract with continuous use. This is an important beneficial effect seen in a drug as the efficiency of some can be reduced with continuous use (Laurence & Bennett, 1992).

The adjuvant-induced arthritis model is considered as the closest animal model for human rheumatoid arthritis which involves several biochemical and immunological complications (Kuby, 1997). The anti-inflammatory effect of E1, shown at 18 h after its administration in the adjuvant model, indicates anti-rheumatoid properties (Singh et al., 1989). The anti-inflammatory activity of E1 at 18 h was greater than that of indomethacin suggesting that E1 could be more effective than indomethacin against human rheumatoid arthritis. Another beneficial effect of E1 is that it did not induce gastric lesions and haemorrhage (Dharmasiri et al., in press) like non-steroidal anti-inflammatory drugs (Laurence & Bennett, 1992).

The strong antihistamine effect of E1, which is not claimed in the traditional medicine, indicates the possibility of using *A. indica* at the pre-flowering stage to treat allergic conditions (Laurence & Bennett, 1992).

In conclusion, this study demonstrates in detail the efficacy and safety of E1 as an anti-inflammatory agent, scientifically justifying use of the decoction as an anti-inflammatory agent in the Sri Lankan traditional medicine. In addition, it shows that anti-inflammatory activity is absent after flowering of the plant as it is the case for its analgesic effect (Dharmasiri et al., 2002). On the other hand, E1 could be developed as a herbal anti-inflammatory drug with mild side effects.

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