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Histamine mediates the pro-inflammatory effect of latex of *Calotropis procera* in rats

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INTRODUCTION: Calotropis procera **is known to produce contact dermatitis and the latex of this plant produces intense inflammation when injected locally. However, the precise mode of its pro-inflammatory effect is not known. In present study we have pharmacologically characterized the inflammation induced by latex of *C. procera* in a rat paw edema model and determined the role of histamine in latex-induced inflammation.**

Methods: **Inflammation was induced in the hind paw of rats by injecting different doses of dried latex (DL) of *C. procera*. The inhibitory effect of phenylbutazone, dexamethasone, celecoxib, cyproheptadine, chlorpheniramine and compound 48/80 on edema volume was evaluated and compared with that against carrageenan. The histamine content of DL was measured fluorometrically.**

Results: **DL produced dose-dependent inflammation of the rat paw. Cyproheptadine and chlorpheniramine effectively inhibited DL-induced inflammation (90%; *p* < 0.01), while anti-inflammatory drugs phenylbutazone, dexamethasone and celecoxib were more effective against carrageenan-induced inflammation. Depletion of mast cell histamine by compound 48/80 produced a significant decrease in DL-induced inflammation as compared with carrageenan (50% versus 25%). DL was also found to contain about 6 µg/g of histamine.**

Conclusions: **Thus, our study shows that the biogenic amines play a significant role in *C. procera* latex-induced inflammation and antihistaminic drugs could be effectively used to inhibit inflammatory response elicited by exposure to latex.**

Key words: Calotropis procera, Carrageenan, Histamine, Inflammation, Anti-inflammatory drugs

Introduction

Calotropis procera (Ait). R. Br., a wild growing plant, has been used in Indian folk medicine for the treatment of various ailments such as rheumatism, aches, gastrointestinal disorders, ulcers, piles and tumors.[1,2] However, accidental exposure to the latex causes congestion of eyes, iridocyclitis and dermatitis.[3,4] Although antihistaminic drugs and corticosteroids have been used in the management of hypersensitive response elicited by latex, the mechanism and the mediators involved in such a response remain to be identified.[5] When injected locally, the latex produces an intense inflammatory response that could be used to screen anti-inflammatory drugs.[6] Other agents that are known to induce inflammation include dextran, formalin, yeast, turpentine and carrageenan.[7–10] Of all these agents the inflammatory response elicited by carrageenan has been well characterized and it has been widely used to induce footpad edema for the screening of anti-inflammatory drugs. In this study we have characterized and compared the inflammation produced by the latex of *C. procera* and carrageenan in a rat paw edema model. Furthermore, we have also evaluated the role of histamine in latex-induced inflammation and measured the content of histamine in the latex.

Materials and methods

Preparation of plant extract

The latex of *C. procera* was collected from the aerial parts of the plant and dried under shade (dried latex (DL)). It was triturated in normal saline (NS) and centrifuged to obtain a clear solution.

Drugs and animals

The drugs used in this study were obtained from SG Pharmaceuticals, Baroda, India (phenylbutazone),

Alembic Ltd, Baroda, India (celecoxib), Wyeth Lab. Ltd, Mumbai, India (dexamethasone) and Sigma, St Louis, Missouri, USA (cyproheptadine, chlorpheniramine). Carrageenan was procured from Spectrochem (Mumbai, India).

The study was carried out on male Wistar rats weighing 120–150 g. They were kept at ambient temperature and had free access to water and diet. The animal experiments were carried out in accordance with the guidelines of Institutional Animal Ethics Committee.

Rat paw edema model

The aqueous extract of DL (0.1 ml) was injected into the plantar surface of the hind paw of rats in different concentrations: 1%, 2.5% and 5% (1 mg, 2.5 mg and 5 mg, respectively, in 0.1 ml of NS). The paw volume was measured up to a fixed mark on the lateral malleolus using a plethysmometer with a sensitivity of 0.01 ml, just before (0 h) and after 30 min, 1 h, 2 h, 3 h, 4 h and 5 h of injecting DL. The edema volume at different time intervals was calculated by subtracting the paw volume at 0 h from the paw volume obtained at different time intervals. The effect was compared with that of 1% carrageenan (1 mg in 0.1 ml of NS). The standard anti-inflammatory drugs, phenylbutazone, a non-selective cyclooxygenase (COX) inhibitor (100 mg/kg), dexamethasone, a glucocorticoid (3 mg/kg), celecoxib, a selective COX-2 inhibitor (30 mg/kg), cyproheptadine, a $5HT_{2A}$ and H_1 receptor antagonist (3 mg/kg), and chlorpheniramine, a H_1 receptor antagonist (3 mg/kg), were given orally as a suspension with 5% gum acacia 1 h before injecting DL. The effect of these drugs was evaluated against DL (2.5 mg) and carrageenan (1 mg) at 90 and 180 min. The anti-inflammatory activity of these agents was expressed as percent inhibition of edema obtained in respective control groups.

Depletion of histamine and 5-HT

To study whether DL-induced inflammatory response involves the release of biogenic amines like histamine and serotonin, the rats were treated with compound 48/80.[10] Compound 48/80 was administered intraperitoneally twice a day for 4 days as a 0.1% solution in NS. The doses employed were 0.6 mg/kg for the first six doses and 1.2 mg/kg for the remaining two injections. The phlogestic agent, DL or carrageenan was injected 6 h after the last dose of compound 48/80. The edema volume was measured at 90 and 180 min and compared with the control group.

Assay of histamine

Extraction of histamine from DL and its estimation was carried out by the method described by Shore *et*

al.[11] Briefly, the aqueous extract of DL was treated with perchloric acid and extracted with *n*-butanol. The extracted histamine was condensed with *o*-phthalaldehyde in strongly alkaline solution and the resulting product was stabilized by acidification and measured fluorometrically.

Statistical analysis

The values were expressed as mean ± standard error of the mean of six observations and statistical analysis was carried out by Student's *t'* test. Differences with $p < 0.05$ were considered statistically significant.

Results

Inflammatory response of the paw to different doses of latex of *C. procera* and carrageenan

DL when injected into the hind paw of rats, at doses ranging from 1 to 5 mg, produced an intense inflammatory response that was dose dependent. The effect was discernible as early as 15 min and a maximum inflammation was obtained at 1 h. The peak effect was maintained until the second hour, followed by a decline. The inflammation induced by 1 mg of carrageenan was comparatively slower in onset and a peak effect was attained in 3 h (Fig. 1).

Inhibition of DL-induced paw edema

The effect of various anti-inflammatory drugs was studied against inflammation induced by 2.5 mg of DL at 90 and 180 min. The drugs were given orally 1 h before injecting DL. Phenylbutazone produced a significant decrease (52%) in edema volumes at 90 min (0.12 ± 0.02 ml versus 0.25 ± 0.02 ml in control, $p < 0.01$). However, the inhibitory effect was only
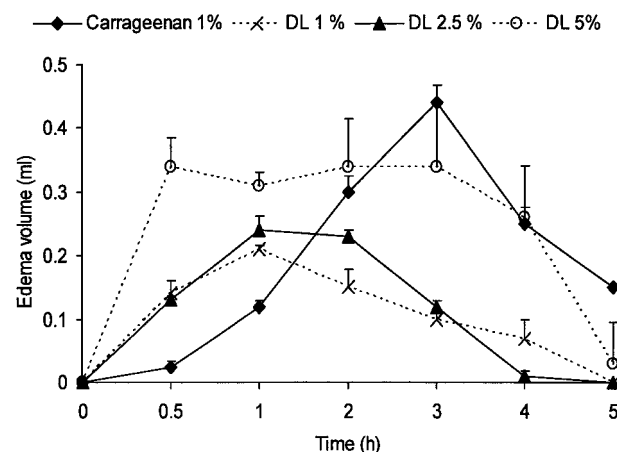

FIG. 1. Time course for inflammation induced by different doses of DL of *C. procera* and carrageenan. Aqueous extracts of DL and carrageenan were injected into the subplantar surface of the rat paw and the paw volume was measured at 0, 0.5, 1, 2, 3, 4, and 5 h ($n = 6$).

15% at 180 min. Dexamethasone and celecoxib were less effective against DL induced inflammation as compared with phenylbutazone. Dexamethasone produced only 24% inhibition of edema formation while the effect of celecoxib was marginal (only 16% at 90 min). The inhibitors of biogenic amines cyproheptadine and chlorpheniramine were more potent in inhibiting DL-induced inflammation. The edema volume in the cyproheptadine-treated group was only 0.03 ± 0.01 ml against 0.25 ± 0.02 ml in the control (88% inhibition; $p < 0.01$), and in the chlorpheniramine-treated group it was 0.09 ± 0.02 ml (64% inhibition; $p < 0.01$) (Table 1).

Inhibition of carrageenan-induced paw edema

Table 2 shows the effect of various anti-inflammatory agents given orally against carrageenan-induced paw edema at 90 and 180 min. Both phenylbutazone and dexamethasone were equi-effective in this regard and produced 61% inhibition of edema ($p < 0.01$). Celecoxib produced only marginal inhibition of 6% at 90 min, which increased to 49% at 180 min. Cyproheptadine and chlorpheniramine were not so effective in inhibiting carrageenan-induced inflammation. Cyproheptadine produced approximately 15% inhibition while chlorpheniramine produced 30% inhibition of carrageenan-induced edema.

Inflammatory response of paw following depletion of biogenic amines

Biogenic amines (namely, histamine and serotonin) were depleted by treating the rats with compound 48/80, and the inflammatory response to DL and carrageenan was compared with respective controls. The edema formation in response to DL was reduced by 52–54% while that of carrageenan was reduced by only 6–25%.

Estimation of histamine in DL and carrageenan

To see whether inflammation induced by DL is due to presence of histamine, extraction and estimation of histamine was carried out. DL was found to contain

6.13 ± 1.3 µg/g of histamine, while it was undetectable in carrageenan.

Discussion

In the present study we have characterized and compared the inflammatory response of rat paw to DL and carrageenan. Both DL and carrageenan, when injected into the paw, produced inflammation with a different time course. The inflammation induced by DL was rapid in onset and short lasting as compared with carrageenan, suggesting thereby that different mediators are involved in these responses. The inhibitory effect of phenylbutazone against DL and carrageenan was comparable at 90 min; however, it persisted up to 180 min in case of carrageenan-induced inflammation. The early inhibitory effect of phenylbutazone on DL-induced inflammation was due to its ability to inhibit vascular permeability, while the inhibition of carrageenan-induced inflammation at 180 min could be due to inhibition of prostaglandin synthesis.[12] Prostaglandins are considered to be released during the later phase of inflammation due to induction of COX-2.[13] Our study indicates that prostaglandins play a significant role in carrageenan-induced inflammation as the effect could be blocked by celecoxib to the extent of 49% at 180 min while it was ineffective against DL-induced edema. This was further substantiated by the effect of dexamethasone, which was found to be more effective against carrageenan-induced edema. Besides inhibiting the prostaglandin pathway, steroids are also known to inhibit other mediators of inflammation; namely, nitric oxide, interleukins and tumor necrosis factor-α.[14] Clearly, dexamethasone was less effective against DL-induced edema.

The acute inflammation induced by DL appears to be similar to dextran-induced and Brewer's yeast-induced inflammation, both in terms of rapid onset and sensitivity to antihistaminic and anti-serotonergic drugs.[7,9] Our study strongly suggests the involvement of histamine in DL-induced inflammation since it was effectively blocked by cyproheptadine (5-HT$_{2A}$ and H$_1$ blocker) and chlorpheniramine (H$_1$ blockers). Cyproheptadine was more effective

Table 1. Anti-inflammatory effect of various drugs administered orally 1 h before injecting DL into the rat paw

Treatment	90 min		180 min	
	Edema volume (ml)	% inhibition	Edema volume (ml)	% inhibition
Control	0.25 ± 0.02		0.13 ± 0.01	
Phenylbutazone	$0.12 \pm 0.02**$	52	0.11 ± 0.01	15
Dexamethasone	0.19 ± 0.04	24	$0.08 \pm 0.03*$	38
Celecoxib	0.21 ± 0.02	16	0.13 ± 0.02	0
Cyproheptadine	$0.03 \pm 0.01**$	88	$0.01 \pm 0.01**$	92
Chlorpheniramine	$0.09 \pm 0.02**$	64	$0.05 \pm 0.01*$	61

The edema volume was measured at 90 and 180 min. * $p < 0.05$, ** $p < 0.01$.

Table 2. Anti-inflammatory effect of various drugs administered orally 1 h before injecting carrageenan into the rat paw

Treatment	90 min		180 min	
	Edema volume (ml)	% inhibition	Edema volume (ml)	
Control	0.36 ± 0.03		0.47 ± 0.02	
Phenylbutazone	0.14 ± 0.04**	61	0.22 ± 0.05**	53
Dexamethasone	0.14 ± 0.04**	61	0.17 ± 0.05**	64
Celecoxib	0.34 ± 0.04	6	0.24 ± 0.02**	49
Cyproheptadine	0.31 ± 0.03	14	0.39 ± 0.03	17
Chlorpheniramine	0.25 ± 0.01	31	0.32 ± 0.01*	32

The edema volume was measured at 90 and 180 min. * $p < 0.05$, ** $p < 0.01$.

against DL as compared with chlorpheniramine as it inhibits various other mediators involved in inflammation.[15] We further studied the role of biogenic amines, histamine and 5-HT stored in mast cells in DL-induced inflammation. Rats were depleted of their histamine and 5-HT stores by treating with compound 48/80 and the inflammagens were injected into the paw. It was interesting to note that the inflammatory response to DL was reduced by more than 50% while a marginal reduction was observed in the case of carrageenan. We also tested the DL for presence of histamine. DL was also found to contain histamine while there was no trace of histamine in carrageenan. A number of other plants like *Tamus communis* and *Urtica urens* are also known to contain histamine and produce a local irritant effect on the skin.[16,17]

Thus, our study indicates that the mediators released in inflammation induced by DL and carrageenan are different. The inflammatory response brought about by carrageenan involves activation of prostaglandin pathway while DL produces inflammation both by releasing histamine from the mast cells and by virtue of presence of histamine in DL itself. Therefore, antihistaminic drugs could be effectively used in the management of inflammation induced by exposure to latex.

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