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Research Article

ANTI-INFLAMMATORY ACTIVITY OF VARIOUS EXTRACTS OF ROOTS OF *CALOTROPIS PROCERA* AGAINST DIFFERENT INFLAMMATION MODELS

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ABSTRACT

The methanolic extract of plant *Calotropis procera* (Asclepiadaceae), has been reported to exhibit potent anti-inflammatory activity against carrageenan induced paw oedema and cotton pellet induced granuloma in albino Wistar rats. In the present study we have evaluated the efficacy of successive soxhlet extracts prepared from the roots of *C.Procera* against inflammation induced by different models. The paw oedema was induced by the sub plantar injection of carrageenan aqueous solution (0.1ml of 1%in saline) into the plantar side of the hind paw and the paw volume is measured plethysmographically immediately after injection, again 0.5 h, 1 h, 2 h, 3 h, 4 h and eventually 5h after challenge. The latter model was characterized for granulomatous lesions were induced by surgically implanting two cotton pellets subcutaneously in the dorsal region of the rats, one near each axilla in rats. The different extracts of the roots of *C procera* and standard anti-inflammatory drugs were administered orally 1 hour before inducing of inflammation. The increase of paw volume after 3 or 5 h is calculated as Mean±SEM, compared with the volume measured immediately after injection of the irritant for each animal. Thus, in preview of this results indicates that methanolic extracts (180mg/kg.p.o) of roots of *C.Procera* has potential to inhibit sub-acute inflammation by interruption of the arachidonic acid metabolism in both paw oedema as well as cotton pellet model and shows inhibition of inflammation ($^{**}p<0.01$and $^{***}p<0.001$) very close to the inhibitory effect of diclofenac sodium (25 mg/kg i.p).The results of this study indicated that the methanolic extracts from roots of *C.Procera* possess significant anti-inflammatory activity in rodent models.

Keywords: *C.Procera*, Anti-inflammatory, Carragenan, Cotton pellet, Paw Oedema.

INTRODUCTION

Calotropis procera Ait. (Asclepiadaceae) is a wild shrub, well known for its medicinal as well as toxic properties. Different parts of the plant have been used as a purgative, anthelmintic, treatment of ulcers, tumors and piles[1]. It has been reported to produce congestion of eyes, iridocyclitis and dermatitis following accidental exposure[2-4].The plant produces milky white latex which irritates mucous membranes has been shown to possess significant anti-inflammatory activity against carrageenan, formalin induced paw oedema and antipyretic effect [5-6]. It was found that, this plant contains flavonoids, alkaloids, cardiac glycosides, tannins, sterols and triterpenes[7]. There are reports showed that flowers possess anti-inflammatory, antipyretic, analgesic, antimicrobial properties and larvicidal activity [8-9].The latex of the plant was reported to possess analgesic and wound healing activity [10-11].The roots are reported to have anti-fertility and anti-ulcer activities[12-13]. However there is no scientific basis or reports in the modern literature regarding its usefulness as anti-inflammatory agent. Thus the present study was conducted to evaluate the anti-inflammatory activity of the methanolic extract of the *C.procera* roots by using paw oedema and cotton pellet-induced inflammation in rats.

EXPERIMENTAL

Plant

The Fresh matured roots of *C.Procera* were collected locally from the suburban out fields of Bangalore, province of India. The identity of *C.procera* was authenticated was authenticated by Dr.Siddamallayya from Regional Research Institute, Bangalore, on the basis of taxonomical characters following routine pharmacognostical studies, including organoleptic macroscopic tests and herbarium specimen was deposited in the department of herbarium. The roots were air dried under shade and further subjected to extraction.

Preparation of extract

The collected *C.Procera* roots were air dried under shade at room temperature and milled to a coarse powder. The obtained dried powder was subjected to successive soxhlet extraction with pet. Ether, benzene, chloroform, methanol and aqueous solvents respectively. The powdered root material was packed in a tumble made of Whatmann's filter paper. It was subjected to extract with various non-polar to polar solvents for 40 cycles each. The extract thus obtained was concentrated to dryness in a flash evaporator under reduced pressure and controlled temperature. The yield of pet. Ether, benzene, chloroform, methanol and aqueous extract of roots of *C. Procera* was 4.11%, 2.55%, 3.22%, 10.11% and 5.25% respectively. The obtained residues were yellowish brown to dark brown colour with thick and sticky paste. The extract was stored in refrigerator (2-8^{0}C) and reconstituted uniformly in 0.1 mL of 1% saline before administration to animals orally using an intragastric feeding tube.

Animals

Adult rats of wistar strain of either sex weighing 125-175 g and 10 - 12 weeks old were obtained from National Institute of Mental Health and Neuro Sciences. They were fed commercial pellet diet and water ad libitum. The diet approximately contained: carbohydrate (55%), fat (5%), protein (24%), fiber (4%), calcium (0.6%), phosphorous (0.3%), moisture (10%) and ash (9%). Before treatment allocation and randomization rats were acclimatized to the laboratory conditions for a week. Animals were housed in polypropylene cages (38×23×10cm) with not more than four animals per cage under standard laboratory conditions (25^0 C ± 2^0 C), relative humidity 55 ± 10%, alternating 10 h dark/ 14 h light photoperiod. Approval from the institutional animal ethical committee for the usage of animals in the experiments was obtained and conducted in accordance to the Indian national science academy guidelines for the use and care of experimental animals.

Rat paw oedema test

The doses of root extracts of *C.Procera* (Table-1) were administered orally using an intragastric feeding tube. The rats of the control group were administered with the same volume of vehicle. The paw volumes up to a fixed mark at the level of lateral malleolus were measured by recording the volume displacement of a water mercury column just before and 0.5, 1, 2, 3, 4 and 5 h after the administration of root extracts. The standard anti-inflammatory drug (Diclofenac sodium, 25 mg/kg.i.p) was given 1 h before the root extracts administration and the oedema volume was compared with the control group after 90 and 180 min [14-15]. Paw oedema was induced by injecting carrageenan (0.1 mL of 1% solution in saline). The anti-

inflammatory effect of different root extracts of *C.Procera* were compared with that of standard anti-inflammatory drug administered intraperitonially. The anti-inflammatory activity was expressed as percent inhibition against the respective control.

Cotton pellet-induced granuloma inflammation

Winter CA et al[16], described a technique, granulomatous lesions were induced by surgically implanting two cotton pellets subcutaneously in the dorsal region of the rats, one near each axilla. Different extracts of *C.Procera* was administered orally. After 20 min, autoclaved sterile pellets of cotton weighing 7 ± 1 mg each were aseptically implanted in the interscapular distance under the skin on the previously shaved back of the rats which were anesthetized with thiopental sodium (25 mg/kg, i.p.). The rats of the control group were administered with the same volume of vehicle. The rats sacrificed on the 8th day and the pellets surrounded by granuloma tissue were dissected out carefully and dried at 70^{0}C.Mean weight of the granuloma tissue formed around each pellet was recorded. The pellets were weighted both moist and dry. The weight of the pellets taken out from *C.procera* extracts administered rats were compared with the weight of pellets taken out from the control group.

Drugs and chemicals

All chemicals used in present study were of analytical grade. Diclofenac sodium, a widely used non steroidal anti-inflammatory drug (NSAID) was used as standard drug. For dosing, the different extracts of *C.procera* roots were suspended uniformly in 0.1 mL of 1% saline and administered orally using an intragastric feeding tube.

Animal experimentation and drug treatment protocol

Rats were randomly divided into seven experimental groups, each consisting of six rats and were treated as follows.

Group I: Vehicle treated control animals received 0.1 mL of 1% solution in saline.

Group II: Animals administered diclofenac sodium, 25 mg/kg, i.p

Group III: Animals received pet. Ether extract of *C.Procera* 200 mg/kg, p.o.

Group IV: Animals received benzene extract of *C.Procera* 200 mg/kg, p.o.

Group V: Animals received chloroform extract of *C.Procera* 200 mg/kg, p.o,

Group VI: Animals received methanol extract of *C.Procera* 180 mg/kg, p.o.

Group VII: Animals received aqueous extract of *C.Procera* 200 mg/kg, p.o.

Statistical analysis

All the grouped data were statistically evaluated by using Graph Pad Prism software. The results are expressed as the Mean±SEM. (n=6).The results were analyzed for statistical significance using one-way analysis of variance fallowed by *Dunnett's t*' with P<0.01 considered significant.

RESULTS

Inflammatory response of the paw oedema to different extracts of roots of *C.Procera* and its inhibition by anti-inflammatory drugs

The anti-inflammatory activity of root extracts of *C.Procera* was evaluated by injection of carrageenan into the sub plantar surface of hind paw in albino rats and produced a marked increase in paw volume as compared to the saline control. In carrageenan-induced paw oedema model, roots of *C.Procera* at doses of 180 mg/kg b.w (methanol extract) and 200 mg/kg (other extracts) were selected and the response was measured at 0.5, 1, 2, 3, 4 and 5 hrs respectively. Diclofenac sodium 25 mg/kg b.w given intraperitonially 1 hr before the injection of extracts, produced significant decrease in the inflammatory response (P<0.01).The oedema volume in diclofenac sodium treated animals was 4.58±0.13, 4.85 ±0.07, 5.45±0.16, 4.17±0.15, 5.17±0.13 and 5.07±0.06 mL at 0.5, 1, 2, 3, 4 and 5 hrs as compared to 5.04±0.08, 5.91±0.05, 6.60±0.08, 6.89±0.04, 6.90 ±0.03 and 5.97±0.04 mL in the control group. Further it has been found that methanolic extract of roots of *C.Procera* shows significant anti-inflammatory activity compared to other extracts. (Table-1, Fig-1)

Table 1: Paw volume of various extracts of roots of *C. procera* against inflammation induced by carrageenan

Group (n=6)	Dose (mg/ kg b.w)	Paw volume in mL Mean ± SEM					
		0.5 hr	1 hr	2 hr	3 hr	4 hr	5 hr
1.	Control	5.04±0.08	5.91±0.05	6.60±0.08	6.89±0.04	6.90 ±0.03	5.97±0.04
2.	Carrageenan +Diclofenac sodium[25]	4.58 ±0.13	4.85 ±0.07**	5.45±0.16**	4.17±0.04**	4.18±0.13**	4.19±0.06**
3.	Pet. Ether extract [200]+Carrageenan	5.02±0.13	5.78±0.23	6.46±0.43	6.69±0.01	6.78.±0.14	5.9±0.13
4.	Benzene extract [200]+ Carrageenan	5.19±0.34	5.69±0.23	5.87±0.14	6.79±0.42	6.49±0.13	5.87±0.01
5.	Chloroform extract[200]+ Carrageenan	5.01±0.04	5.61±0.01*	5.41±0.13**	5.01±0.23**	6.43±0.01	5.69±0.04
6.	Methanol extract [180]+ Carrageenan	5.1±0.16	5.66±0.06*	5.21±0.21**	4.87±0.09**	6.25±0.16**	5.32±0.32
7.	Aqueous extract [200]+ Carrageenan	5.19±0.32	5.58±0.11**	5.34±0.43**	4.64±0.11**	5.0±0.13**	5.35±0.21

All the values were analysed using ANOVA followed by *Dunnett's t*' test and expressed as Mean ± SEM, *p < 0.05, **p < 0.01. All the groups were compared with control.

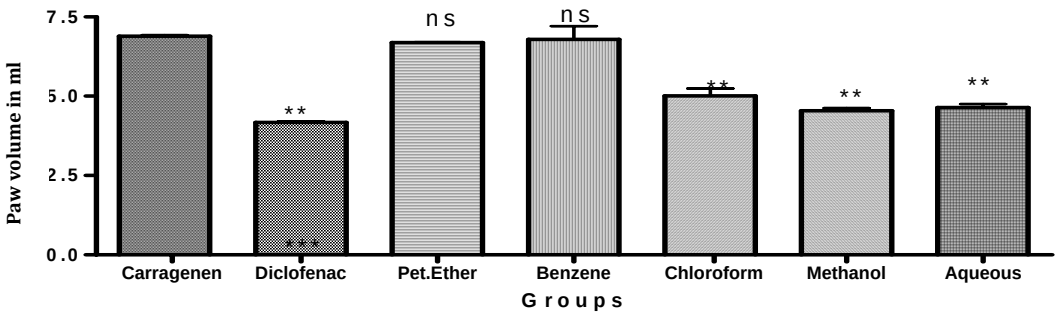

Fig. 1: Anti-inflammatory effect of various extracts of roots of *C.Procera* against inflammation induced by carragenan

Inflammatory response of cotton pellet-induced granuloma to different extracts of roots of *C. procera* and its inhibition by anti-inflammatory drugs

The extracts of roots of *C.Procera* has been found to reduce the weight of cotton pellet granuloma in a dose of 180 mg/kg.b.w (Methanol extract) and 200 mg/kg.b.w for other extracts in the cotton pellet induced model of inflammation. The reduction in the weight of cotton pellet granuloma with different extracts of pet. Ether, benzene, chloroform, methanol and aqueous was found 10.25, 7.06, 29.47, 50.74 and 44.11 % respectively, (Fig-2, Table-2). However, the decrease in inflammation by *C.Procera* of methanolic extract of the root at 180 mg/kg.b.w was comparable to diclofenac sodium 25 mg/kg b.w, which reduced the weight of cotton pellet granuloma by 55.68% .In pharmacological studies, number of medicinal plants demonstrated anti-inflammatory properties, which helped into the management of inflammatory diseases, especially rheumatism through inhibition of synthesis of cellular prostates[17]

Table 2: Percentage inhibition in mean weight of cotton pellet in various extracts of roots of *C.Procera*

Group n=6	Dose(mg/kg b.w.)	Mean ± SEM	% Inhibition
1	Control	14.01 ± 0.07	--
2	Diclofenac sodium(25)	6.21 ± 0.05***	55.68
3	Pet. Ether extract (200)	14.00 ± 0.78	10.25
4	Benzene extract (200)	13.02 ± 0.73	7.06
5	Chloroform extract (200)	9.88 ± 0.43**	29.47
6	Methanol extract (180)	6.9 ± 0.01***	50.74
7	Aqueous extract (200)	7.83± 0.12**	44.11

All the values were analyzed using ANOVA followed by Dunnett's t'' test and expressed as Mean ± SEM, *p < 0.05, **p < 0.01. ***p < 0.001.All the groups were compared with control

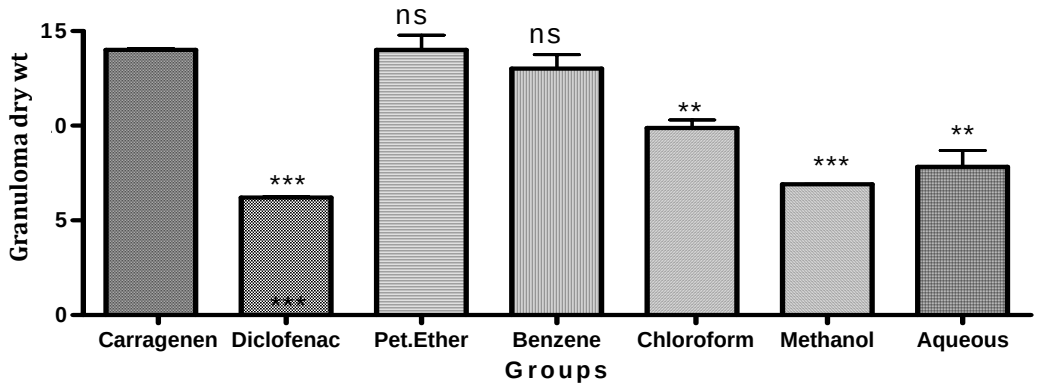

Fig. 2: Anti-inflammatory effect of various extracts of roots of *C. procera* against inflammation induced by Cotton pellet technique

DISCUSSION AND CONCLUSION

The present study was carried out to characterize the anti-inflammatory activity of different extracts of roots of *C.Procera* against various inflammatory mediators using pharmacological reagents. Carrageenan has been widely used as an inflammagen to induce experimental inflammation for the screening of compounds possessing anti-inflammatory and anti-rheumatic activity[18]. The cotton pellet-induced granuloma is widely used to assess the transudative and proliferative components of chronic inflammation[16]. The weight of the wet cotton pellets correlates with transude material and the weight of dry pellet correlates with the amount of granulomatous tissue. It is well known fact that diclofenac sodium act by inhibiting the prostaglandins synthesis at the late phases of inflammation. This effect may be due to the cellular migration to injured sites and accumulation of collagen an important mucopolysaccharide[19].The greater efficacy of methanolic extracts of roots of *C.Procera* could be attributed to constituents present in methanol extract other than those present in Pet.Ether, benzene, chloroform and aqueous extracts. Thus, in preview of this results indicates that methanolic extracts of roots of *C.Procera* has potential to inhibit sub-acute inflammation by interruption of the arachidonic acid metabolism in both paw oedema as well as cotton pellet model and shows inhibition of inflammation very close to the inhibitory effect of diclofenac sodium. Present study finding supports the traditional claims and provides a scientific basis for anti-inflammatory.

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