Anti-inflammatory effect of the methanol extract from *Anthocephalus cadamba* stem bark in animal modelsa

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Abstract

Anthocephalus cadamba (Rebox) Miq. (Rubiaceae) is widely distributed throughout the greater part of India, especially at low levels in wet places. Traditionally the bark is used as a tonic and a febrifuge, and to reduce pain and inflammation. The anti-inflammatory effect of methanol extract obtained from *Anthocephalus cadamba* aerial parts, MEAC, were investigated in this study. The effects of MEAC on the acute and chronic phases of inflammation were studied in carrageenan, dextran and mediators (histamine and serotonin) induced paw edema and cotton pallet-induced granuloma, respectively. The anti-edema effect of MEAC was compared with 10 mg/kg of indomethacin orally. Results suggested that MEAC possess potent anti-inflammatory activity. The acute inflammatory model showed that all the doses of MEAC effectively suppressed the edema produced by histamine, so it may be suggested that its anti-inflammatory activity is possibly backed by its antihistaminic activity. In a chronic inflammatory model, the effect may be due to the cellular migration to injured sites and accumulation of collagen and mucopolysaccharide. On the basis of these findings, it may be inferred that *Anthocephalus cadamba* is an anti-inflammatory agent and the results are in agreement with its traditional use.

Introduction

Anthocephalus cadamba (Rebox) Miq. (Rubiaceae) is widely distributed throughout the greater part of India, especially at low levels in wet places. The stem bark is pungent, bitter, sweet, acrid, and cooling, a galactogogue, astringent, and aphrodisiac, it has a positive effect in uterine complaints and blood disease. The bark is also used as a febrifuge and tonic. In the konkan, the fresh juice of the bark is used for inflammation of the eyes. The fruit is aphrodisiac, and causes biliousness when ripe.[1] *Anthocephalus cadamba* has been reported for its antimicrobial[2] activity. Isolation of a triterpenoid saponin cadambagenic acid[3] and chlorogenic acid[4] has been previously reported.

The present study was designed to investigate and evaluate the pharmacological basis for the use of *Anthocephalus cadamba* in folk medicine for the treatment of inflammation.

Materials and Methods

Plant material

The stem bark of the plant *Anthocephalus cadamba* was collected from Mangalore of Karnataka, India during December 2007. The plant material was authenticated by Dr. Gopalkrishna Bhat, Department of Botany, Poorna Prajna College, Udupi. A voucher specimen CH/NGSM-IF17 has been kept in the herbarium of the college.

Extraction

The stem barks were shade-dried and then powdered with a mechanical grinder and stored in an airtight container. The dried powder material of the bark was extracted with methanol (yield 9.25%), in a Soxhlet apparatus.

Animals

Swiss albino male mice and male rats weighing 18-22 g and 180-200 g, respectively, were obtained from an animal colony of NGSM Institute of Pharmaceutical Sciences, Paneer, Deralakatte, Mangalore, India. They were housed in polypropylene cages in an air-conditioned area at 25±2°C with 10:14 h light and dark cycle and maintained on Amrut brand balanced animal feed and water ad libitum. All procedures described were reviewed and approved by the University's animal ethics committee.

Chemicals

The drugs and chemicals used in the study were carrageenan (S.D. Fine Chemicals Limited, Bombay), 5-hydroxytryptamine hydrochloride (Serotonin), histamine (Sigma, USA), indomethacin (BPRL Bangalore), aspirin (USV Bombay), paracetamol (Cipla, Bombay) and morphine (M.M.Pharma, New Delhi).

Toxicity study

The LD50 was determined using the graphical method[5] in mice. Briefly, geometric doses of the extract (100-1750 mg/kg) were administered i.p. to 10 groups of mice. The control group received normal saline (5 mg/kg i.p.). Signs of toxicity and mortality within 24-72 h were noted. A confirmatory test was carried out and the LD50 was calculated from the graph of percent mortality against profit log dose of the extract.

Anti-inflammatory activity

Carrageenan-induced rat paw edema

The rats were divided into five groups of six animals each. The different groups were treated with crude MEAC (50, 100 and 200 mg/kg b.w.,p.o.), indomethacin (10 mg/kg, p.o.) and vehicle control (10% propylene) p.o., and the paw volume was measured at 0 h and 3 h after carrageenan injection using a plathysmometer.[6] The animals were pre-treated with the extract 1 h before the administration of carrageenan. Acute inflammation was produced by the subplantar administration of 0.1 mL of 1% carrageenan in normal saline in the right paw of the rats. The anti-inflammatory activity (%) was (1-D/C) x100, where D represents the percentage difference in paw volume after

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Contributions: KSC carried out practical work and wrote the paper; BA has helped in collecting the plant material, carrying out the extraction and administering the drug to the animals; KSP carried out data analysis, statistical work and results.

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Table 1. Effect of the methanol extract of *Anthocephalus cadamba* stem bark on cotton pellet-induced granuloma in rats and on leukocyte migration in peritoneal exudation in carrageenan-induced mice.

Treatment (mg/kg)	Dose	Weight of cotton pellet (mg) (moist)	Percentage of inhibition	Weight of cotton pellet (mg) (dried)	Percentage of inhibition	Leukocytes (10^5 mL^{-1})	Leukocyte inhibition	Neutrophils 10^5 mL^{-1}	Change in neutrophils
Control		205.63±14.2	---	45.83±1.1	----	4.98±0.32	---	2.61±0.36	---
Indomethacin	10	96.45±8.6	53.08	20.13±0.7*	56.05	2.07±0.13*	58.72	0.91±0.27*	34.87
MEAC	50	145.35±11.1	29.32	32.46±1.2*	29.24	2.92±0.06*	41.68	0.97±0.06**	37.16
MEAC	100	121.60±9.9*	40.85	28.17±0.8*	38.58	2.55±0.15**	49.29	0.31±0.05**	11.87
MEAC	200	111.31±10.5*	45.85	21.21±0.5*	53.68	1.67±0.07**	66.33	0.29±0.06*	11.11

Values are mean ± SEM (n=6); *Experimental groups were compared with control (P<0.01); **Experimental groups were compared with control (P<0.05)

MEAC was administered to the rats and C represents the percentage difference of volume in the control groups.[7]

Dextran-induced paw edema

The rats were divided into five groups of six animals each. The animals were treated in a manner similar to that of the carrageenan-induced paw edema models; dextran (0.1 mL, 1% W/V in normal saline) was used in the place of carrageenan.[6]

Histamine- and serotonin-induced inflammation

The rats were divided into five groups of six animals each. The anti-inflammatory activity of MEAC was measured with phlogistic agents (i.e. histamine, 5-HT) that mediate inflammation. The paw edema was induced in rats by subplantar injection of freshly prepared histamine (1 mg/kg b.w.) and serotonin (1 mg/kg b.w.) solutions, respectively. The paw edema was measured as described earlier.[8]

Cotton pellets-induced granuloma

The normal paw volumes of all the rats were measured initially and were divided into five groups of six animals each. The different groups were treated with MEAC (50, 100 and 200 mg/kg b.w.,p.o.), indomethacin (10 mg/kg, p.o.) and control vehicle were administered orally for seven consecutive days from the day of cotton pellet implantation. The animals were anesthetized on the eighth day, cotton pellets were removed surgically and freed of extraneous tissues. The moist pellets were weighed and then dried at 60°C for 24 h after which the dried pellets were weighed again. Any increment in the dry weight of the pellets was taken as measure of granuloma formation. The antiproliferative effect of MEAC was compared with the control.

Mouse carrageenan peritonitis

The mice were divided into five groups of six animals each. Inflammation was induced by modification of the technique as previously described.[8] The extract was administered orally at doses of 50, 100 and 200 mg/kg p.o., and carrageenan (0.25 mL, 0.75% in saline) was injected intraperitoneally 1 h later. After 4 h the animals were sacrificed by cervical dislocation for further investigation. Ca^{2+} and Mg^{2+} free phosphate buffered saline was used during the collection of peritoneal fluids. The total leukocyte count was determined in a Neubauer chamber and the differential cell count was determined.[9] The percentage of the leukocyte inhibition was (1–T/C)x100, where T represents the treated groups' leukocyte counts. Changes in neutrophil were calculated by the following equation: changes in neutrophil counts was neutrophils counts of treated groups/neutrophil counts of control groups x 100.

Statistical analysis

The statistical analysis was carried out to calculate mean (SEM). Further analysis was carried out by Student's t-test to calculate significance of results. Values with $P < 0.05$ were considered statistically significant.

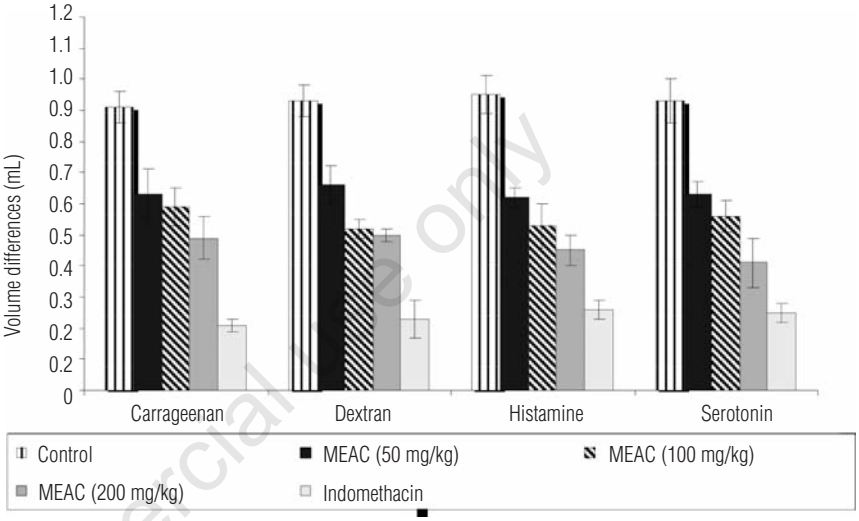

Figure 1. Effect of methanol extract of *Anthocephalus cadamba* (MEAC) stem bark on carrageenan-, dextran-, histamine- and serotonin-induced pedal edema in rats. Difference of mean of edema volume (mL) between control and treatment values at different doses ± S.E.M. Variation compared to the control animals. ANOVA followed by Student's t-test. P<0.001.

Results

Toxicity study

The LD_{50} value of MEAC was estimated to be 961.05 mg/kg (914.80-994.75 mg/kg) body weight i.p. in mice.

Anti-inflammatory studies

The anti-inflammatory activity of MEAC was measured at doses of 50, 100 and 200 mg/kg b.w. against acute paw edema induced by carrageenan, dextran and mediators (histamine and serotonin), and is summarized in Figure 1. The extract was found to significantly (P<0.001) inhibit the carrageenen-induced rat

paw edema, a test which has significant predictive value for anti-inflammatory agents acting by inhibiting the mediators of acute inflammation. MEAC at the doses 50, 100 and 200 mg/kg showed an inhibition of 29.1, 38.2 and 45.5%, 27.3, 36.6 and 44.6% and 29.1, 40.2 and 46.7% against acute paw edema induced by carrageenan, dextran, histamine and serotonin, respectively.

The inflammatory granuloma is a typical feature of established chronic inflammatory reaction. The effects of MEAC and indomethacin on the proliferative phase of inflammation are summarized in Table 1. It was seen that MEAC was responsible for an anti-inflammatory effect, which would be calculated depending on the moist and dry weight of cotton pellets. According to these results, the antiproliferative effects of MEAC (200 mg/kg b.w.) and indomethacin (10 mg/kg b.w.) were calculated as 44.85 and 53.09% ($P<0.05$), respectively. After they were dried, the antiproliferative effects were calculated on the basis of dry weight pellets; the inhibition of inflammation by MEAC and indomethacin were established as 53.68 and 56.05% ($P<0.05$), respectively.

Mouse carrageenan peritonitis

The MEAC also inhibited peritoneal leukocyte migration at the rate of 41.68, 49.29 and 66.33% at the doses of 50, 100 and 200 mg/kg, respectively, whereas the inhibition produced by indomethacin (10 mg/kg) 58.72% was found in a carrageenan-induced peritonitis model as shown in Table 1.

Discussion

Anti-inflammatory activity

The MEAC showed a dose-dependent anti-edematogenic effect on paw edema induced by carrageenan at 3 h. Dextran-induced paw edema is known to be mediated both by histamine and serotonin. The MEAC also exhibited significant ($P<0.001$) anti-inflammatory effect in dextran-induced paw edema.

Histamine is one of the important inflammation mediators since it is a potent vasodilator and vascular permeability action.[10] This study showed that all the doses of MEAC effectively suppressed the edema produced by histamine at all the doses assayed, so it may be suggested that its anti-inflammatory activity is possibly backed by its antihistaminic activity. The MEAC also effectively suppressed the inflammation produced by serotonin-induced hind paw edema, which indicates that the MEAC may exhibit its anti-inflammatory action by means of either inhibiting the synthesis or releasing the action of inflammatory mediators, i.e. histamine, serotonin and prostaglandins. From the above results it is suggested that the crude MEAC act through the inhibition of kinin and prostaglandin synthesis, since the extract was effective during the mediator release.

The cotton pellet granuloma method is widely used to evaluate the transudative and proliferative components of chronic inflammation. The moist weight of the cotton pellet correlates with transudes; the dry weight of the pellet correlates with the amount of the granulomatous tissue.[11] Administration of MEAC (50,100 and 200 mg/kg b.w.) and indomethacin (10 mg/kg b.w.) appears to be effective in inhibiting the moist weight of the cotton pellets. On the other hand, the MEAC effect on dry weight of the cotton pellet was similar to that of indomethacin. These data support the hypothesis of the greater effect of the MEAC on the inflammation mediators in the immediate response to inflammation in rats that may be due to cellular migration to injured sites and accumulation of collagen and mucopolysaccharide.

Mouse carrageenan peritonitis

Leukocyte aggregation at the site of inflammation is a fundamental event in the inflammatory process.[12] In the present investigation, we compared the effect of the extract at the doses of 50, 100 and 200 mg/kg b.w. and indomethacin on cell migration. The MEAC also inhibited the carrageenan-induced leukocyte migration in a peritonitis model in mice. The MEAC was found to more potently inhibit leukocyte migration than indomethacin. The extract (in a peritonitis model) drastically reduced migration of neutrophils.

Conclusions

There have been a number of reports on the anti-inflammatory activity of triterpenoids.[13,14] The effect of the MEAC in the inflammation process induced by stimulus injection indicates that they act by affecting a time-delayed system in a similar fashion to glucocorticoids. This suggests that the active principle (triterpenoid) of the extract has some degree of affinity for glucocorticoid receptors. This mechanism was confirmed by other triterpenes isolated from *D. leucomelas.*[14] To conclude, the results showed the anti-inflammatory effect of methanol extract of *Anthocephalus cadamba* and the possible mechanism might be inhibition of mediator release and PG biosynthesis. All findings corroborate the traditional use of the plant in inflammatory conditions.

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