Vol. 9/2 (2009) 209 - 215

JOURNAL OF NATURAL REMEDIES

Anti-inflammatory activity of *Delonix elata* (L.) gamble

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Abstract

Objectives: To investigate anti-inflammatory activity of *Delonix elata (D.elata)* leaf, by carrageenan induced paw edema and cotton pellet granuloma models, along with the antioxidant potential underpinning its role as traditional medicine for joint disorders. Materials and methods: Methanol extract and its, ethyl acetate soluble and insoluble daughter fractions were evaluated for anti-inflammatory activity. Rats were divided into seven groups (n=6), including control and standard. Oral doses of methanol extract (100, 200 and 300mg/kg) and its daughter fractions (300mg/kg) were given to animals after carrageenan challenge. The best performing methanol extract was forwarded for cotton-pellet induced granuloma model. The same was also subjected to antioxidant assays like DPPH free radical scavenging activity, reducing power assay and nitric oxide scavenging activity. Moreover, isolation and HPTLC quantification of a marker compound was also attended from methanol extract derived ethyl acetate fraction. Results: In comparison to control, methanol extract of *D. elata* leaf at 300 mg/kg showed significant reduction in area under curve of rat paw edema in the later phases of inflammation (45.83%), which was comparable to that of standard (valdecoxib, 33.525%). The extract was also effective in producing 28.125% inhibition of granuloma formation which was comparable to that of standard (23.88%). Apart from nitric oxide scavenging assay (IC_{50} 157.08µg/ml), the activity of methanol extract in other assays were not as significant as the respective standard drugs. Of the ethyl acetate soluble and insoluble fractions evaluated, both showed marginal reduction in paw edema. From the ethyl acetate fraction, luteolin was isolated as a marker compound and quantified by HPTLC. Conclusion: Methanol extract of *D. elata* leaf was found to be active in the last phase of paw edema at 300 mg/kg, compared to other doses and fractions. At the same dose it was found to have potent antioxidant capacity, which may play its role in reducing inflammation. The claims regarding anti-inflammatory activity of *D. elata* leaf can be considered valid as explicated by its methanol extract.

Keywords: Antioxidant, *Delonix elata*, edema, inflammatory joint disorders, leaf, luteolin.

1. Introduction

Delonix elata (Caesalpiniaceae) is a small sized tree found in Gujarat, Western Peninsular and Southern India. Trunk of the tree is smooth, ash coloured, leaves compound, rachis 15-30 cm long, bipinnate, leaflets 10-20 pairs, flowers yellowish white in terminal corymbiform racemes, pods small, 12-18 cm long, seeds 4-8 [1,2]. *D. elata* is native to Madagaskar, later

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introduced and naturalized in India, commonly known as "Sandesaro". The leaves of which are used both internally and for external application in cases of inflammatory joints by applying paste or by taking the expressed juice by local people. Medicated oil prepared from the leaves is marketed under the name of "Vathanarayana". Leaves are used as a folklore remedy for inflammatory joint disorders [3].

2. Materials and methods

2.1 Plant materials and preparation of extracts

Identity and authenticity of the plant *D. elata* was confirmed by the experts of the Institute of Post Graduate Teaching & Research in Ayurveda (IPGT&RA), Gujarat Ayurved University, standard Herbarium samples of the museum of M. S. University, Baroda with the aid of its morphological characters in the literature [1,4]. Mature leaves were collected in the month of September just before flowering and were dried in shade, powdered for phytochemical and pharmacological studies. Powder of the plant material (100 gm) was continuously extracted under reflux with methanol (200 ml x 5).The resultant extracts were filtered, pooled and concentrated to dryness to yield dark brown solid (MeOH Ext, 30% w/w). The methanol extract was dissolved in 20% methanol in water and extracted with ethyl acetate to separate the ethyl acetate soluble (EtOAC Ext) and insoluble (EtI Ext) fractions.

2.2 Phytochemical analysis

For the isolation of chemical markers from *D. elata* leaf, MeOH Ext was subjected to acid hydrolysis (2N HCl; 5h on boiling water bath), followed by extraction using ethyl acetate (50ml x 3). Dried ethyl acetate extract (3.14 %w/w) was subjected to column chromatography. 300 mg of the ethyl acetate extract was loaded in a glass column (20 x 1cm) using silica gel (200-400#) as a stationary phase. Gradient elution was performed using toluene and ethyl acetate as mobile phase (100:0 to 0:100). Fractions eluted were subjected to Co-TLC separation with various standards (luteolin, kaempferol, isorhamnetin and apigenin; Sigma Chemicals, USA), using silica gel G as adsorbent and Toluene : Ethyl acetate : Formic acid (5 : 4 : 1) as mobile phase. The HPTLC was done on precoated TLC plates of silica gel 60 F_{254} (Merck) and Toluene : Ethyl acetate : Formic acid (5 : 4 : 1) as solvent system using CAMAG LINOMAT IV (semiautomatic spotting device) equipped with Camag TLC Scanner 3 and Camag CATS 4 integration software. The plates were visualized and scanned at 254 nm. Calibration curve of accurately weighed standard (1 mg/ml) was prepared in methanol in a volumetric flask. A fixed volume of standard solution (1, 2, 3, 4, 5, 6 µl) was spotted. Calibration curve of peak area vs. concentration of standard was plotted. The concentration of the marker compound in the EtOAC was calculated from the calibration curve.

2.3 Anti-inflammatory activity Methods

Male and female albino Wistar rats weighing around (150-250 g) were kept under standard conditions with free access to food and water. The protocols were permitted by the institutional animal ethical committee.

2.3.1 Carrageenan-induced paw edema

Animals were divided into seven groups of 6 rats. Group 1 served as control (vehicle only), group 2 to 7 received, MeOH Ext100 (100 mg/kg), MeOH Ext200 (200 mg/kg), MeOH Ext300 (300 mg/kg); EtOAC Ext300 fraction (300 mg/kg), EtI Ext300 (300 mg/kg) and standard (valdecoxib, 0.14 mg/kg) respectively. The doses were given one hour before the rats were challenged by carrageenan as reported earlier [6]. The paw volume was measured using plethysmometer from 0 to 5 h after the challenge.

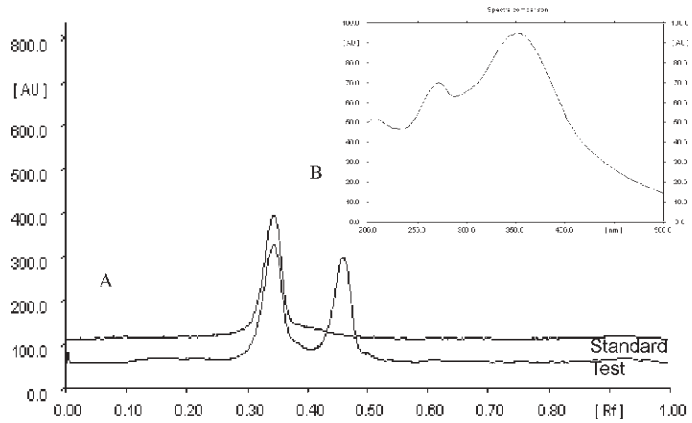

Fig. 1. A. HPTLC densitometric chromatogram and *B. Overlay* of UV absorption spectra of luteolin in standard and test solution tracks scanned at 254 nm.

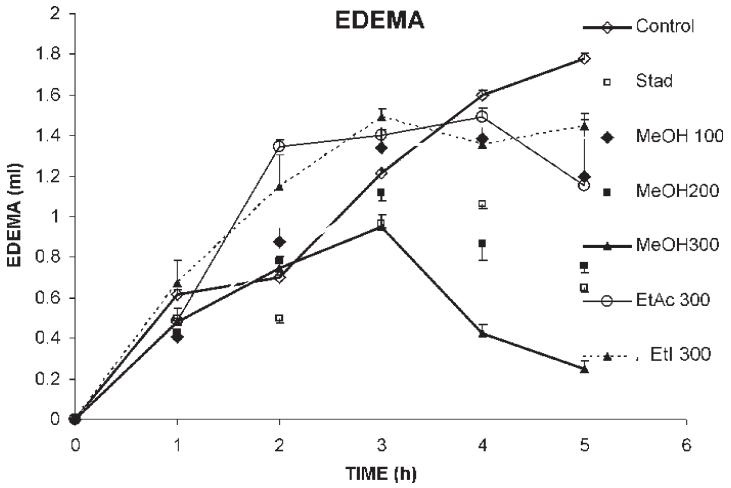

Fig 2. Carrageenan induced paw edema by time. MeOH Ext100 (100 mg/kg), MeOH Ext200 (200 mg/kg), MeOH Ext300 (300 mg/kg), EtOAC Ext300 fraction (300 mg/kg), EtI Ext300 (300 mg/kg) and Standard (valdecoxib, 0.14 mg/kg).

Table 1. Free radical scavenging activity of *Delonix elata* leaf

(n=3)	IC_{50} (µg/ml)	SD	*p*	R^2
DPPH radical scavenging activity				
Ascorbic	7.76	0.35	0.01	0.6672
MeOH Ext	160.355	3.9	0.03	0.878
Reducing power assay				
Ascorbic acid	31.77	1.4	0.01	0.8571
MeOH Ext	155.399	6.2	0.03	0.7484
Nitric oxide scavenging activity				
Ascorbic acid	160.38	5.8	0.01	0.8427
MeOH Ext	157.086	7.3	0.045	0.7958

The percentage reduction of edema was determined as reported earlier [5]. The area under curve (AUC) of edema at 0 to 5h for each dose was also determined to calculate % reduction in AUC. From AUC at different doses, median effective concentration (EC_{50}) was also determined.

2.3.2 Cotton pellet-induced granuloma formation

Pre-weighed autoclaved cotton pellets were surgically implanted in the subcutaneous region below abdomen [5] of the rats. The animals divided in four groups were treated for 7 days orally with MeOH Ext100 (100 mg/kg), MeOH Ext200 (200 mg/kg), MeOH Ext300 (300 mg/kg), apart from control receiving water. The removal and processing of pellets thereafter was done to calculate percentage inhibition as reported earlier [5].

2.4 In vitro free radical scavenging

Antiradical activity (free radical scavenging activity) by DPPH method was done as reported earlier [6]. Ascorbic acid (10 to 100 µg/ml, at an equal increment of 10) was used as a standard to compare with methanol extract of leaf at concentrations of 100, 200, 400 µg/ml. The reducing power assay was done as reported elsewhere [7], wherein the standard ascorbic acid and test were applied at concentrations of 10, 20, 40, 60 and 100 µg/ml. Nitric oxide scavenging activity was done as reported [8], where NO generated by sodium nitroprusside and estimated by Griess reagent is comparable to the amount quenched by ascorbic acid and extract at 10, 20, 40, 60 and 100 µg/ml.

2.5 Statistical analysis

Results are presented as mean ± SEM. Statistical differences between the means of the various groups were evaluated using one-way analysis of variance (ANOVA) followed by Bonferroni *t*-test against control. Data were considered statistically significant at $p < 0.05$ and highly significant at $p < 0.001$. Statistical analysis was performed using Sigmastat statistical software.

3. Results and discussion

Leaves of *D. elata* is traditionally known to possess anti-inflammatory properties. Elfin reports [25] regarding phytochemical and pharmacological studies led us to investigate the folklore claims.

Thin layer chromatography (TLC) of the methanol extract of the leaf was carried out by using Toluene : Ethyl acetate : Formic acid (9 : 4 : 1) as a solvent system. Luteolin was isolated from hydrolyzed methanol extract and its identity was confirmed by Co-TLC using standard luteolin. Further, HPTLC method was developed and luteolin was found to be 0.1% w/w in the leaf powder (Fig. 1).

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 [9]. Mechanism of induction of carrageenan edema has been extensively investigated [9]. In this test, development of edema (inflammatory response) is a biphasic event with a maintenance phase in between [9]. The initial phase (12 h) is primarily mediated by histamine and serotonin [9], but platelet activating factor and arachidonic acid metabolites also play important roles [10]. The control animals showed significant and continuous increase in edema up till the 5^{th}h (Fig. 2). The biphasic response was evident from the pattern of increase. The standard drug, valdecoxib at 0.14 mg/kg showed significant decrease in edema visible in all quarters of time prolific in the 5^{th}h. The area under curve of control was around 50.217, which was higher than that of standard AUC of 33.525, exhibiting a 33.24% reduction in AUC. The methanol extract of *D. elata* leaf at 100 mg/kg showed

significant reduction in edema at 1st (34.32%, P = 0.001), 4th (13.73%, P = 0.015) and 5th (32.77%, P = 0.001) h in comparison to control. The MeOH Ext of *D. elata* leaf at 200 mg/kg also showed highly significant reduction in edema at 1st (30.76%, P = 0.001), 4th (51.57%, P = 0.001) and 5th (57.48%, P = 0.001) h. The MeOH Ext of *D. elata* leaf at 300mg/kg showed significant but lower reduction in edema at 1st and 3rdh, which drastically changed in the 4th (73.57%, P = 0.001) and 5thh (86.14%, P = 0.001) in comparison to control. EtAOC and EtI were administered the dose at which MeOH was found to perform the best (300 mg/kg). In the 1sthe reduction by MeOH can be attributed to EtAOC giving 20.39% ($P < 0.05$) reduction, which is not observed in EtI fraction. In the 4thh the reduction of EtAOC (6.8%, $P < 0.05$) and EtI (15.5%, $P < 0.05$) was minor. In the concluding hour EtAOC (37.9%, P = 0.001) showed better performance than EtI (18.8%, $P < 0.05$). Global effect of EtAOC (6.75%) and EtI (9.06%) is negligible as compared to standard and its mother extract as revealed by percent reduction in AUC. Overall, this indicates EtAOC is more competent than EtI, but still falling short of MeOH 300 mg/ml makes space for possible synergistic interaction between both in their mother extarct.

The dose dependent activity of the drug could be predicted by EC_{50} of methanol extract around 318.88 mg (R^2 =0.9965) which is evident from the pattern of time wise reduction in edema in most of the phases. At lower doses the methanol extract seems to inhibit histamine and serotonin mediated early inflammatory response. But at higher doses the inhibition of prostaglandin and arachidonic metabolites mediated phase was found to be prolific in comparison to the standard and control. The cotton-pellet test is considered as a model for studies on chronic inflammation [11], and inflammatory granuloma is considered as a typical feature of established chronic inflammatory reaction [12]. Proliferation of fibroblasts, synthesis of new tissue and repair of the damaged area mark this phase [6]. It represents both exudative and proliferative phases of inflammation. Formation of fibrous tissue predominates over fluid accumulation. The inflammatory mass consists of inflammatory cells, area of granulation and fibrous tissue thus representing intermingling of healing and inflammation, which is the main feature of chronic inflammation [13]. Besides, lymphokines are also reported to increase fibroblast migration and division. Subcutaneous implantation of cotton pellets induced formation of granuloma exhibiting characteristic features of a typical chronic inflammatory lesion. Of all the doses tested, in comparison with control, the methanol extract at 300 mg/kg was found to be significant (28.125%, p=0.001) and comparable with that of standard (valdecoxib, 0.14 mg/kg, 23.88%, p=0.001). Methanol extract at the dose of 100 mg/kg gave only 10.16%, while 200 mg/kg (-5.86%), did not show inhibition, and the values were statistically insignificant. Thus, it can be said that the drug showed considerable anti-inflammatory activity against histamine, serotonin, prostaglandin, arachidonic acid metabolites and tissue damage phases.

An uncontrolled oxidative activity is accepted as a general mechanism of tissue damage in a variety of pathological conditions like joint inflammations, carcinogenesis, aging, atherosclerosis, diabetes mellitus and gastro-duodenal ulceration [14]. Several studies have shown that biologically derived oxidants like superoxide anion, hydrogen peroxide, hypochlorous acid, and peroxidase generated oxidants play major role in the tissue injury that result as a consequence of inflammatory response [15]. Further, metabolic disruptions caused by diverse toxic agents acting at various sites may in part, be expressed by abnormal

levels of reactive oxygen species (ROS) [16]. Thus, agents such as antioxidants that can control states of oxidative stress represent a major line of defense regulating general health status [17]. Flavonoids, phenols, glycosides, terpenoids, lignans, anthraquinones, etc. act as natural free radical scavengers [17-19].

In the light of the known involvement of free radicals in tissue damage involved particularly with inflammatory conditions, the methanol extracts of *D. elata* was tested for its interaction with reactive oxygen species (ROS) in various ROS-generating chemical reactions. The extract also showed significant nitric oxide radical scavenging activity (Table 1). The leaves of *D. elata*, contain high amount of phenolics [20], ascorbic acid (295 mg/100 g), total carotene (60 mg/100 g) and tannin (1330 mg/100 g) [21]. Such components may play important role underlying the potent antioxidant activity of *D. elata*. Certain flavonoids viz. flavonol, flavonones and flavones (luteolin) are reported to harbor antiinflammatory activity [22-24].

Thus, it can be said that the methanol extract of leaves of *D. elata* posses anti-inflammatory activity pillared by its free radical scavenging potential, possibly contributing to its multiple action against inflammation.

4. Acknowledgement

The authors are heartily thankful to Ms Bakula K. Shah, Dr. Rajani Mandapati and Prof. M. S. Baghel for their kind help.

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