

ANTI-INFLAMMATORY AND ANTI-ARTHRITIC ACTIVITIES OF *DELONIX ELATA* BARK EXTRACTS

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ABSTRACT

Delonix elata (*D. elata*), has long been used in traditional herbal medicine for the treatment of arthritis pain. In the present study an attempt was made to study the effect of *D. elata* barks for its anti-inflammatory and anti-arthritic effect in animal models. Barks were subjected for extraction with pet. ether, chloroform and 40% hydroalcohol successively and evaporated under rotary evaporator to get the concentrated extract. All the extracts were subjected for acute oral toxicity studies in rats and found to be safe up to the dose of 5g/kg body weight. Anti-inflammatory screening by carrageenan-induced paw oedema and cotton pellet induced granuloma method, the hydro alcohol extract of *D. elata* barks showed significant protection against the inflammation. In Complete Freund's Adjuvant induced arthritis model also the hydro alcohol exhibited significant protection on day 7 onwards.

Key words: *Delonix elata*, Antiinflammatory, Antiarthritic, Extracts.

INTRODUCTION

Inflammatory diseases including different types of rheumatic conditions are a major and worldwide problem. Rheumatoid arthritis (RA) is a chronic, inflammatory, systemic autoimmune disease that affects about 1% of the general population in Western countries and is two to three times more common in women than in men¹. According to Ayurveda, the first requirement for healing oneself is a clear understanding of the three doshas. The concept of Vata-Pitta-Kapha is unique to Ayurveda and it holds the potential for revolutionizing the healing systems of the West. However, the concept of the three principles and the Sanskrit words, Vata-Pitta-Kapha, are very difficult to translate into Western terms. The leaves and barks of the plant are widely used by Siddha and Ayurveda practitioners for treating several conditions. *D.elata* used as anti-inflammatory², rheumatism², anti-microbial^{3,4}, and possess antioxidant⁵ activities. Hence attempt was made to study the bark for anti-inflammatory and anti-arthritic activity.

MATERIALS AND METHODS

Collection of plant

Fully grown *Delonix elata* barks were collected from outskirts of Tiruchengode, Tamil Nadu, India and voucher specimens have been authenticated by Dr. Sreenath (Taxonomist), Department of Botany, Bangalore University Bangalore, India. Barks were washed with tap water to remove the dirt, shade dried, and powdered.

Extraction of plant material

The powdered plant material was loaded in Soxhlet apparatus and was extracted with pet. ether, chloroform and 40 % hydroalcohol successively. The solvent from the extract was subjected to vacuum evaporator to collect the crude extract.

Pharmacological studies

Pharmacological studies on animals were followed according to OECD notified guidelines after the approval from Institutional Animal Ethical Committee (IAEC).

Acute oral toxicity study of the extracts

Acute oral toxicity study of the extracts was performed as described by Eva S⁶ et al., Three groups of each 3 rats were selected and they were housed in 12h light dark cycle for a period of one week. 2000mg/ kg of pet. ether, chloroform and hydro alcohol were administered orally to the groups respectively. The animals were observed at a regular interval for a period of 48h. All the animals were found safe and healthy. This procedure was repeated by increasing the dose up to 5000mg/ kg and the results were noted.

After 48h animals were found safe, hence all the three extracts of *Delonix elata* bark were found to be safe up to 5000mg/ kg.

Carrageenan-induced paw oedema in rats

Male wistar rats weighing 250±20 g were taken for the study. The animals were divided into five groups of six each. Group I served as control received only the vehicle (1% Tween 80). Group II served as standard, received diclofenac sodium at dose of 10 mg/kg ip. Group III to V received pet. ether, chloroform and hydro alcoholic extracts of *Delonix elata* at doses of 250 mg/kg respectively. The animals were pretreated with extracts or diclofenac sodium one hour before and they were injected with 0.1 ml of 1% carrageenan (in 1% CMC) solution into the sub-plantar region of right hind paw. Paw volume was measured by plethysmograph immediately after carrageenan application at 0, 1, 2, 4 and 24 h after the stimulus. Reduction in the paw volume compared to the vehicle-treated control animals was considered as anti-inflammatory response^{7,8,9}. The results are shown in table 1.

Cotton pellet induced granuloma method

Five groups of each six healthy male wistar rats weighing 250±20 g were taken for the study. They were housed in 12 h light dark cycle for a period of one week. One group served as negative control. They were anaesthetized with Ketamine (75mg/kg), fur was removed and an incision was made in the scapular region and one in the lower back region under aseptic precautions. Using a blunt forceps, subcutaneous tissue (fascia) was dissected and a tunnel was formed. Non absorbable Cotton pellets (which were prepared earlier, weighed to exactly 20 mg with digital weighing balance and sterilized with hot air oven) was inserted into the end of each tunnel. Care was taken so that the cotton pellets did not absorb blood. Thus, two cotton pellets were placed into one rat¹⁰. On the other rat two pellets were put; each pellet was put in either side of groin region. The wounds were sutured with 2.0 non absorbable silk threads. The rats were observed carefully and caged individually till they started moving around normally. The rats were given standard pellet food, water and they were housed for 7 days. On the 8th day, the rats were sacrificed and incisions were made around the area where the pellets were placed, the granuloma tissue around the cotton pellet identified by colour changes in the area and by palpation and dissected out. The dissected granuloma was placed in hot air oven at 50°C for 48 h in order to remove all the water content and to achieve the dry weight. The pellets were taken out and weighed with weighing balance. The actual weight of the granuloma was found out by

subtracting the initial cotton pellet weight^{11,12}. The results are shown in table 2.

Adjuvant induced arthritis

Complete Freund's Adjuvant (CFA) induced arthritis was carried out as described by Chakraborty M et. al¹³. Wistar albino rats of either sex weighing 200 ± 20 g were used for the study. The animals were divided into five groups of six animals each. The standard drug diclofenac sodium was dissolved in water whereas extracts of *Delonix elata* were triturated with tween 80 in a glass mortar, made as a suspension and administered immediately. Arthritis was induced in rats by the intraplantar injection of 0.1 ml of CFA in the left hind paw. The adjuvant contained heat killed *Mycobacterium tuberculosis* (H37Rv strain, Tuberculosis Research Centre, ICMR, Chennai) in sterile paraffin oil (10 mg/ml). The paw volume of all the animal groups was measured by plethysmograph at 0, 7, 14, 21 and 28 days after the injection of CFA. Group I served as control (without treatment), Group II served as arthritic control (negative control), Group III was treated with diclofenac sodium (positive control) the standard anti - arthritic drug, Group IV, V & VI were treated with pet ether, chloroform and hydro alcoholic extracts of *Delonix elata*. The body weight changes were also observed every week¹⁴. The results of anti arthritic activity are shown in table 3.

Statistical Analysis

All data in the figures or table are presented as the Mean±S.E.M. and analyzed by SPSS version. 18.0 statistical packages. The data were analyzed by One Way ANOVA followed by Tukey's test. p≤0.05 was considered as statistically significant.

RESULTS AND DISCUSSION

The oral toxicity revealed that all the extracts were found to be safe up to 5000 mg/kg. The hydro alcohol extract of *D.elata* showed potent anti-inflammatory by significantly preventing the paw oedema from the 1st h to 4th h. The results of cotton pellet granuloma model revealed that oral administration of 250 mg/kg of hydro alcohol extract prevented 39.66% inhibition of granuloma as compared to standard diclofenac (10mg/kg) 50%. In adjuvant induced arthritis model the hydro alcohol extract shows potent activity. Oral administration of 250 mg/kg of hydro alcoholic extract significantly inhibited CFA induced rat paw oedema after 14 and 21 days. The results were significant when compared with diclofenac (10 mg/kg). Present study revealed that there is a close relationship between the extent of joint inflammation and the degree of weight loss. In the first week after adjuvant injection, the arthritic rats showed marked weight loss, followed by normal weight gain in the subsequent weeks, whereas the plant extract and standard drug treated groups did not show any weight loss.

Flavonoids are present in *D.elata* bark, which are reported to possess potent anti-inflammatory activity by targeting COX, LO and AT, leading to blockage of their action thereby preventing generation of inflammatory mediators. The enzyme deactivation is may be reversible hence it is to be justified through appropriate experimental models.

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Table 1: Anti-inflammatory activity of extracts of *Delonix elata* bark by carregen induced paw oedema method

Carregen induced paw oedema of <i>Delonix elata</i> Bark.				
Difference in paw Volume from 0hr				
Treatment	1 h	2 h	4 h	24 h
Control	0.210±0.006	0.250±0.008	0.259±0.008	0.138±0.0168
(Diclofenac 10mg)	0.062±0.004**	0.052±0.015**	0.035±0.012**	0.013±0.005**
Pet.Ether(250mg)	0.204±0.002	0.205±0.021	0.238±0.004	0.129±0.011
Chloroform (250mg)	0.205±0.003	0.207±0.020	0.241±0.001	0.042±0.006*
Hydro Alcohol (250mg)	0.077±0.005**	0.045±0.006**	0.030±0.004**	0.014±0.003**

n = 6 per group. Values are Mean±SEM. *P< 0.05, ** P < 0.001

Table 2: Anti-inflammatory activity of extracts of *Delonix elata* bark by cotton pellet granuloma method

Cotton pellet Granuloma by <i>Delonix elata</i> Bark extracts		
Treatment	Weight of granuloma Mean \pm SEM	Percentage Inhibition
Control	96.67 \pm 3.333	----
(Diclofinac 10mg)	48.33 \pm 3.073*	50.00*
Pet.Ether (250mg)	95.00 \pm 2.236	1.73
Chloroform (250mg)	91.67 \pm 3.073	5.18
Hydro Alcohol (250mg)	58.33 \pm 3.073*	39.66*

n = 6 per group. Values are Mean+SEM. * P < 0.001

Table 3: Anti-arthritic activity of extracts of *Delonix elata* Bark by adjuvant induced Arthritis Method

Anti-Arthritic activity of <i>Delonix elata</i> Bark extracts					
Treatment	DAY 0 Mean \pm SEM	DAY 7 Mean \pm SEM	DAY 14 Mean \pm SEM	DAY 21 Mean \pm SEM	DAY 28 Mean \pm SEM
Control	0.69 \pm .011	1.50 \pm .034	1.74 \pm .031	1.72 \pm .024	1.70 \pm .017
(Diclofinac 10mg)	0.67 \pm .017	1.16 \pm .034**	1.25 \pm .029**	0.95 \pm .020**	0.80 \pm .022**
Pet.Ether (250mg)	0.70 \pm .009	1.49 \pm .373	1.72 \pm .249	1.77 \pm .171	1.79 \pm .194
Chloroform (250mg)	0.66 \pm .004	1.49 \pm .045	1.70 \pm .193	1.75 \pm .192	1.75 \pm .194*
Hydro Alcohol (250mg)	0.70 \pm 0.014	1.31 \pm .026**	1.36 \pm .035**	1.02 \pm .018**	0.94 \pm .022**

n = 6 per group. Values are Mean+SEM. *P< 0.05, ** P < 0.001

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