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Research Article

Evaluation of Anti-inflammatory Activity of Methanolic and Toluene Extract of *Dipteracanthus prostratus* Nees.

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ABSTRACT

The present study was conducted with the objective of phytochemical investigation, safe dose determination and evaluation of anti-inflammatory activity of the toluene and methanolic extract of whole plant of *Dipteracanthus prostratus* Nees.. Preliminary phytochemical investigation was performed after the successive extraction of whole plant of *Dipteracanthus prostratus* and found to contain a rich amount of important bioactive compounds. The dose determination studies were carried out according to OECD guidelines no. 425 on Wistar albino rats and the safe dose determined was 2000 mg/kg. Hence on the basis of toxicity studies two dose level i.e. 100 mg/kg and 200 mg/kg, body weight were selected for the present anti-inflammatory activity using carrageenan induced paw edema model. The percentage reduction in paw volume observed against carrageenan induced paw edema for toluene extract was found to be 20.68% and 25.86% at the dose level of 100 mg/kg and 200 mg/kg, body weight respectively whereas in methanolic extract it was 17.24 % and 22.41% at the dose level of 100 mg/kg and 200 mg/kg, body weight respectively. The data were found statistically significant by using one way ANOVA ($P<0.05$). Although both the extracts were able to exhibit anti-inflammatory activity as compared with standard drug Diclofenac Sodium but the overall effect of toluene extract of whole plant of *Dipteracanthus prostratus* at the specified dose level of 200 mg/kg, body weight was found to be more statistically significant compared to methanolic extract.

Keywords: Anti-inflammatory activity, paw edema, carrageenan, Dipteracanthus *prostratus* Nees.

INTRODUCTION

Inflammation is an element of the multifaceted natural response of vascular tissues to injurious stimuli, such as pathogens, damaged cells or irritants. Inflammation is the body defense reaction in order to eliminate or limit the spread of injurious agent as well as to remove the consequent necrosed cells and tissue[1]. A variety of molecules are released from cells and plasma proteins during acute inflammation whose net overall effect is to increase vascular permeability, resulting in tissue edema. The released molecules include histamine, prostaglandins, ecosanoids, bradykinin, platelet activating factor, and serotonin[2]. Chronic inflammatory diseases stay one of the world's major health problems[3]. Presently, both steroidal anti-inflammatory drugs and non-steroidal anti-inflammatory drugs are used in the management of inflammation[4]. Steroids comprise an apparent role in the management of inflammatory disorders, but due to their toxicity, their use is limited excluding in very severe cases where the risks are acceptable. Prolonged use of non-steroidal anti-inflammatory drugs is also linked with rigorous side effects, remarkably gastric hemorrhage leading to formation of gastric ulcers[5, 6].

In recent years, a lot of work has been carried out on natural drugs to elucidate their potential effectiveness in inflammation. Herbal medication is promising as an alternative treatment to available synthetic drugs for the treatment of inflammation probably due to availability, affordability, lesser adverse effects and proved effectiveness. Many natural herbs have been pharmacologically reported possessing potent anti-inflammatory activity[7, 8]. Currently there are huge variety of anti-inflammatory drugs are available in the market. These anti-inflammatory drugs are associated with some or the other side effects. Therefore, the development of potent anti-inflammatory drugs with fewer side effects is necessary. Alternatively, we can use the herbal plant extracts for the treatment of inflammation, because these herbal plants shows the better activity and lesser side effect**.**

Dipteracanthus prostratus Nees (Family: Acanthaceae) is an erect hoary pubescent, up to 50 cm tall, basally woody and much branched shrub locally known as Haadjud by tribal peoples. It is widely distributed plant in Africa, Arab, Srilanka, Pakistan and India. In India it is generally found in Tamil Nadu, Western Ghat and Andhra Pradesh[9]. The stem of plant is greenish and rounded, becoming angular with age. Leaves are 4-10 mm long, lamella elliptic ovate, densely pubescent on both sides. Flowering period is July-November. Flowers are sessile, 3-4 cm long, pale white in

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Table 1. Percentage yield, color, odour and consistency of different extracts

S. No.	Extract	Color	Odour	Consistency	% Yield
1	Toluene	Green	Characteristic	Solid	8.37 %
2	Methanolic	Green	Characteristic	Semi Solid	12.4 %

Table 2. Phytochemical screening of toluene and methanolic extract of *Dipteracanthus prostratus*

S. No.	Chemical Test	Toluene extract	Methanolic extract
1	Carbohydrates	+	+
2	Tannins	+	+
3	Alkaloids	+	+
4	Glycosides	+	+
5	Flavonoids	+	+
6	Steroids and sterols	+	+
7	Proteins and amino acids	+	+
8	Saponins	-	+

+ Sign indicates presence and – sign indicates absence of the compound

colour, usually solitary

axillary, and 2-3 in cymes. Fruit capsule is elliptic clavate, glabrous, 1.4-1.8 cm in length, 8-10 seeded. Seeds are flat and orbicular[10].

Previous phytochemical studies confirmed that *Dipteracanthus prostratus* contains a rich amount of bioactive compounds including flavonoids, saponins, steroids, phenols, tannins, and lignin[11]. In traditional medicinal system of India different parts of the plant have been used in the treatment of a variety of diseases. It is used as cardiotonic, antiulcer, antioxidant, paranychia, venereal diseases, rheumatic complaints, eye diseases, insect bite and healing of wounds[12, 13].

In the light of above mention facts about plant, present study was designed to investigate the anti-inflammatory activity of the methanolic and toluene extract of the whole plant of *Dipteracanthus prostratus* Nees.

MATERIALS AND METHODS

Plant material

Dipteracanthus prostratus (whole plant) was collected in the month of December from the ABS Botanical Conservation, Research & Training Centre, Kaaripati, Salem, Tamil Nadu, India. Herbarium of plant was prepared and authenticated by Dr. A. Balasubramanian (Executive Director) Former Siddha Research Consultant (Ayush), Ministry of Health & Family Welfare, New Delhi, India. The specimen voucher number (AUT/JNU/029) deposited with the herbarium in the Department of Pharmacognosy, ABS Botanical Conservation, Research & Training Centre, Kaaripati, Salem, Tamil Nadu, India. For future reference.

Processing of the plant material and extraction

Whole plant of *Dipteracanthus prostratus* was shade dried for four weeks, pulverized to coarse powder, passed through sieve no. 20 to maintain uniformity and coarsely dried powder was first defatted with petroleum ether (60-80ºC) to remove fatty materials and then successively extracted with toluene and then finally extracted with methanol using Soxhlet apparatus. After complete extraction extracts were collected, and concentrated in vacuum under reduced pressure using rotary flash evaporator and the dried crude extracts were stored in air tight glass containers at 4ºC for further study. The percentage yield, color, odour and consistency of both extracts were recorded.

Phytochemical screening

Both the crude extracts (toluene and methanolic) of whole plant of *Dipteracanthus prostratus* were subjected to qualitative phytochemical investigation for the identification of the different phytoconstituents using standard tests and procedures[14].

Preparation of test formulation of extracts

Suspension formulations of both the crude extracts (toluene and methanolic) were prepared separately in 0.5% carboxy methyl cellulose (CMC) solution in distilled water stored at 2-8ºC for further studies.

Chemicals and reagents

Diclofenac sodium was obtained as a gift sample from Sapience Bio-analytical Research Lab, Bhopal, Madhya Pradesh, India. All the drugs, solvents and chemicals used in the study were of analytical grade. Petroleum ether, methanol, toluene were purchased from S.D. Fine Chemicals, Mumbai, India. Carragenan was purchased from Hi-Media Pvt. Ltd., Mumbai, India.

Animal care and handling

The experiment was carried out on healthy Wistar albino rats, weighing between 140-200g Animals were provided by the authorized animal house of Sapience Bio-analytical Research Lab, Bhopal, Madhya Pradesh, India. The animals were acclimatized to the standard laboratory conditions at temperature 25±2°C relative humidity 44-56% and 12:12 hours light and dark cycles, fed with standard pellet diet and water *ad libitum* during experiment. The experiment was approved by the Institutional Animal Ethics Committee (IAEC) as per the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) guidelines (Approval no. 1413/PO/a/11/CPCSEA)

Acute oral toxicity study[15]

Acute oral toxicity study was evaluated as per Organization for Economic Cooperation and Development (OECD) guidelines no. 425 on Wistar albino rats, weighing between 140-200g. Before experiment rats were fasted overnight with water *ad libitum*. Six animals were selected for the assessment of maximum tolerable dose of toluene and methanolic extract of *Dipteracanthus prostratus*. First three animals received 2000 mg/kg body weight dose of toluene extract of *Dipteracanthus prostratus* and other three animals received 2000 mg/kg body weight dose of methanolic extract of *Dipteracanthus prostratus*. Both extracts were given in suspension form by gavage using oral canula. Animals were observed

Table 3. Mortality data of acute oral toxicity study of toluene and methanolic extract of *Dipteracanthus prostratus*

S. No.	Group	No. of animal	No. of animals dead	Mortality Ratio
1.	Toluene extract (2000 mg/kg, body weight, p.o.)	3	Nil	Nil
2.	Methanolic extract (2000 mg/kg, body weight, p.o.)	3	Nil	Nil

Table 4. Effect of toluene and methanolic extract of *Dipteracanthus prostratus* on carrageenan induced paw edema in rats

Group	Paw volume (mm)					% Inhibition
	0 h	1 h	2 h	3 h	4 h	
I	0.2±0.0	0.41±0.03	0.48±0.03	0.55±0.02	0.58±0.01	-
II	0.21±0.01	0.25±0.02a**	0.26±0.02a***	0.31±0.01a***	0.33±0.02a***	43.10
III	0.23±0.02	0.33±0.02	0.4±0.02b*	0.43±0.02a*, b*	0.46±0.02a**, b***	20.68
IV	0.23±0.02	0.3±0.03a*	0.35±0.02a*	0.4±0.02a**	0.43±0.02a***,b*	25.86
V	0.18±0.01	0.36±0.02b*	0.43±0.02b***	0.45±0.02b**	0.48±0.01a*, b***	17.24
VI	0.2±0.02	0.33±0.02	0.38±0.03b*	0.41±0.03a**	0.45±0.02a***, b**	22.41

Values are represented as mean ± SEM, n = 6 animals in each group. *P<0.05, **P<0.01, ***P<0.001. Where a represented the Significance difference as compared to group-I (Control) and b-Significance difference as compared to group-II (Standard)

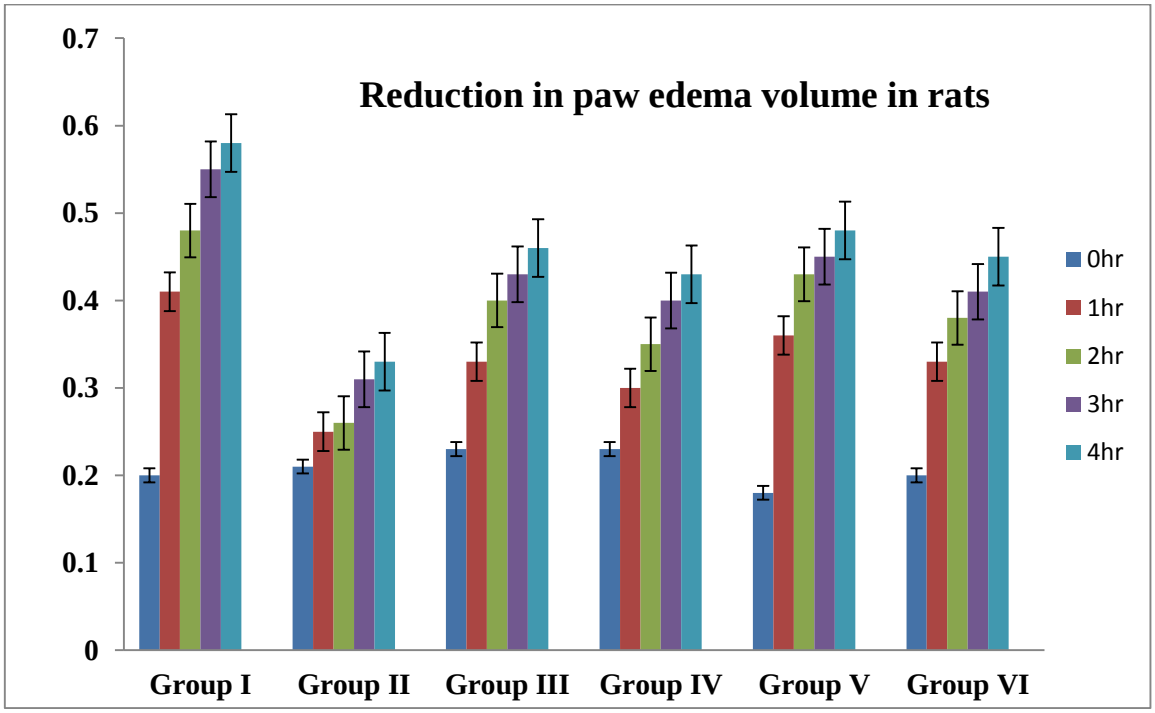

Figure 1. Effect of toluene and methanolic extract of *Dipteracanthus prostratus* on carrageenan induced paw edema in rats

individually for any toxicity sign of gross changes like convulsion, tremor, circling, depression, and mortality after dosing for 24 hours, with special attention given during the first 4 hours and daily thereafter, for a total of 14 days. All observations were systematically recorded with individual records being maintained for each animal.

In-vivo screening of anti-inflammatory activity (Carragenan induced paw rat edema model)

The anti-inflammatory activity of toluene and methanolic extract of *Dipteracanthus prostratus* was assessed using carrageenan induced rat paw edema. Wistar albino rats weighing between 140-200 g were used to evaluate the anti-inflammatory activity using carrageenan induced rat paw edema. 36 rats were divided in to six groups consisting of six rats in each group. Group I animals received vehicle (CMC 1.0% w/v in normal saline). Group II animals received Diclofenac sodium (10 mg/kg b. w., p. o.). Groups III and IV animals received toluene extract of *Dipteracanthus prostratus* (TEDP) (100 and 200 mg/kg b. w., p. o., respectively) once daily for 7 days, while Groups V and VI animals received methanolic extract of *Dipteracanthus prostratus* (MEDP) (100 and 200 mg/kg b.

w., p. o., respectively) once daily for 7 days. On 7th day, after 1 h of extracts administration, inflammation was induced by injecting 0.1 ml of 1% (w/v) carrageenan in saline into the sub-plantar region of the right hind paw of each rat. Inflammation was calculated by measuring paw volume using plethysmometer (Ugo Basil Plethysmometer, 7150) initially at 0, and 1, 2, 3, and 4 h after the carrageenan injection. Edema was expressed as mean increase in paw volume relative to control animals. The percentage inhibition of edema was calculated by the following[16, 17].

% Inhibition of edema = 100 (1-Vt/Vc)

Where Vc is the edema volume in the control group and Vt is the edema volume in test group.

STATISTICAL ANALYSIS

The results are expressed as the mean ± SEM for each group. Statistical differences were evaluated using a One-way analysis of variance (ANOVA) followed by posthoc Tukey-Kramer Multiple Comparisons Test, employing GraphPad InStat 3 software. Difference between groups was considered significant at $P < 0.05$ levels.

RESULTS

Extraction and Phytochemical screening

After the extraction, percentage yield, color, odour and consistency of both extracts were recorded (Table 1)

The phytochemical screening revealed that toluene and methanolic extract of *Dipteracanthus prostratus* whole plant contain a rich amount of flavonoids, alkaloids, glycosides, tannins, carbohydrates and phenolic compounds. Result of the phytochemical screening is shown in table 2.

Acute toxicity study

There was no change in the behavioral pattern and not any sign of toxicity and mortality observed on administration of TEDP and MEDP at the dose level of 2000 mg/kg, body weight, during the overall toxicity studies (OECD 425). Both the extracts were found nontoxic and safe as no death occurs. Thus, the final doses for further studies selected were 100 mg/kg and 200 mg/ kg. Result of the acute toxicity study is shown in table 3.

Carragenan induced paw edema in rats

The effects of toluene and methanolic extract of *Dipteracanthus prostratus* on paw edema induced by carrageenan are shown in Table 4. The reduction in the volume of paw edema with different doses of toluene extract at 100 and 200 mg/kg, body weight was found to be 20.68 & 25.86% respectively while the reduction in the volume of paw edema in case of methanolic extract at the dose of 100 and 200 mg/kg, body weight was found to be 17.24 & 22.41% respectively. Standard drug Diclofenac sodium reduced the paw volume significantly 43.10%.

The anti-inflammatory effect of the toluene extract at 200 mg/kg, body weight was more potent and significant during the three phases of inflammation, compared to control and with other extract. The anti-inflammatory effect produced by Diclofenac sodium progressively increased and reached a maximum (43.10%) after four hours. However, the decrease in inflammation by toluene extract at 200 mg/kg as compared to Diclofenac sodium, which reduced the volume of paw oedema by 43.1%, was more significant and reproducible.

DISCUSSION

Carrageenan induced paw edema is the standard experimental model for acute inflammation. Carrageenan is the phlogistic agent of choice for testing anti inflammatory drugs as it is not known to be antigenic and is devoid of apparent systemic effects. Moreover the experimental model exhibits high degree of reproducibility. The development of edema has been described as biphasic[18]. 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 prostanes[19, 20].

Herbal medication is promising as an alternative treatment to available synthetic drugs for the treatment of different diseases probably due to availability, affordability, lesser adverse effects and proved effectiveness[21]. Previously studied also stated that flavonoids are responsible for the reduction of deleterious effects of free radicals and reactive oxygen species in the prevention of inflammation[22]. Phytochemical studies of both extracts of *Dipteracanthus prostratus* confirmed the presence of important phytoconstituents such as flavonoids and tannins thus on the basis of findings of phytochemical studies and results of the anti-inflammatory activity we can conclude that the protective mechanism produced by *Dipteracanthus prostratus* in carrageenan induced paw edema model may be due to the multiple mechanisms provoked by the flavonoids and other important phytoconstituents.

CONCLUSION

In conclusion, toluene extract of *Dipteracanthus prostrates* at the specified dose level of 200 mg/kg, body weight showed maximum reduction in rats paw edema volume as compared to other extract and their respective dose in carrageenan induced paw edema model of anti-inflammatory activity.

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