



EVALUATION OF ANTI-INFLAMMATORY ACTIVITY OF *PEDILANTHUS TITHYMALOIDES* (L.) POIT. LEAVES IN MALE ALBINO RATS

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ABSTRACT

The *Pedilanthus tithymaloides* has been used in folk medicine to treat several ailments including inflammation. The anti-inflammatory activity of different extracts such as hexane extract (HEPT), methylene chloride extract (MCEPT), chloroform extract (CEPT), ethyl acetate extract (EAEPT) and methanol extract (MEPT) of *Pedilanthus tithymaloides* leaves were studied in male albino rats using the carrageenin induced paw edema model with the help of Plethysmometer. The different extracts at the dose of 200 mg/kg body weight suspended in 0.03% Tween 80 in 0.9% sodium chloride aqueous solution were administered orally 30 minutes prior to the injection of carrageenin (0.1 ml of 1%) into sub-plantar region of the right hind paw. Inflammation was expressed in terms of paw edema volume displaced by the inflamed rat paw. The percentage inhibition of inflammation was measured. Among the different extracts, methanol extract (MEPT) at the dose of 200 mg/kg body weight showed significantly more anti-inflammatory activity (percentage inhibition 81.44%) in comparison with other extracts using the carrageenin induced paw edema model as compared to the standard drug, Phenylbutazone with percentage inhibition of 82.72%. After that the anti-inflammatory activity of MEPT was also observed using histamine-induced rat paw edema, and dextran-induced rat paw edema model. The MEPT exhibited anti-inflammatory activity in a dose dependant manner in these models when compared to the standard drug, phenylbutazone. It is concluded that methanol extract of *Pedilanthus tithymaloides* showed a significant anti-inflammatory effect.

KEYWORDS: *Pedilanthus tithymaloides*; anti-inflammatory activity; carrageenin; histamine; dextran



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INTRODUCTION

Herbal medicines derived from plant extracts are being increasingly utilized to treat a wide variety of clinical diseases, though relatively little knowledge about their mode of action is available. There is a growing interest in the pharmacological evaluation of various plants used in Indian traditional systems of medicine. Thus, the present investigation was carried out to evaluate the anti-inflammatory potential of *Pedilanthus tithymaloides* in experimental animal models. The *Pedilanthus tithymaloides* (L.) Poit. belonging to the family Euphorbiaceae is commonly known as "Rangchita" and Plants of Hawaii in Bengali and English language respectively. The plant is a low tropical succulent shrub with milky juice, stems green, widespread, ranging from southern Florida and Mexico to northern South America, the Caribbean to India. It is reported wide of range of healing properties, namely emetic, anti-inflammatory, antibiotic, antiseptic, antihemorrhagic, antiviral, antitumoral, and abortive^{1, 2}. There is growing awareness in the investigation of natural products for the discovery of new anti-inflammatory agents and to think an alternative route to avoid synthetic compounds, side effects of which is always in question. The facts encouraged us for comparative study of different extracts of leaves of the plant for anti-inflammatory potential and also for evaluation of this activity of the extract having good potency in histamine-induced rat paw edema and dextran-induced rat paw edema models. The effect of the extracts were compared with the standard non-steroidal anti-inflammatory drug (NSAID), phenylbutazone.

MATERIALS AND METHODS

Plant Material

The leaves of the plant were collected from Jalpaiguri, India during September- October. The herbarium of the plant was authenticated by a Botanist of Botanical Survey of India, Howrah, India (voucher specimen no CNH/-1-1(56) /2006/ Tech-11/ 1450).

Extraction Procedure

The dried leaves of *Pedilanthus tithymaloides* were successively extracted with Hexane, methylene chloride, chloroform, ethyl acetate and methanol by continuous hot extraction process using Soxhlet apparatus. The solvent was completely removed under reduced pressure from the different extracts such as hexane extract (HEPT), methylene chloride extract (MCEPT), chloroform extract (CEPT), ethyl acetate extract (EAEPT) and methanol extract (MEPT) and stored in vacuum dessicator.

Chemical reagents

Hexane (Ranbaxy Chemical, India), methylene chloride (Ranbaxy Chemical, india),, chloroform (Ranbaxy Chemical, India), ethyl acetate (Ranbaxy Chemical, India) and methanol (Ranbaxy Chemical, India) extracts of *Pedilanthus tithymaloides*, Tween 80 (Qualigens Fine Chemicals, India), Phenylbutazone (Qualigens Fine Chemicals, India), carrageenin (Sigma Chemical Co., USA), histamine (Qualigens Fine Chemicals, India) and dextran (Qualigens Fine Chemicals, India).

Experimental animals

Male albino Wistar rats weighing 140 – 170 g supplied by M/s. B.N. Ghosh & Co., Calcutta, India, were placed in wire netted cages in a controlled room temperature $22 \pm 1^{\circ}\text{C}$, relative humidity 60 - 70% and with 12 hrs light and dark cycle. The animals were maintained with pellet diet and water *ad libitum*. The animals were deprived of food for 24 hrs before experimentation but allowed free access to tap water throughout. Diet pellet was obtained from Hindustan Lever Ltd. The animals were divided into different groups using six rats in each group.

Carrageenin-induced rat paw edema

Carrageenans are complex group of polysaccharides made of repeating galactose related monomers. Cardinal signs of inflammation immediately follow on subcutaneous injection resulting in edema, hyperalgesia and erythema due to the action

of pro-inflammatory agents like bradykinin, histamine, tachykinin, complement, reactive oxygen and nitrogen species¹¹. Edema was induced by sub-planter injection of 0.1 ml of 1% freshly prepared suspension of carrageenin (Sigma Chemical Co., USA) in 0.9% sodium chloride into the right hind paw to each animal of seven groups. The different extracts such as HEPT, MCEPT, CEPT, EAEPT and MEPT were suspended in 0.03% Tween 80 in 0.9% sodium chloride aqueous solution. The test group animals received the different extracts (200 mg/kg; orally), standard group animals received Phenylbutazone 100 mg/kg orally and control group received vehicle (0.03% Tween 80 in 0.9% sodium chloride aqueous solution) only. All the doses were administered orally 30 minutes prior to the injection of carrageenin. The paw volumes were measured before of carrageenin administration (normal paw volume) and after 4 hours of carrageenin administration (inflamed paw volume). Inflammation was expressed in terms of paw edema volume displaced by the inflamed rat paw in Plethysmometer³ and the inhibition rate was calculated for comparison.

Histamine-induced rat paw edema

In this model, 0.05 ml of 1% freshly prepared solution of histamine was given by sub-planter injection of right hind paws of the rats to induce edema⁹. The animals were divided into five groups each containing six rats. The standard groups received phenylbutazone and the control groups received the vehicle only. The test groups received the extract (50, 100 and 200 mg/kg; orally). The animals were treated like Carrageenin model.

Dextran-induced rat paw edema

In this model, edema was induced by sub-planter injection of 0.05 ml of freshly prepared 1% solution of dextran into the right hind paw of the rats⁹. The animals were divided into five groups each containing six rats. The treatment of the animals in tests, standards and control groups were the same as that of histamine model. In all the above cases, the degree of edema formation was assayed by measuring the hind paw volume Plethysmographically before and 4 hours after injecting the irritants like carrageenin, histamine and dextran. The inhibition rate was calculated as follow¹⁰. Inhibition rate (I) % = $[(V_c - V_t) / V_c] \times 100$
 V_c is the paw volume of the control group and V_t is the paw volume of the treated group.

Statistical analysis

The results are expressed as mean \pm S.E.M. The significance analysis was performed by student's t test and the p values $p < 0.001$ implied significance.

RESULTS AND DISCUSSION

The dried leaves of *Pedilanthus tithymaloides* was successively extracted with Hexane, methylene chloride, chloroform, ethyl acetate and methanol using Soxhlet apparatus. The average percentage yield of hexane, methylene chloride, chloroform, ethyl acetate and methanol extracts were found to be 7.27%, 4.42%, 2.12%, 3.50% and 3.35% W/W respectively. The results obtained with hexane, methylene chloride, chloroform, ethyl acetate, and methanol extracts of *Pedilanthus tithymaloides* (L.) on carrageenin-induced rat paw edema are shown in Table-1.

Table 1
Anti-inflammatory activity of various extracts of *Pedilanthus tithymaloides* leaves on carrageenan induced rat paw edema model (n=6)

Treatment	Dose (mg./kg, orally)	Paw volume in ml.	Percentage Inhibition of edema
Control	Vehicle	0.625 \pm 0.025	----
HEPT	200	0.250 \pm 0.028*	60.00
MCEPT	200	0.308 \pm 0.024*	50.72
CEPT	200	0.333 \pm 0.021*	46.72
EAEPT	200	0.233 \pm 0.011*	62.72
MEPT	200	0.116 \pm 0.012*	81.44
Phenylbutazone	100	0.108 \pm 0.014*	82.72

* $P < 0.001$, P – value was calculated by student's t test. Values are expressed as Mean \pm SEM; Vehicle -0.03% v/v aqueous tween 80 solution.

The extracts significantly ($p < 0.001$) inhibited the inflammatory edema. The inhibition was highest in methanol extract among the different extracts. The percentage of inhibition of methanol extract is 81.44 and result obtained was compared to that of the phenylbutazone (% inhibition 82.72), a prototype of a non-steroidal anti-inflammatory drug. The anti-inflammatory activity of methanol extracts at the different doses (50, 100 and 200 mg/kg of body weight) was also observed in histamine-induced rat paw edema

as well as dextran-induced rat paw edema model. The administration of MEPT at doses 50, 100 and 200 mg/kg, caused 40.00%, 56.67% and 83.33% inhibition of histamine-induced rat paw edema and 34.62%, 58.46% and 78.46% inhibition of dextran-induced rat paw edema (Table 2&3). The methanol extract of *Pedilanthus tithymaloides* exhibited anti-inflammatory activity significantly in a dose dependent manner that is reflected from both models.

Table 2
Effect of Methanolic extract of *Pedilanthus tithymaloides* leaves on histamine induced paw oedema in rats (Values are Mean \pm SEM, n = 6)

Treatment	Dose (mg./kg, orally)	Paw edema volume in units after 4 hrs	Percentage Inhibition of edema
Control	Vehicle	0.750 \pm 0.034	-
	50	0.450 \pm 0.040	40.00
MEPT	100	0.325 \pm 0.035*	56.67
	200	0.125 \pm 0.025*	83.33
Phenylbutazone	100	0.150 \pm 0.014*	80.00

* $P < 0.001$, P - value was calculated by comparing with by control by student's t - test
Vehicle - 0.03% v/v aqueous tween 80 solution.

Table 3
Effect of Methanolic extract of *Pedilanthus tithymaloides* leaves on Dextran induced paw oedema in rats (Values are Mean \pm SEM, n = 6)

Treatment	Dose (mg./kg, orally)	Paw edema volume in units after 4 hrs	Percentage Inhibition of edema
Control	Vehicle	0.650 \pm 0.540	-
	50	0.425 \pm 0.035	34.62
MEPT	100	0.270 \pm 0.040*	58.46
	200	0.140 \pm 0.025*	78.46
Phenylbutazone	100	0.130 \pm 0.020*	80.00

* $P < 0.001$, P - value was calculated by comparing with by control by student's t - test.
Vehicle - 0.03% v/v aqueous tween 80 solution.

Indigenous drug systems can be source of variety new drugs that can provide relief in inflammation. Carrageenin-induced rat paw edema model has been used as an inflammatory model in order to investigate the anti-inflammatory agents³. Carrageenin-induced inflammation is a biphasic phenomenon^{4, 5}. The first phase of edema is attributed to release of histamine and 5-hydroxytryptamine. Plateau phase is maintained by kinin-like substance and second accelerating phase of swelling is attributed to prostaglandin-like substance. The knowledge of these mediators involved in different phases is important for interpreting mode of drug action⁶. The result of the present study has shown that methanol extract of the

investigated plant *P. tithymaloides* (L.) exhibited very high anti-inflammatory activity. Preliminary phytochemical screening showed the presence of flavonoids or flavonoidal compound in methanolic extract of *Pedilanthus tithymaloides* (L.) leaves. Flavonoids found in many plants have been shown to have diuretic, laxative, antispasmodic, anti hypertensive and anti-inflammatory action^{7, 8}. So we can conclude that the present study supports the claims by traditional medicine practitioners about the usefulness of *Pedilanthus tithymaloides* (L.) leaves in inflammatory diseases^{1, 2}.

CONCLUSION

MEPT at the different doses in histamine-induced rat paw edema and dextran-induced rat paw edema model showed significant anti-inflammatory property in a dose dependent manner. Thus this studies conclude that the methanol extract of leaves of *Pedilanthus tithymaloides* (L.) possess significant anti-inflammatory potential in rats.

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