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EVALUATION OF ANTI-INFLAMMATORY ACTIVITY OF LEAVES OF AVERRHOEA CARAMBOLA IN EXPERIMENTAL ANIMAL MODELS

Pharmacology

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ABSTRACT

To evaluate the anti-inflammatory activity of the Ethanolic Extracts of leaves of Averrhoea carambola (EEAC) on experimental animal models. **Materials and Methods:** The ethanolic extract of Averrhoea carambola leaves was prepared by percolation method using 95% ethanol. For each of the studies four groups of albino ratsof either sex, weighing 100-150g were taken (n = 5). Group A was taken as control, group B and C as test groups, group D as standard. Acute oral toxicity test of the extract was performed as per OECD 425 (OECD Guidelines, 2001). Acute inflammation was studied by carrageenan-induced rat paw oedema method and chronic inflammation was studied by Freund's complete adjuvant-induced arthritis method. Aspirin 100 mg/kg was taken as a standard drug. **Results:** The results were analysed by ANOVA followed by Dunnett's multiple comparison test. EEAC at the doses of 200mg/kg and 400 mg/kgshowed significant ($p< 0.01$) anti-inflammatory activity in carrageenan induced acute inflammation when compared to the control. EEAC was also effective in chronic arthritis model in dose-dependent manner. **Conclusion:** The present study indicates that EEAC has significant anti-inflammatory activity against both acute and chronic inflammation.

KEYWORDS

Ethanolic Extract, Averrhoea Carambola, Anti-inflammatory, Carrageenan, Freund's Complete Adjuvant.

INTRODUCTION

Inflammation is a biological response of the immune system that can be triggered by a variety of factors, including pathogens, toxic compounds and damaged cells.[1] The steps of the inflammatory response are recognition of the injurious agent, recruitment of leukocytes, removal of the agent, regulation (control) of the response, and resolution (repair).[2]

Since ancient times, man has familiarized himself with plants and used them in variety of ways throughout the ages. The growth of knowledge to treat and cure diseases continued at a steady pace and a number of new plant derived drugs has increased.[3]

Averrhoea carambola plant is widely distributed around the world especially in tropical countries.[4] Each part of the plant has different therapeutic value. Ripe fruit and juice are used as appetite stimulant, laxative, roots in the treatment of headache, arthralgia, epistaxis., leaves in chicken pox, ring worm and headache, boiled flowers as vermifuge in fever and in malaria, powdered seeds in the treatment of asthma, colic, bark on prickly heat.[5] The plant is rich in flavonoids, tannins, saponins, alkaloids, oxalic acid and steroids.[6]

MATERIALS AND METHODS

Plant Material

The study was conducted in the Department of Pharmacology, Assam Medical College Dibrugarh. Fresh leaves of *Averrhoea carambola* were collected from the college campus. Leaves were authenticated by Prof. L. R. Saikia, (voucher no. DULSc 471) Department of Life Sciences, Dibrugarh University, Assam.

Plant Extract

Fresh leaves of *Averrhoea carambola* were air-dried, ground to fine powder and stored in air tight container. The ethanolic extract of leaves of *Averrhoea carambola* were obtained by method of percolation described by SS Handa *et al.* (2008).[7]

Animals

Healthy albino rats of the species *Rattus norvegicus* of either sex weighing 100 - 150 gm were procured from Chakraborty Enterprise, Kolkata. A total of 40 animals were used for the study with 5 animals in each group. The study was conducted in accordance with CPCSEA (Committee for the Purpose of Control and Supervision of Experiment on Animals) guidelines and the study was approved by the Institutional Animal Ethical Committee (Registration No. 634/G0/Re/S/02/CPCSEA). The animals were provided with standard diet and water *ad libitum*.

Acute Toxicity Study

Acute toxicity test was done for the ethanolic extract of *Averrhoea carambola* following OECD 425 guidelines.[8] There were no signs of toxicity and mortality recorded among the rats at 2000mg/kg. The doses for the experiment were taken arbitrarily at 200 mg/kg and 400 mg/kg.

Anti-inflammatory studies

For each experiment, the animals were divided into 4 groups with 5 animals in each group.

- Group-A (control) received Normal saline 10 ml/kg p.o.
- Group-B (Test-1) received EEAC 200 mg/kg p.o.
- Group-C (Test-2) received EEAC 400 mg/kg p.o.
- Group-D (standard) received aspirin 100 mg/kg p.o.

All the drugs were administered orally and the volume of medicaments kept constant at 10 ml/kg body weight of the animals.

(a) Anti-Inflammatory Study against Acute Inflammation

The acute anti-inflammatory activity of EEAC was tested by carrageenan-induced rat paw oedema method. 1 hour after administration of normal saline, test drug and aspirin, acute inflammation was induced by injecting 0.1 ml of 1% (w/v) carrageenan in the plantar region of the left hind paw of rats of control, test as well as standard drug groups. The paw volume was measured plethysmometrically, as described by Chattopadhyay *et al*. 1986 just before carrageenan injection i.e. at 0 hr and at 1st, 2nd, 3rd and 4thhr after carrageenan injection.[9]

(b) Anti-Inflammatory Study against Chronic Inflammation

The chronic anti-inflammatory activity of EEAC was tested by Freund's adjuvant-induced arthritis method in rats. On Day 1, the animals were injected into the sub-plantar region of the left hind paw with 0.1 ml of complete Freund's adjuvant. Dosing with the test compounds or the standard to the respective groups was started on the same day and continued for 12 days. The paw volumes of both sides were measured plethysmographically and the body weights were recorded on the first day of injection. On Day 5 the volume of the injected paw was measured again. The severity of the disease was followed by measurement of the non-injected paw volume (secondary lesions). From Day 13 to 21, the animals were not administered with the test compound or the standard.[10]

The non-injected paw volume and the body weight were measured again on 21st day and the polyarthritis severity score was graded on 0 to 4: 0= No swelling; 1= Phalanx joint involvement; 2= Phalanx joint and all digits involvement; 3=involvement of the entire region down to the ankle; 4= involvement of entire paw, including ankle.[11,12]

Evaluation :[10]

a. For primary lesions: The percentage inhibition of paw volume of the injected left paw over vehicle control was measured at day 5.

b. For secondary lesions: The percentage inhibition of paw volume of the non-injected right paw over control was measured at day 21.
c. An arthritic index was calculated as the sum of the scores as indicated above for each animal. The average of the treated animals was compared with the control group.

RESULTS

Table-1 Anti-inflammatory activity of EEAC against acute inflammation

Group	Drug dose p.o.	Mean increase in Paw volume (Mean ± S.E.M) (ml) with Percentage Inhibition			
		1st hour	2nd hour	3rd hour	4th hour
Group A (Control)	Normal saline 10 ml/kg	0.26 ± 0.0093	0.45 ± 0.0089	0.58 ± 0.015	0.47 ± 0.011
Group B (Test drug)	EEAC 200mg/kg	0.23 ± 0.0060[a] (11.53%)	0.36± 0.012[a] (20.0%)	0.44 ± 0.018[a] (24.14%)	0.38 ± 0.016[a] (19.14%)
Group C (Test drug)	EEAC 400mg/kg	0.19 ± 0.0071[a] (26.90%)	0.24± 0.0084[a] (46.66%)	0.38 ± 0.0071[a] (52.63%)	0.23 ± 0.012[a] (51.06%)
Group D (Standard)	Aspirin 100mg/kg	0.14 ± 0.0066[a] (46.15%)	0.17± 0.0058[a] (62.22%)	0.19 ± 0.0051[a] (67.24%)	0.15 ± 0.0055[a] (68.08%)
One Way Anova	F	47.72	189.7	164.1	146.6
	df	16,3	16,3	16,3	16,3
	p	<0.01	<0.01	<0.01	<0.01

n = 5 rats in each group; [a]p <0.01 when compared to group A; ANOVA followed by Dunnet''s multiple comparison test

Table- 2 Anti-inflammatory activity of EEAC against chronic inflammation

Name of the group	Drug dose p.o.	Mean increase in Paw volume (Mean ± S.E.M) (ml)with Percentage Inhibition		Weight change on 21st day	Arthritis index
		On 5th Day (injected paw)	On 21st Day (Non injected paw		
Group A (Control)	Normal saline 10 ml/kg	1.23±0.011	0.35±0.0073	-31.40± 1.5	10.40±0. 40
Group B (Test Drug)	EEAC 200mg/kg	1.19±0.012 3.25%	0.25±0.010 28.57%	-20.80± 1.1[a]	8.80±0.3 7[b]
Group C (Test Drug)	EEAC 400mg/kg	0.91±0.0086 26.01%	0.16±0.0037 54.29%	-11.00± 0.71[a]	6.00±0.4 5[a]
Group D (Standard drug)	Aspirin 100mg/kg	0.71±0.032 42.27%	0.09±0.0071 74.29%	-54.80 ±0.86[a]	2.80±0.3 7[a]
One Way Anova	F	171.3	229.9	305.1	69.67
	df	16,3	16,3	16,3	16,3
	p	<0.01	<0.01	<0.01	<0.01

n = 5 rats in each group; [a]p <0.01, [b]p <0.05 when compared to group A; ANOVA followed by Dunnet"s multiple comparison test.

DISCUSSION

Paw oedema induced by carrageenan has two phases. Carrageenan, stimulates phospholipase A_2 thereby initiating the early phase of inflammation, and the cytotoxic effects progress the inflammation. In the process several pro-inflammatory mediators are released.[13]

Swelling of the rat paw reached a peak in 3 to 5 hours, and then remained about the same degree of oedema for several hours.[14] In the present study EEAC at the doses 200 mg/kg, 400 mg/kg showed significant percentage of inhibition of carrageenan induced paw oedema when compared to the control which was maximum at 3rd hour. The anti-inflammatory activity of EEAC may be due to the inhibition of release of some mediators of inflammation which are released within three hours of carrageenan injection. The maximum paw oedema was seen at the end of 3rd hour of carrageenan injection i.e. after the release of the mediators of acute inflammation.

Freund's complete adjuvant was used to induce arthritis for evaluation of chronic anti-inflammatory activity. One of the reasons for the wide utilization of this model is due to the strong correlation between the efficacy of therapeutic agents in this model and in rheumatoid arthritis in humans.[15]

EEAC at the doses 200 mg/kg, 400 mg/kg showed significant reduction of adjuvant induced paw oedema. Leaves of *Averrhoea carambola* is reported to have flavonoids.[6] Flavonoids are reported to exhibit anti-inflammatory activities, against both acute and chronic inflammation.[16]

Flavonoids inhibit phopholipase A_2, cyclo-oxygenase, lipoxygenases thereby reduce the concentrations of prostanoids and leukotrienes.[17]

The presence of important phytochemicals like flavonoids in the leaves may be also responsible for the significant antiinflammatory activity of EEAC.

Adjuvant arthritis is characterized by weight loss.[18] Weight loss is associated with increased production of pro-inflammatory cytokines such as TNF-α and interleukin-1.[19] In the present study EEAC has significantly prevented the weight loss probably inhibiting these cytokines.

EEAC has significantly down regulated the arthritis index in adjuvant arthritis of rats.

CONCLUSION

All the findings observed in the present study suggest that ethanolic extract of *Averrhoea carambola* leaves possess significant anti-inflammatory activity against acute and chronic inflammation in experimental animal models.

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