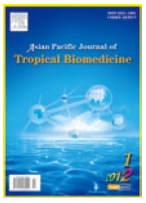
Contents lists available at ScienceDirect

Asian Pacific Journal of Tropical Biomedicine

journal homepage:www.elsevier.com/locate/apjtb



Document heading

Anti-inflammatory activity of root, leaves and stem of *Dipteracanthus patulus* (Jacq.) Nees (Acanthaceae)

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ARTICLE INFO

Article history:
Received 19 February 2012
Received in revised form 25 February 2012
Accepted 12 April 2012
Available online 28 April 2012

Keywords:
Anti-inflammatory activity
Dipteracanthus patulus
Acanthaceae
Carrageenan
Animal model

ABSTRACT

Objective: To screen and evaluate the anti-inflammatory activity of methanolic and aqueous extracts of root, leaves and stem of *Dipteracanthus patulus* (Jacq.) Nees in animal models to support its traditional uses. **Methods:** The anti-inflammatory activity using carrageenan was examined. Acute paw edema was induced by injecting 0.1 mL of 1 % (w/v) carrageenan solution, prepared in normal saline in sub-plantar region of the left hind paw of the rat. Measurements were taken at 0, 1, 2, 3 & 4 hours after the administration of the carrageenan. The extract which showed best activity were further evaluated by egg white, xylene and TPA (12-O-tetradecanoylphorbol-13-acetate) induced inflammation in rat models. **Results:** Methanolic extract (26.4%) and aqueous extract (22.8%) of stem showed the best anti-inflammatory activity in carrageenan induced paw edema as well as in the other methods at a dose of 250 mg/kg body weight. **Conclusions:** Present study, for the first time, confirms the significant anti-inflammatory activity potential of methanolic and aqueous extracts of stem of *Dipteracanthus patulus* on animal models.

1. Introduction

Dipteracanthus patulus (Jacq.) Nees (Syn. *Ruellia patula* Jacq) belongs to family Acanthaceae. It is an erect hoary pubescent, up to 50 cm tall, taproot, basally woody, much branched shrublet. The stem of plant is greenish and rounded, becoming angular with age. Flowering period is July to November. Leaves are 4-10 mm long, lamella elliptic ovate, densely pubescent on both sides. Flowers are pale-white, sessile, 3-4 cm long, usually solitary axillary, rarely 2-3 in cymes. Fruit capsule elliptic- clavate, 1.4-1.8 cm long, glabrous, 8-10 seeded. Seeds are flat and orbicular[1]. It is widely distributed in Africa, Arabia, Srilanka, Pakistan and India[2]. In India it is found in Tamil Nadu, Western Ghat, Andhra Pradesh, Rajasthan and Haryana. In Haryana it grows widely on rocky soil of Aravali hills during rainy season and disappears in the beginning of winter season. This plant is commonly known as Haadjud by local people. Previous phytochemical investigations on this plant revealed the occurrence of flavonoids, saponins, steroids, phenols, tannins, and lignan[3]. This plant is widely consumed by cattle and humans. In traditional medicinal system different parts of the plant have been mentioned to be useful in a variety of diseases. It is used as cardiotonic, antiulcer, antioxidant, insect bite, paranychia, venereal diseases, rheumatic complaints, eye diseases, insect bite and healing of wounds. Traditionally in Haryana and Rajasthan decoction of stem with cow milk is taken orally for the treatment of bone fracture and paste of stem with mustered oil is applied topically. Whole plant extract is also used to cure syphilis, gonorrhea and renal infections[4,5].

The attention of pharmacologists throughout the world has been focused on finding out safer and potent anti-inflammatory drug. The natural products today symbolize safety in contrast to the synthetic drugs that are regarded as unsafe to humans and environment. So, people are returning to the natural products with the hope of safety and security[6]. However, so far there is no systematic study on anti inflammatory activity has been reported in the literature. Hence the present study focuses on evaluating the anti-inflammatory activity of root, leaves and stem of *Dipteracanthus patulus*.

2. Materials and methods

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Foundation project: It is supported by University Grant Commission (UGC), New Delhi, Grant NO. 34-220/2008 (SR).

2.1. Plant material collection and identification

The plant material (leaves, stem and roots) were collected from the rocky soil of Aravali hills (Narnaul) of South Haryana immediate after the rainy season of 2008. The specimens of the collected material was authenticated and deposited in Botanical Survey of India (BSI), Northern circle, Dehradun with voucher specimen no BSD-112193. The plant specimens were also deposited in the herbarium of Department of Genetics, M. D. University, Rohtak with a voucher specimen no (MDU 5604).

2.2. Preparation of extract

The collected plant materials was thoroughly washed with deionized water, shade dried and chopped into fine powder in Willey mill. The methanolic extract of shade dried plant material (500 g each) was prepared using soxhlet apparatus and the same material (500 g each) was percolated with hot water to get the aqueous extract. The obtained extracts were then filtered by using Whatmann No. 1 filter paper and then concentrated under vacuum at 40℃ by using a rotary evaporator. The extract was then lyophilized (Allied Frost lyophilizer) to powdered form at -55℃ under vacuum conditions.

2.3. Drugs and chemicals

Indomethacin (Cayman chemical, USA), carrageenan (Himedia, India), cyproheptadine (Sigma Chemicals, USA), xylene (Merck, Germany), phenyl butazone (Cayman chemical, USA), 12-O-tetradecanoylphorbol-13-acetate (TPA) (Enzo life Sciences, Switzerland), ethanol 70 % (Bangal Chemicals & Pharmaceuticals, India) and egg white.

2.4. Experimental animals

Male albino rats (100-150 g) were procured from the disease-free small animal house of CCS Haryana Agricultural University, Hisar (Haryana), India. The animals had free access to food and water, and they were housed in a natural (12 h each) light-dark cycle. Food given to animals consisted of wheat flour kneaded with water and mixed with a small amount of refined vegetable oil. The animals were acclimatized for one week to the laboratory conditions before doing experiments. The animals were divided into groups of six animals each and fasted for 12 hours before the experiment. The experimental protocol was approved by the Institutional Animals Ethics Committee and the care of laboratory animals was taken as per the guidance of CPCSEA, Ministry of Forests and Environment, Government of India.

2.5. Acute oral toxicity studies

Acute oral toxicity studies were performed according to OECD-423 guidelines (acute toxic class method). The animals were fasted for 4 h with free access to water only. The extract (5 mg/kg b.w. in normal saline) was administered orally initially and mortality was observed for 3 days. If mortality was observed in 4/6 or 6/6 animals, then the dose administered was considered toxic dose. However, if mortality was observed in only one mouse out of three animals then the same dose was repeated again to confirm the toxic effect. If mortality was not observed, the procedure was then repeated with higher doses such as 50, 300 and up to 20 00 mg/kg b.w. of rats[7].

2.6. Methods

The methanolic and aqueous extracts of all three parts (root, leaves and stem) of *Dipteracanthus patulus* were used for the screening of anti-inflammatory activity by using carrageenan induced hind paw edema. The extract which shows best results were further analyzed for anti-inflammatory activity by using other methods like egg white, xylene and TPA induced inflammation in rat models.

2.7. Carrageenan induced hind paw edema

The animals were fasted overnight before the experimentation. The rats were divided into five groups (n=6). Rats in Group I were given normal saline and were treated as negative control. Rats in Group II were administered with indomethacin in normal saline at the dose of 10 mg/kg b.w. orally and were kept as standard. Rats in Group III to Group V were administered orally with the crude extract in normal saline at the doses of 100, 150 & 250 mg/kg b.w., respectively[8]. Since the LD_{50} has not been determined during the acute toxicity study, the doses for this study were selected by trial and error method. The standard and the extracts were given orally to the animals one hour prior to carrageenan injection. Acute paw edema was induced by injecting 0.1 mL of 1 % (w/v) carrageenan solution, prepared in normal saline in sub-plantar region of the left hind paw of the rat. The perimeter of paw was measured by using screw gauge. Measurements were taken at 0, 1, 2, 3 & 4 hours after the administration of the carrageenan.

% Inhibition of edema = [(C-T)/C]×100

Where, C = Control paw edema; T = Test paw edema

2.8. Egg white induced hind paw oedema

The rats were divided into four groups of six animals each. The methanolic extract of stem of *Dipteracanthus patulus* at a concentration of 100 and 200 mg/kg was administered orally to last two groups of rats. The first and second group of rats received 5 mL/kg propylene glycol as vehicle control and 8 mg/kg cyproheptadine as drug control, respectively. All the drugs and vehicle were given 1 h prior to the study. Freshly taken egg white (0.1 mL) was injected into the sub plantar tissue of the left hind paw of the rat. The volumes of the injected paws were measured at 0, 1, 2, 3 and 4 hours. The percent increase in paw oedema of the treated group was compared with that of the control and the inhibitory effects of the drugs were studied[9]. Percentage inhibition was

calculated for both models by using the following formula:

Percent inhibition = $(V_C - V_T)/ V_C \times 100$

V_C = Average control (% increase in paw volume),
V_T = Average test (% increase in paw volume)

2.9. Xylene induced ear edema

In xylene induced ear edema four groups of six (6) albino rats were used. Group I served as normal control and received no treatment. Group IV served as positive control with Phenyl butazone 1 mg/kg as reference. Group II and III were test groups and extract (50 and 100 mg/kg) were applied onto the pinna of the right ear using a micropipette. After an interval of about 1 h, xylene (0.03 mL) was then applied to the same portions of the right ear[10]. Ear thickness was measured initially and after 30 min of xylene application using screw gauge and edema expressed as an increase in ear thickness due to the inflammatory challenge.

2.10. TPA induced ear edema

Each mouse received 2.5 μg of TPA dissolved in 20 μL of ethanol 70 %.This was applied by an automatic pipette in 20 μL volumes to both anterior and posterior surfaces of the right ear. The left ear (control) received the same volume of the solvent (ethanol, 70%), simultaneously with TPA. Indomethacin (0.5 mg/ear) was used as reference drug. The extracts (5 and 10 mg/ear) were used. For the evaluation of the activity the thickness of each ear was measured at 4th hour after induction of inflammation using a screw gauge. The edema was expressed as the difference between the right and left ears due to TPA application and consequently inhibition percentage was expressed as a reduction thickness with respect to the control group[11].

2.11. Statistical analysis

The results were expressed as percent inhibition. Statistical significance was determined by analysis of variance and subsequently followed by Dunnett's multiple comparison test where á value at 99% significance level. The analysis was performed using Graphpad Prism version 5.04 statistical software.

3. Results

3.1. Carrageenan induced paw edema

Only two extract (methanol and aqueous) of leaves, stem and root were used for screening. The results are represented as percent inhibition. All the extracts of *Dipteracanthus patulus* showed anti-inflammatory activity. The extracts showed maximum inhibition at a concentration of 250 mg/kg during 4th hour of study (Table 1). During preliminary testing, it was observed that the aqueous stem extract and methanol stem extract showed good inhibition against paw edema as compared to other extracts However, stem methanol extract shows excellent (maximum inhibition) results and this was subjected to further anti-inflammatory tests after measuring their acute toxicity at higher doses.

3.2. Egg-white induced paw edema

The anti-inflammatory activity of stem methanol extract of *Dipteracanthus patulus* by egg-white induced paw edema

Table 1.
Effect of different extracts of leaves, root and stem of *Dipteracanthus patulus* on carrageenan-induced rat paw edema.

Plant part	Extract	Concentration (mg/kg b.w.)	Percent inhibition (%)			
			1 hour	2 hours	3 hours	4 hours
Leaves	Methanol	100	3.9	7.1	9.4	11.0
		150	5.9	7.5	9.5	11.1
		250	15.6**	16.3**	17.6**	18.5**
	Aqueous	100	0.5*	1.9*	4.2*	7.7*
		150	4.5*	4.5*	6.8*	9.3*
		250	13.0**	13.9**	17.5**	19.7**
Root	Methanol	100	1.5	5.7	8.4	12 .0
		150	10.9**	13.2**	14.2**	17.2**
		250	14.1***	16.9***	17.1***	18.6***
	Aqueous	100	11.3*	14.6*	15.0*	20.6*
		150	13.1**	15.5**	18.3**	22.6**
		250	13.9**	17.0**	21.9**	22.8**
Stem	Methanol	100	9.6*	15.7*	15.6*	15.0*
		150	13.6**	18.2**	20.1**	21.4**
		250	18.5***	23.6***	25.3***	26.4***
	Aqueous	100	0.9	7.5	9.4	11.0
		150	1.5	12.5	15.3	16.0
		250	5.7*	14.5*	18.2*	22.8*
Indomethacine (Strandard)		10	16.0	23.3	26.5	33.0

Note – Values are in percent inhibition and we applied Dunnett's multiple comparison tests where α value at 99% significance level. * = $P<0.01$, ** = $P<0.001$, *** = $P<0.000\ 1$.

Table 2.
Effect of methanol stem extract of *Dipteracanthus patulus* on egg white induced rat paw edema.

Groups	Treatment	Dose (mg/kg)	No. of animals	Percent inhibition (%)			
				1 hour	2 hours	3 hours	4 hours
I	Propylene glycol (−ve control)	–	6	–	–	–	–
II	Cyproheptadine (+ve control)	8	6	12.8	22.3	24.6	33.5
III	Methanol extract	100	6	3.1	5.8	8.4	12.6
IV	Methanol extract	200	6	8.8	11.3	13.2	15.9

Note – Values are in percent inhibition and we applied Dunnett's multiple comparison tests where ∝ value at 99% significance level.

showed maximum inhibition (15.9%) at a concentration of 200 mg/kg (Table 2).

3.3. Xylene induced ear edema

In xylene induced ear edema, stem methanol extract of *Dipteracanthus patulus* showed good results at a concentration of 100 mg/kg (Figure 1).

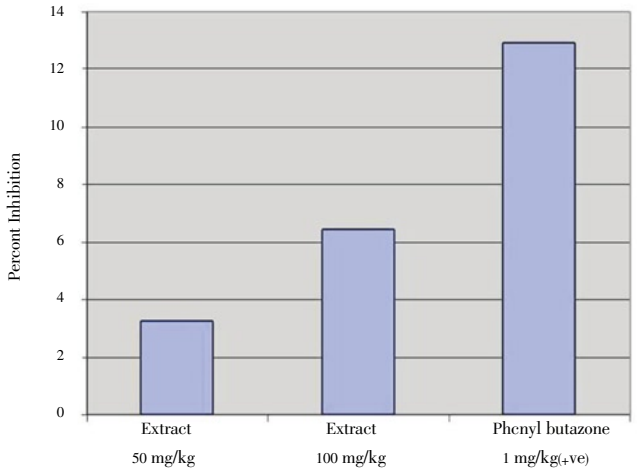

Figure 1. Anti-inflammatory activity of methanol stem extract of *Dipteracanthus patulus* by xylene induced ear edema.

3.4. TPA-induced ear edema

Anti-inflammatory activity of methanol stem extract of *Dipteracanthus patulus* by TPA induced ear edema was also good (Figure 2).

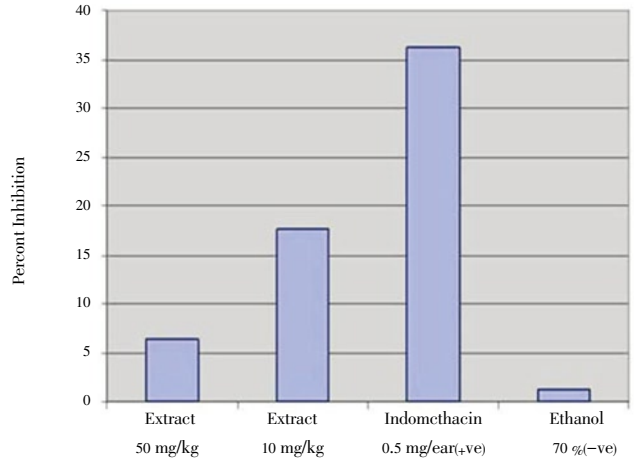

Figure 2. Anti-inflammatory activity of methanol stem extract of *Dipteracanthus patulus* by TPA induced ear edema.

4. Discussion

Inflammation is a common phenomenon and it is a reaction of living tissues towards injury[12-15]. The carrageenan-induced paw edema test is widely accepted as a sensitive phlogistic tool for investigating potential anti-inflammatory agents, particularly the non-steroidal type. The development of oedema in the paw of the rat after the injection of carrageenan is due to the release of histamine, serotonin, prostaglandin and the like. Acute hind paw oedema is induced in rats by injecting 0.1 mL of 1% v/v carrageenan which reaches a peak edema levels at 3-5 hours after carrageenan injection. Prostaglandin-E_2, a powerful vasodilator, synergizes with other inflammatory vasodilators such as histamine and bradykinin and contributes to the redness and increased blood flow in areas of acute inflammation[16-18]. In the present study the extracts were tested at three different dose levels to know if they were dose dependent. From the results obtained the stem methanol extract showed highly significant activity ($P < 0.0001$) comparable to the reference drug used. At the different dose range used (100, 150 and 250 mg/kg), there was a significant differences in their anti-inflammatory activity hence they were found to be dose-dependent. The significant level of anti-inflammatory activity of the methanolic extract could be attributed to high amount of flavonoids present in the extract. More importantly, the research work justified the traditional use of the plant in the treatment of bone fracture. Further studies will be carried out to isolate and characterize other anti-inflammatory chemical constituents present in the methanolic extract of this plant.

The results indicate that the methanol stem extract of *Dipteracanthus patulus* have significant anti-inflammatory activity as compared to corresponding leaves and root extracts. However, the extract was found to be effective in acute inflammation studies. Further molecular and cellular experiments are warranted to explore its action mechanisms. Identification of its active constituents is also necessary for further use as anti-inflammatory medicines.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgements

Authors are very thankful to University Grant Commission

(UGC), New Delhi for financial support vide Grant NO. 34-220/2008 (SR).

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