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Research Article

POTENTIAL ANTI-INFLAMMATORY ACTIVITY OF *PLUMBAGO ZEYLANICA*

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ABSTRACT

Objective: To determine the anti-inflammatory activity of dichloromethane extract of *Plumbago zeylanica* (DMEPZ), and its possible mechanism of action.

Methods: Male Wistar rats (180-200 g) under controlled standard conditions (24±1°C, 55-58 humidity and 12 hrs light/dark cycle). The groups were divided into 5 groups (n=6/group) and assigned as positive control, negative control, and standard and two different test dose groups of *P. zeylanica.* Paw edema induced by subplantar injection of 0.1 mL of carrageenan (suspended in 1% carboxymethyl cellulose) into the right hind paw in all groups except negative control group. Granuloma induced by cotton pellets (10±1 mg) were implanted into groin region of each rat. The groups were divided into 4 groups (n=6/group) and assigned as possitive control, two different test dose groups of *P. zeylanica* and standard.

Results: Oral administration of DMEPZ shown a significant ($p<0.05$) dose-dependent protection against carrageenan-induced paw edema. At 1st hr, *P. zeylanica* shown an inhibition effect of edema in the different doses of 250 mg/kg and 500 mg/kg were found to be 28.57 and 31.79%, respectively. At 3rd hrs, the paw edema inhibition was found to be 30.70 and 40.15%, respectively. Diclofenac (25 mg/kg) had effect of 34.10 and 41.73% ($p<0.001$) inhibition of paw edema at in 1 and 3 hrs. *P. zeylanica* 500 mg/kg showed percentage inhibition of wet and dry cotton pellet granuloma in rats 55.84% and 47.92%, respectively.

Conclusion: Thus, the present study revealed that the DMEPZ offered significant protection against inflammation.

Keywords: *Plumbago zeylanica*, Inflammation, Anti-inflammatory, Anti-granuloma activity.

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INTRODUCTION

Plumbago zeylanica is a family of Plumbaginaceae and commonly known as "chittiramulam or vellai" in Tamil and widely distributed in southern parts of India. In the traditional system of medicine, different parts of the plant used a variety of diseases [1,2]. *P. zeylanica* is widely used as a gastrointestinal disease [3], respiratory disease [4], gonorrhea and syphilis [5], inflammatory diseases [6], scabies [7], blood coagulation profile activity [8], antiallergic activity [1], central nervous system (CNS) stimulant activity [9], antioxidant [10], anti-infertility activity [11], lipid metabolism activity [12], and cytotoxicity activity [13]. There is no documentary evidence of contraindication and interaction. Subcutaneous injection of the carrageenan is to promote hyperalgesia and to develop erythema. This response due to proinflammatory mediators such as bradykinin, histamine, tachykinins, reactive oxygen, and nitrogen species [14]. These mediators readily migrate to sites of inflammation and proven with current study. After administration of the carrageenan showed significant inflammatory response in paw edema model [15]. Inflammation is a disorder involving swelling associated with multiple complex mediators [16]. Inflammation is a pathological state and characterized by concurrent active inflammation, tissue destruction, and attempts at repairing stage [17]. The natural system of medicines is believed that one of the important source of health-care field [18]. However, we investigated the protective effect of dichloromethane extract of *P. zeylanica* (DMEPZ) influence on regulating complex mediators in inflammatory rats to provide a definite experimental basis for the clinical medication.

METHODS

Preparation of the extracts

The roots of *P. zeylanica* were collected in Nellore District, India. Botanical identification and voucher specimen No. RIP/2013/120 has been deposited in the museum of the Department of Pharmacognosy at Ratnam Institute of Pharmacy, Nellore, India. The roots were dried under shade, segregated, and pulverized by a mechanical grinder and passed through a 40 mesh sieve. The powdered 1 kg of the material was soaked in solvent dichloromethane (4000 mL) for 48 hrs and repeats the process for thrice to get complete extraction. The solvent was removed in a rotary vacuum and stored in an airtight container.

Drugs and chemicals

Carrageenan was obtained from SD. Fine Chemicals Limited, Bombay. All other chemicals were of analytical grade procured from reputed Indian manufacturers.

Experimental animals

The experimental design was approved by the Institutional ethical committee of Ratnam Institute of Pharmacy, Nellore (Ethical Approval No. 13/(Institutional Animal Ethics Committee/Pharma/RIP/2013). Male albino Wistar rats weighing 180-200 g (6-8 weeks) were supplied from animal facility and housed six animals per cage at 23-25°C and relative humidity between 55 and 58%, respectively. They had access to food and water *ad libitum* and were exposed to alternate 12 hrs light and dark cycles.

Acute toxicity study

The acute toxicity study was carried out albino Wistar rats. The experiment made into six groups containing six animals in each group. DMEPZ was suspended in critical micelle concentration + dimethyl-sulfoxide and starting dose from 5, 50, 100, 200, and 400 to 2000 mg/kg body weight (b.w) to all groups, respectively. These animals were observed for a 72 hrs period. The number of deaths was expressed as a percentile and the lethal dose 50 (LD50) was determined by probit a test using the death percentage versus the log dose [19].

The time at which signs of toxicity appear and disappear was observed systematically and recorded for each animal.

Carrageenan-induced rat paw oedema

A total of 30 animals were equally divided into 5 groups of six each. Before the experimental study allowed for overnight fasting in the rats. All the groups of rats, hind paw volume measured by the plethysmograph instrument (Yukui *et al*.,). All the groups were injected 0.1 mL of a suspension of 1% carrageenan under the subplantar aponeurosis of the right hind paw of rats except Group I. Group I is the positive control and injected 0.1 mL saline. Group II is a negative control and injected 0.1 mL of a suspension of 1% carrageenan under the subplantar region. Group V served as positive control and received diclofenac sodium was injected intraperitoneally at 25 mg/kg b.w 1 h before carrageenan injection. Group III and IV were orally administered with DMEPZ 250, 500 mg/kg b.w, respectively. After carrageenan injection, paw volume was measured at 1, 2, and 3 hrs to determine the inflammatory activity.

In the rats, percentage of inhibition of edema calculated using the following formula,

% of inhibition of oedema=Vc−Vt/Vc × 100

Where, Vc is the edema in the disease control group and Vt is the edema in the treatment group.

Cotton pellet-induced granuloma

A total of 24 were equally divided into four groups of six each. The sterile cotton pellets in milligram of 10±1 were implanted to subcutaneously into both sides of the groin region of each rat, and before the pellets implantation rats were anesthetized. Group I received the vehicle (0.9% NaCl, 10 mL/kg b.w) and served as control. The dose of 250 and 500 mg/kg b.w of DMEPZ was orally administered as Group II and III rats for seven consecutive days from the first day of cotton pellet implantation. Diclofenac at a dose of 25 mg/kg b.w received group IV animals. Rats were anesthetized on the 8th day and pellets with the granuloma tissues carefully removed and made free from extraneous tissues. The wet pellets dried an oven at 60°C for 24 hrs. Before and after dry cotton pellets were weighed. This assessment was to determine the granuloma formation in rats. DMEPZ effect was compared with control and standard drug-treated animals.

Statistical analysis

All the data were expressed as means±standard error mean. The measurement data of multiple groups were compared with one-way ANOVA, the comparison between normal control versus other groups, and a value of $p<0.05$ was considered significant.

Table 1: HPLC profiles of the DMEPZ

Retention time	Area (mV.s)	Height (mV)	Area (%)	Height (%)
3.580	46.3701	2.7367	0.3227	0.3232
4.860	121.0003	3.9909	0.8420	0.4714
6.713	91.5804	5.8665	0.6373	0.6929
7.447	419.5180	22.7855	2.9192	2.6913
8.233	137.2056	13.0742	0.9547	1.5443
8.880	4067.7216	316.7515	28.3048	37.4133
10.147	21.8959	1.5991	0.1524	0.1889
10.867	11.8915	0.8879	0.0827	0.1049
13.247	54.9761	3.3796	0.3825	0.3992
14.400	44.8306	2.3031	0.3119	0.2720
15.180	8925.8227	461.7467	62.1094	54.5395
24.833	128.1171	3.6027	0.8915	0.4255
27.660	195.0064	5.6933	1.3569	0.6725
38.853	105.1888	2.2108	0.7320	0.2611

Values are expressed as mean±SE (n=6). Data were analyzed using one-way analysis of variance followed by Dunnett's multiple comparison test.
SE: Standard error, *P. zeylanica: Plumbago zeylanica*, HPLC: High-performance liquid chromatography

RESULTS

Acute toxicity test

There are no significant physiological, behavioral, and biochemical alterations at different dose group of DMEPZ-treated rats. These effects revealed extract was safety as non-toxic and no mortality in rats. The median LD50 was determined highest dose tested, i.e., 2000 mg/kg b.w. Hence, *P. zeylanica* at doses of 250 and 500 mg/kg, p.o. was selected for further pharmacological study.

Instrumental analysis

DMEPZ was subjected to high-performance liquid chromatography (HPLC). The result obtained by gradient chromatography on C-18 column with U.V. detection at 254 nm and eluted with 70:30:1 (Methanol:Water:Acetic acid). There was retention time in crude extract content for the 14 different samples as shown in Table 1.

Carrageenan-induced rat paw oedema

DMEPZ against the inflammatory effect in significant ($p<0.001$) at the different dose groups such as 250 and 500 mg/kg b.w (Fig. 1). These results were comparable to reference drug of diclofenac, the doses 250 and 500 mg/kg b.w in 3 hrs inhibited 31.79 and 40.15%, respectively, in carrageenan-induced rat paw oedema.

Cotton pellets-induced granuloma

Decreased level of granuloma reflected in DMEPZ-treated groups as shown in Table 2. As the results dose group of 250 and 500 mg/kg b.w weight of the cotton pellets was significantly reduced. Moreover, the anti-inflammatory effect of DMEPZ slightly less than diclofenac but statistically significant.

DISCUSSION

Acute toxicity studies of *P. zeylanica* up to 2000 mg/kg were found to be non-toxic and did not cause any death of the tested animals. Previous data indicated that polyphenolic compounds may protect against oxidative damage and have anti-inflammatory activity [18]. Tilak *et al.* study reported plumbagin one of the major constituent of *P. zeylanica* to protect oxidative stress [20]. As Fig. 2 showed that HPLC analysis showed that retention time in crude extract content for the 14 different samples (Table 1). There are several mediators involved in inflammation including histamine, serotonin, and bradykinin. In the late phase to produce the inflammation through increased vascular permeability. Inflammation in local or systemic to assess with levels of pro-inflammatory cytokines tumor necrosis factor -α, interleukin

Table 2: Effect of DMEPZ on cotton pellets-induced granuloma in rats

Treatment	Dose (mg/kg)	Weight of wet cotton pellets (mg)	Percentage inhibition	Weight of dry cotton pellets (mg)	Percentage inhibition
Control	Saline 2 mL	182.34±1.44		48.33±0.79	
DMEPZ	250	106.40±1.97[a]	41.64	33.66±0.62[a]	30.35
DMEPZ	500	80.52±1.62[a]	55.84	25.17±0.62[a]	47.92
Diclofenac	25	77.59±1.10[a]	57.44	23.54±0.58[a]	51.29

Values are expressed as mean±SE (n=6). Data were analyzed using one-way analysis of variance followed by Dunnett's multiple comparison test. [a]$p<0.001$; $p<0.05$ considered as significant; NS: Non-significant; All groups are compared with normal control. SE: Standard error, DMEPZ: Dichloromethane extract of *Plumbago zeylanica*

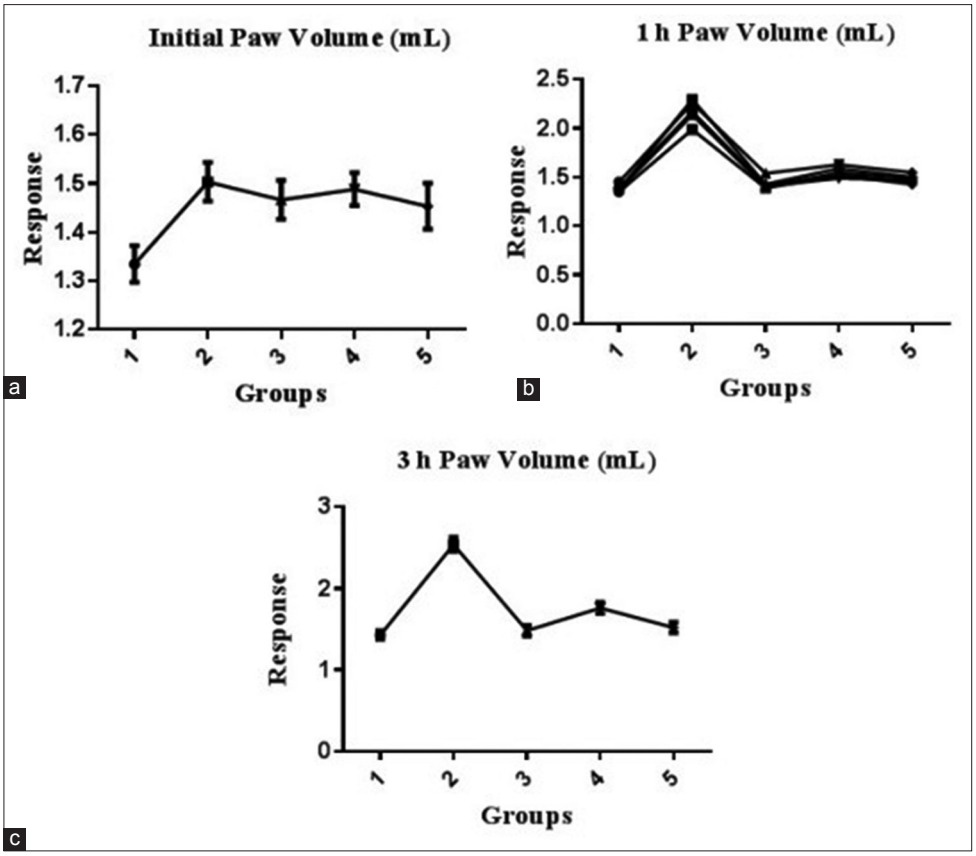

Fig. 1: (a) Effect of dichloromethane extract of *Plumbago zeylanica* (DMEPZ) on carrageenan-induced rat (b) effect of DMEPZ on carrageenan-induced paw edema (basal time) rat paw edema (after 1 hr) (c) effect of DMEPZ on carrageenan-induced rat paw edema (after 3 hrs)

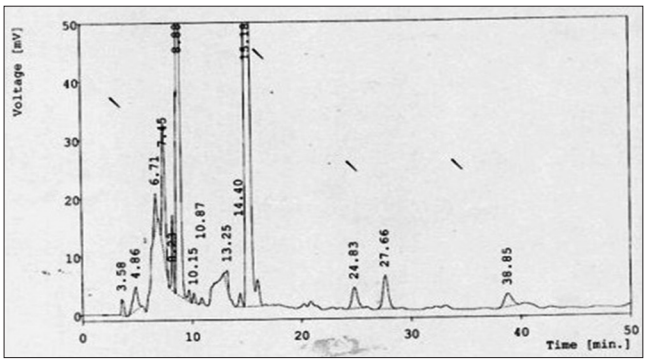

Fig. 2: High-performance liquid chromatography profiles of the dichloromethane extract of *Plumbago zeylanica*

IL - 6, and IL - 1 [21]. Non-steroidal anti-inflammatory drug including indomethacin or aspirin is not inhibiting initial phase of edema and has been attributed to the release of chemical mediators. The second phase of swelling attributed to the production of cyclooxygenase -2 in the hind paw as revealed in previous study [22]. In the recent years, the biological effect of phytosterols emphasis on their *in vitro* and *in vivo* immune modulatory activity [23].

Some of the chemotactic and chemokinetic agents reported to be involved topical inflammation through arachidonic acid by lipoxygenase activity such as 12-hydroxy-6,8,11,14-eicosatetraenoic acid from platelets, leukotriene B4 from polymorphonuclear leukocytes, and 5-hydroxy-6,8,11,14-eicosatetraenoic acid [24]. Carrageenan-induced edema has been commonly used as an experimental animal model for acute inflammation. In the carrageenan-induced rat paw edema model, except control group, and all examined with DMEPZ administered orally. The results showed significant anti-inflammatory activity, where dose 500 mg/kg exhibited the highest effect. Initially, 1-2 h, carrageenan mainly mediated by histamine, serotonin, and increased synthesis of prostaglandins in the damaged tissue surroundings [25]. After sustained by prostaglandin release and mediated by bradykinin, leukotrienes, and polymorphonuclear cells [26]. The findings of the present study confirmed carrageenan causes the production and release of nitric oxide (NO) at the injured site NO, which alerts pathological conditions of NO synthesis, this could be involved in tissue injury, including edema and hyperalgesia condition [27].

Treatment with *P. zeylanica* extract showed significant action against paw edema in a dose-dependent manner. At 500 mg/kg dose of DMEPZ was quite comparable to diclofenac (25 mg/kg). The present study results indicate that a dose of 250 and 500 mg/kg b.w influencing against the inflammatory process. The inflammation due to arachidonic cofactors also revealed a previous study [28]. Among groups, cotton pellet granuloma tissue compared with wet and dry weight of the cotton pellets. Different dose of 250 and 500 mg/kg b.w of DMEPZ showed curing effect of inflammation comparable to diclofenac treatment. The results demonstrated that herbal medicine has ability to treat inflammatory diseases. Hence, it needs further detailed pharmacological and clinical investigations to prove it as an effective therapeutic agent for inflammation.

CONCLUSION

P. zeylanica extract showed active against carrageenan-induced rat paw edema in a dose-dependent manner. At 500 mg/kg *P. zeylanica* was comparable to diclofenac (25 mg/kg) in the inhibition of paw edema. The effect of DMEPZ may be attributed to its free radical scavenger activity and protection of apoptosis. In the experimental models, DMEPZ was found to exhibit significant ($p<0.001$) anti-inflammatory activity,

and the results were comparable to standard drug of diclofenac. Thus, the present study revealed DMEPZ phytoconstituents exerts the desired effects against hypersensitivity and inflammation.

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