



RESEARCH ARTICLE

PHARMACOLOGY

EVALUATION OF PROTECTIVE EFFECT OF *DELONIX ELATA* ON CHRONIC INFLAMMATION AND COMPARISON OF ITS ULCEROGENIC POTENTIAL WITH IBUPROFEN.

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ABSTRACT

Antinociceptive property of ethanolic extract of plant *Delonix Elata* was screened by acetic acid induced writhing in mice while the anti-inflammatory property against chronic inflammation was studied using cotton pellet granuloma in rats. The gastric irritant action was tested by administering the drug orally and screening the gastric mucosa macroscopically and microscopically in rats. The severity of ulcers were scored from zero to five. Plant extract of *Delonix elata*, at the dose 100 and 200 mg/kg showed significant reduction in the number of writhing with 55.56% and 54.76% of inhibition respectively along with significant dose dependent reduction in the weight of cotton pellet from 48.7 mg to 31.3 mg and 28.5 mg respectively. The results were comparable to the standard drug, Ibuprofen. The histological section after administration of Ibuprofen indicated superficial erosion, in contrast to intact surface epithelium after treatment with *D.elata*.



KEYWORDS

Delonix elata, Cotton pellet, Anti-nociceptive, chronic inflammation, Acetic acid.

INTRODUCTION

Inflammation is the protective mechanism employed by the tissues against endogenous and exogenous antigens¹. It is the protective attempt by the organism to remove injurious stimuli as well as initiate the healing process, however if runs unchecked lead to the onset of the diseases like rheumatoid arthritis and atherosclerosis^{2,3}. Non steroidal anti-inflammatory drugs are one of the commonly used drugs against the diseases like rheumatoid arthritis, gout where there is chronic inflammation. On chronic use, the most harassing side effect is NSAIDs induced gastric ulceration⁴. Though Ibuprofen has been rated as the safest NSAID by the spontaneous adverse drug reaction system in U.K. gastric discomfort is the most common side effect^{5,6}. Therefore, the development of potent anti inflammatory drugs with fewer side effects is necessary⁷. On the contrary, many medicines from the plant origin has been used for inflammation since long time without any adverse effects^{8,9}. *Delonix elata* (*D. elata*), is one of the plants that has long been used in traditional herbal medicine for the treatment of arthritis pain. Though *D.elata* has been earlier studied for its anti-inflammatory action, its effect on gastric mucosa and ulcerogenic potential has not been investigated^{10,11,12}. Hence, the present study was designed to validate the effect of *D. elata* on chronic inflammation using different animal models as well as its effect on gastric mucosa after chronic administration.

MATERIAL AND METHODS

Chemicals

Acetic acid was obtained from Sigma chemicals, Dorset, England. Ibuprofen was obtained from Cipla Ltd.

Plant material and extraction

Collection of the Plant Materials

The leaves of *D. elata* were collected in the month of the September from the Chennai, Tamil Nadu, India and was then authenticated by local Botanist of Science College.

Preparation of extract

Fresh leaves were carefully cleaned, dried in shade, powdered and stored in airtight containers until it was used for further studies. Alcoholic extract was prepared according to the procedure reported by Mahanta & Mukharjee¹³. Forty grams of dried powder was packed in the timple of soxhelet apparatus and was extracted using 70% ethanol refluxing at 50-70° C which yielded an extract which was dark brown in colour. The stock extract was preserved in airtight glass container and kept inside the refrigerator at 4° C.

Animals

Adult Swiss albino mice weighing 20-25 gm and Wistar albino rats weighing 120-130 g of either sex were used for the study. The animals were maintained under standard laboratory conditions (light period of 12 h/day and temperature 27° C ± 2° C) with access to water ad libitum. The animals were used in groups of 6 for all the studies.

Ethical Clearance

The experimental procedures were carried out in strict compliance with the Institutional Animal Ethics committee regulations.

Acute toxicity study in mice

In an attempt to arrive at suitable doses for pharmacological studies, a pilot study



was conducted by the method of Miller and Tainter¹⁴. The LD₅₀ of the plant extract was found to be 1.995 gms/kg using probit analysis so the doses 100 mg/kg, p.o. and 200 mg/kg, p.o. which were ten times less the lethal dose of the plant extract were chosen for further studies.

Acetic acid induced writhing

The antinociceptive activity of *D. elata* was assessed using acetic acid induced writhing test (abdominal constriction test)¹⁵. Acetic acid solution (0.6%) in a dose of 10 ml/kg was injected intraperitoneally (i.p) to all the animals, 30 min after the administration of the test drug. Group 1 received 1% CMC as vehicle orally and was considered as control. Group 2 and group 3 received plant extract at the dose of 100 mg/kg and 200 mg/kg orally. Group 4 received Ibuprofen 100mg/kg and served as the standard control. In all the groups, the time taken for the onset and the number of the contraction of abdominal muscles together with stretching of hind limbs (writhes) were noted for 15 mins.. All the groups received same volume of preparations.

Cotton pellet granuloma

The effect of ethanolic extract of leaves of plant *D. elata* on chronic inflammation was assessed using cotton pellet granuloma model¹⁶. Wistar albino rats were used for this procedure and they were divided into four groups of six animals. Each animal in all the groups were implanted with sterile cotton pellets (10 mg) subcutaneously in arm pits and groins under light ether anaesthesia. Group 1 received 1% CMC as vehicle orally for seven days and served as the control after the commencement of experiment.. Group 2 and Group 3 received plant extract at the dose of 100 mg/kg and 200 mg/kg orally for seven days and Group 4 received Ibuprofen 100 mg/kg for seven days and served as the standard control. All the animals were sacrificed on the 8th day and cotton pellets were removed with surrounding granulomatous

tissues. The cotton pellets were dried at 50°C for 24 hrs and weighed.

Effect of *D. elata* on gastric mucosa

For this study Wistar albino rats were taken and the animals were divided into four groups of six animals in each. Group 1 received vehicle as 1% CMC orally for seven days. Group 2 and Group 3 received ethanolic extract of leaves of *D. elata* at the dose of 100 mg and 200 mg respectively in the same pattern. On 8th day the animals were sacrificed and the stomach were excised and opened along the greater curvature. The gastric mucosa was examined using a magnifying lens for any visible mucosal damage. The severity of ulcers were scored from zero to five depending on the size and severity of ulcers¹⁷. Necrohemorrhagic spots greater than 2 mm diameter are taken as ulcers. These are graded as follows.

0 = normal mucosa

1 = minute sporadic punctuate lesions

2 = several small lesions

3 = one lesion of large extension or multiple moderate sized lesions.

4 = several large lesions

5 = perforation

Ibuprofen, a standard analgesic and anti-inflammatory agent was also studied for its ulcerogenic potential by the above methods. Group 4 received Ibuprofen 100 mg/kg orally for the seven days and the same procedure was followed on 8th day.

Statistical analysis

The statistical analysis were carried out using one way Analysis of Variance (ANOVA) using unpaired student's t test for analgesic and anti-inflammatory experiments and Mann Whitney 'U' test for gastric irritant effect. P value < 0.05 was considered as statistically significant.



RESULTS

Effect of *D. elata* extract on the acetic acid induced writhing

Ethanollic extract of the *D. elata* (100 and 200 mg/kg) showed significant dose dependent reduction in the number of writhing with

approximately 55.56% and 54.76% of inhibition respectively. Maximum inhibition was observed at a dose of 200 mg/kg which proved to be significantly similar to the standard control drug Ibuprofen (100 mg/kg). Ibuprofen treatment resulted in 51% inhibition of acetic acid induced writhing. (Table 1)

Table 1
Effect Of *D. Elata* And Ibuprofen On Acetic Acid Induced Writhing In Mice

Groups	Dose (mg/kg, p.o.)	No. of abdominal contractions (Mean \pm SEM)	Percent Inhibition
Group 1	Vehicle	30.0 \pm 0.58	
Group 2	<i>D.elata</i> 100	13.33 \pm 0.71*	55.56%
Group 3	<i>D.elata</i> 200	13.57 \pm 1.29*	54.76%
Group 4	Ibuprofen 100	14.67 \pm 1.33*	51%

Each value represents the mean \pm SEM of six observations (Student 't' test) *P < 0.001 compared to vehicle treatment

Effect of *D. elata* extract on the Cotton pellet granuloma

The mean weight of the cotton pellet in vehicle treated group was 48.7 mg. When the Ethanollic extract of the *D. elata* was given at the dose 100 mg/kg and 200 mg/kg, the recorded weight was 31.37 mg (35.53 % reduction) and 28.52 (41.38 % reduction) mg respectively, showing

significant dose dependent reduction. Maximum reduction observed at a dose of 200 mg/kg proved to be significantly similar to the standard control drug Ibuprofen (100 mg/kg). In Ibuprofen treated group the weight of the cotton pellet was 28.03 mg (42.39 % reduction). (Table2)

Table 2
Effect Of *D.Elata* And Ibuprofen On Cotton Pellet Granuloma In Rats.

Groups	Dose (mg/kg)	Weight of Cotton Pellets (mg) (Mean \pm SEM)	Percent Inhibition
Group 1	Vehicle	48.66 \pm 0.26	
Group 2	<i>D.elata</i> 100	31.37 \pm 0.15*	35.53
Group 3	<i>D.elata</i> 200	28.52 \pm 0.35*	41.38
Group 4	Ibuprofen 100	28.03 \pm 0.56*	42.39

Each value represents the mean \pm SEM of six observations (Student 't' test) *P < 0.001 compared to vehicle treatment

Effect of *D. elata* on gastric mucosa

The histological section after acute administration of Ibuprofen indicated

superficial erosion, which denotes the loss of the superficial epithelium, generating a defect in the mucosa which did not cross



the muscularis mucosa. (Fig 3) On the contrary, after acute treatment of *D.elata*, it was found that the surface epithelium was intact. (Fig 2) However, the presence of neutrophils above the basement membrane was indicative of mild inflammation even in *D.elata* treated animals. The mean ulcer

score in Ibuprofen treated animals was increased to 2.33 which was statistically significant. However, in *D.elata* (200mg/kg) treated animals the ulcer score observed was only 0.33 which was similar to that of vehicle treated (Fig 3) control animals 0.2. (Table 3)

TABLE 3

Ulcer Scores Observed In Rats After D.Elata And Ibuprofen Chronic Study

Groups	Dose (mg/kg)	Mean Ulcer Score (Mean \pm SEM)
Group 1	Vehicle	0.33
Group 2	<i>D.elata</i> 100	0.33
Group 3	<i>D.elata</i> 200	0.33
Group 4	Ibuprofen 100	1.50

Each value represents the mean of six observations.

Statistical analysis done by using the Mann – Whitney 'U' test.

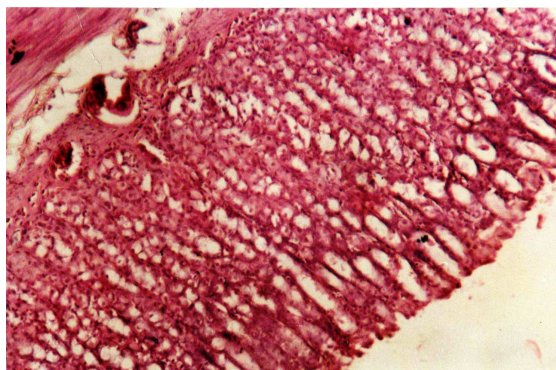


Figure 1

Effect of Control on gastric mucosa

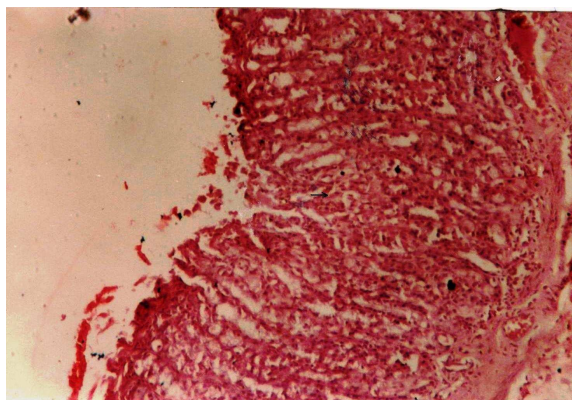


Figure 2

Effect of D. elata on gastric mucosa

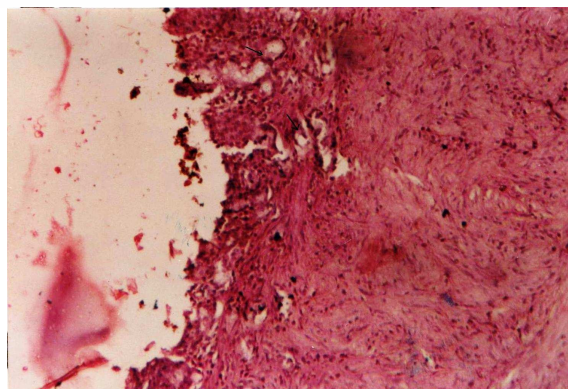


Figure 3
Effect of Ibuprofen on gastric mucosa

DISCUSSION

The results of the above studies identify a novel source of analgesic anti-inflammatory from natural source. However, no information is presently available pertaining to the mechanism of the above mentioned actions of *D.elata*. In general etiology of pain and inflammation mainly encompasses the involvement of the arachidonic acid metabolites notably prostaglandins. Since *D.elata* extract has been proved to inhibit pain and inflammation there is a likelihood of its intervention in prostaglandin synthesis. When compared to Ibuprofen the gastric mucosal damage was very mild with *D.elata*. This may indicate a predominant effect of *D.elata* on cyclooxygenase II than cyclooxygenase I¹⁸. However, this possibility remains to be investigated in detail by future studies. have reported that The anti-inflammatory activity of *D.elata* flowers may be correlated to the presence of bioflavonoids like rutin and quercetin-3-O-galactoside, which have been isolated from these plant. Moreover, many flavanoid compounds have been reported to inhibit prostaglandin synthetase and this property has been considered responsible for their analgesic and anti-inflammatory properties^{10,19}. These evidences are suggestive of the presence of active compounds in *D.elata*

which may be specifically effective against cyclooxygenase II enzyme and exhibit potent anti-inflammatory and analgesic effect with very minimal gastric irritant properties. Analgesic activity was studied by the acetic acid induced writhing test using Swiss albino mice (20-25 g). The time taken for the onset of writhing reflex and number of writhes counted for 15 minutes were noted¹². The percentage inhibition of writhing produced by different pre-treatment was calculated. *D. elata* treatment in doses of 100 and 200mg/kg produced 55.78 and 66.31% of inhibition respectively. Ibuprofen treatment resulted in an inhibition of 65.26%. The potent anti-inflammatory effect of *D. elata* has been studied and its significant analgesic property was also brought out. After chronic treatment of *D.elata* the gastric mucosal damage were mild when compared to that observed after Ibuprofen treatment.

CONCLUSION

Thus, it can be concluded from the study that *D. elata* leaves extract has antiinflammatory activity. Results are comparable with the Ibuprofen. Also it is less ulcerogenic than Ibuprofen. Further elaborative work is necessary for the better understanding of the mechanism of its antiinflammatory activity. Detailed clinical studies in this direction are required to potentiate this claim in humans



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