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Review Article

Anti-Inflammatory Activity Of *Canthium Angustifolium* Roxb Leaves By Carrageenan Induced Paw Oedema And Cotton Pellet Granuloma Method

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ABSTRACT

The total ethanolic extract of Canthium angustifolium Roxb leaves screened for anti-inflammatory activity by carrageenan induced paw odema and cotton pellet granuloma. Anti-inflammatory activity of the leaf extract of Canthium angustifolium Roxb at a dose of 200mg/kg & 400mg/kg was evaluated against the standard drug, diclofenac sodium. The higher dose of the extract (400 mg/kg) exhibited the anti-inflammatory effect better than the effect of the lower dose of the extract (200 mg/kg). In the present study, administration of C. angustifolium Roxb extract (200 mg/kg and 400 mg/kg, p.o.) was found to inhibit the weight of cotton pellet and the higher dose of the extract (400 mg/kg) exhibited inhibition of inflammation close to the inhibitory effect of dexamethasone and better than the effect of the lower dose of the extract (200 mg/kg)

INTRODUCTION

Inflammation is a protective, response involving host cells, blood vessels, and proteins and other mediators that is intended to eliminate the initial cause of cell injury, as well as the necrotic cells and tissues resulting from the original insult, and to initiate the process of repair. Inflammation can be acute or chronic. Acute inflammation is rapid in onset and of short duration, lasting from a few minutes to as long as a few days, and is characterized by fluid and plasma protein exudation and a predominantly neutrophilic leukocyte accumulation. Chronic inflammation may be more insidious, is of longer duration (days to years), and is typified by influx of lymphocytes and macrophages with associated vascular proliferation and fibrosis (scarring)1. Inflammation results in the liberation of endogenous mediators like histamine, serotonin, bradykinin, prostaglandins etc. These mediators even in small quantities can elicit pain response. The greatest disadvantage in presently available

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potent synthetic drugs for the treatment of inflammation lies in their toxicity and reappearance of symptoms after discontinuation. Therefore the screening and development of drugs for their anti-inflammatory activity is still in progress and there is much hope for finding anti-inflammatory drugs from indigenous medicinal plants. There is a growing interest in the pharmacological evaluation of various plants used in Indian traditional systems of medicine.2

Canthium angustifolium Roxb is extensively used in Indian traditional medicine for several diseases including inflammatory disorders but there is no scientific evidence available for such activity. Based on the traditional uses the aim of present study was to investigate the anti-inflammatory activity of leaf extract of Canthium angustifolium Roxb on wistar rats.

MATERIALS AND METHODS

Plant material:

The plant material was collected from Cheruvandoor, Kerala and taxonomic identification of sample was confirmed by C.M.S College, Kottayam (Voucher specimen no:274).



Fig 1: Canthium angustifolium

Preparation of Plant extract:

Fresh leaves were collected and dried at room temperature to remove moisture, and size reduced. Extraction of the dried leaves of Canthium angustifolium Roxb was carried out by soxhalation by ethanol (TEE).

Animals used:

Female albino Wistar rats (220-250)g and Male Wistar albino rats (150-200)g were housed in standard environmental conditions. Food and water were available ad libitum. Female albino Wistar rats were used for the acute toxicity study in accordance with OECD guidelines 423. Male Wistar albino rats were used for screening anti-inflammatory activity of Canthium angustifolium Roxb. All the experiments were done after approval by the IAEC (Institutional animal ethical committee) of College of Pharmaceutical Sciences, Cheruvandoor. (IAEC No: 020/MPH/UCP/CVR/14).

Carrageenan induced paw oedema:

Male Albino wistar rats with a body weight between 150-200g were used. The animals were starved overnight, water given ad libitum. Group I receives vehicle (CMC), Group II receives Diclofenac Na 10mg/Kg and group III and group IV receives 200mg/Kg and 400mg/kg of total ethanolic extract of Canthium angustifolium Roxb respectively. Thirty minutes later, the rats were challenged by subcutaneous injection of 0.1ml of 1% solution of carrageenan in saline into the plantar region of the left hind paw. The paw was marked with ink at the level of lateral malleolus. The readings were measured by immersing the paw in mercury upto the mark. The paw volume were measured plethismographically immediately after the injection and again 1, 2 and 3hour after challenge.

Percentage inhibition of paw edema

$$= \frac{(Vt-V0)control - (Vt-V0)test \times 100}{(Vt-V0)control}$$

Vt is the rat paw volume at time t, V0 is the initial rat paw volume (before carageenan injection), (Vt-Vo) is edema produced in control group and (Vt-V0) treated is edema produced in treatment groups. The difference in the average values between treated animals and control groups were calculated for each time interval and statistically evaluated3.

Cotton pellet granuloma:

The rats were anaesthetized with diethyl ether and a sterilized cotton pellet weighing approximately 10±1mg was inserted in the subcutaneous layer of groin. The incised skin was properly sutured. The control group receives vehicle (CMC); Standard group receives dexamethasone (0.5 mg/Kg) p.o; Test group animals were treated with the total ethanolic extract of Canthium angustifolium Roxb (200mg/Kg & 400mg/kg p.o) once a day for 7 continuous days. On 8th day animals were sacrificed by cervical dislocation and the pellets along with granuloma mass were removed, washed and taken the wet weight. Then the granuloma mass were dried at 60°C for 24 h in an oven and taken the dry weight. On 0th day and 8th day (before sacrificing) blood was withdrawn from each animal by retro orbital puncture for evaluating the leucocyte count4. Weight of the granuloma mass was calculated as percentage and compared the weight of treated group with control group.

$$\% \text{ inhibition} = \frac{Wc - Wd}{Wc} \times 100$$

Wc – Difference in pellet weight of control

Wd- Difference in pellet weight of treated group

Statistical analysis

All data were expressed as mean ± SEM and analyzed by One-way analysis of variance (ANOVA) followed by Dunnet's test. P< 0.001 was considered statistically significant.

Table 1: Anti-inflammatory activity study using carrageenan induced paw oedema model

Treatment	Mean paw edema volume in M±SEM				Percentage inhibition		
	0 hr	1 hr	2 hr	3 hr	1 hr	2 hr	3 hr
Control	0.85 ±0.0226	1.6± 0.03651	1.767±0.03333	1.883± 0.03073	- -	- -	- -
Diclofenac (10mg/kg)	0.85 ±0.0226	1.333* ±0.07032	1.117*** ±0.05456	0.8833*** ±0.03073	39.33	62.583	96.776
Low dose (200mg/kg)	0.85 ±0.0226	1.483 ±0.07032	1.333** ±0.08433	1.083*** ±0.07032	15.6	39.028	80.744
High dose (400mg/kg)	0.85 ±0.0226	1.4* ±0.04472	1.15*** ±0.05627	1.033*** ±0.06146	26.667	58.985	85.585

Values are Mean+SEM, n=6. ANOVA followed by multiple comparison by Dunnet's test. *P<0.05, **P<0.01, ***P<0.001 was considered as significant when compared to positive control

Graph1 : Inhibition of paw edema by std and extracts:

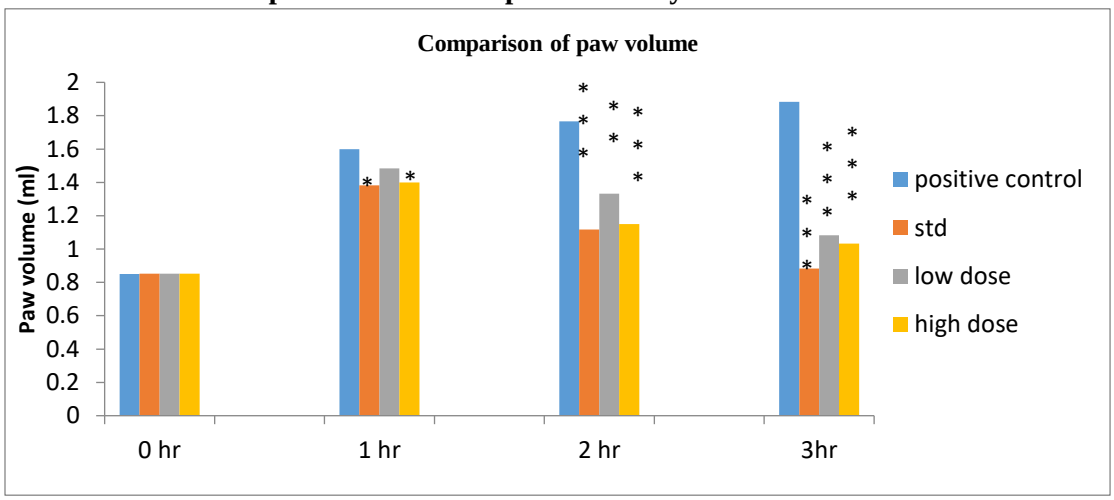
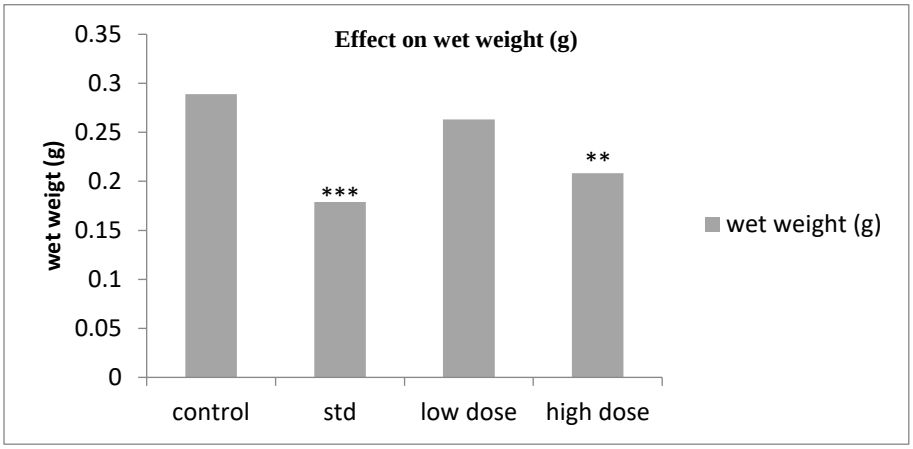


Table2: Effect of TEE on weight of granuloma in cotton pellet inserted animals

Group	Wet weight (g)	% Inhibition	Dry weight (g)	% Inhibition
Control	0.289±0.01076	-	0.09833±0.00833	-
Dexamethasone treated	***0.1788±0.003525	38.1314	*** 0.045±0.00224	54.23
Low dose (200mg/kg)	0.2633±0.01764	8.89	* 0.0733±0.00882	25.45
High dose (400mg/kg)	** 0.2083±0.00833	25.45	** 0.055±0.00428	44.065

Values are mean ± SEM, n=6. ANOVA followed by multiple comparison by Dunnet's test. *p< 0.05, **p<0.01, ***p<0.001 was considered as significant when compared to positive control.

Graph2 : Effect OF TEE on wet granuloma weight:



Graph 3: Effect on dry granuloma weight

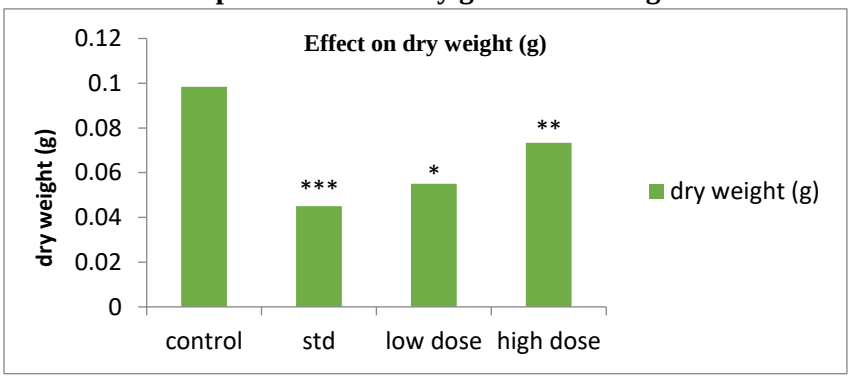
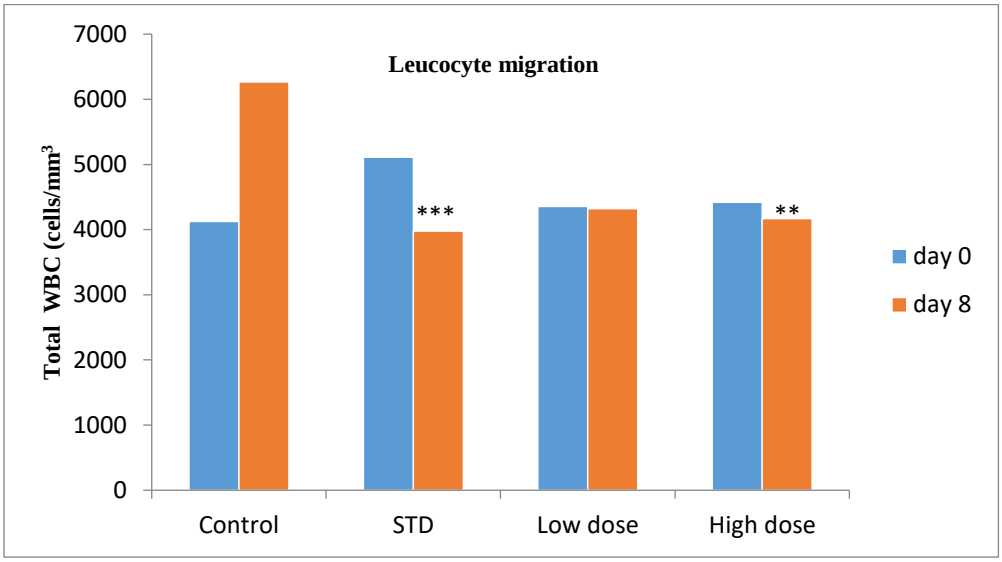


Table3 : Effect of extract on leucocyte migration

Groups	Day 0	Day 8
Control	4195±129.7	6266±62.31
STD- Dexamethasone	5110±34.86	3977±39.18***
Low dose (200mg/kg)	4356±98.77	4321±95
High dose (400mg/kg)	4419±67.44	4168±26.39**

Values are mean ± SEM, n=6. ANOVA followed by multiple comparison by Dunnet's test. *p< 0.05, **p<0.01, ***p<0.001 considered as significant when compared to positive control.

Graph 4 : Effect on leucocyte migration



RESULTS AND DISCUSSIONS

The phytochemical analysis of Canthium angustifolium Roxb leaf extract revealed the presence alkaloids, carbohydrates, saponins, flavonoides, tannins and phenolic compounds. The result shows that the extract at dose of 200 mg/kg and 400 mg/kg has significant reduction in the carrageenan induced paw edema (P< 0.001) in a dose dependent manner when compared to control. In cotton pellet induced granuloma model of inflammation, the results show a marked protection in granuloma by markedly reducing the weight of the cotton pellet at a dose of 200 mg/kg and 400 mg/kg when compared to control (P< 0.001). The higher dose of the extract (400 mg/kg) exhibited inhibition of inflammation close to the inhibitory effect of dexamethasone and better than the effect of the lower dose of the extract (200 mg/kg). The most widely used primary test to screen new anti-inflammatory agents measures the ability of a compound to reduce local edema induced in the rat paw by an injection of an irritant agent. This edema depends on the participation of kinins and polymorphonuclear leucocytes with their pro-inflammatory factors including prostaglandins. Carrageenan induced rat paw edema is a suitable test for evaluating anti-inflammatory drugs which have frequently been used to assess the anti-edematous effect of natural products Development of edema in the paw of rat after carrageenan injection is a biphasic event. Initial phase observed during the first hour is attributed to the release of histamine and serotonin. The second phase of edema is due to the release of prostaglandins, protease and lysosome. Carrageenan-induced edema is characterized by the presence of PGs and other compounds of slow reaction. COX-2 is an inducible isoform found in activated inflammatory cells that generates prostanoid mediators of inflammation. The result of the present study indicates that C.angustifolium Roxb (200mg/kg and 400 mg/kg, p.o.) plays a crucial role as protective factors against the carrageenan-induced acute inflammation. The cotton pellet-induced granuloma is widely used to assess the transudative and proliferative components of chronic inflammation. The weight of the wet cotton pellets correlates with transude material and the weight of dry pellet correlates with the amount of granulomatous tissue2. In the present study, administration of C. angustifolium Roxb extract (200 mg/kg and 400 mg/kg, p.o.) was found to inhibit the weight of cotton pellet in a dose dependent manner.

During the inflammatory process migration of WBC takes place which is the biological marker.

Extract and Dexamethasone treated animals showed a significant reduction in the leukocytes migration as compared with control. These findings also strengthen the anti-inflammatory activity of TEE of Canthium angustifolium Roxb.

CONCLUSION

The plant Canthium angustifolium Roxb was traditionally claimed for a large number of pharmacological action and medicinal use. In the present study it was found that the ethanolic extract of the leaves of plant is safe up to 2000mg/kg. The significant and better anti-inflammatory activity was attributed at a dose 400mg/kg to the phytoconstituents present in it. Further phytochemical studies are needed to isolate the active compounds responsible for these pharmacological activities.

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