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

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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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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