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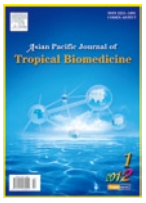

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Antinociceptive activity of *Delonix elata* leaf extract

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ABSTRACT

Objective: To evaluate antinociceptive property of the leaf extract of *Delonix elata* (Linn.). **Methods:** Antinociceptive activity was evaluated by abdominal writhing and tail flick methods in Swiss albino mice. Acetyl salicylic acid was used as standard drug. **Results:** A dose dependent significant ($P<0.01$) antinociceptive activity was observed in three different concentrations 100, 200 and 300 mg/kg. Among them dose at 300 mg/kg, significantly ($P<0.01$) inhibited (74.94%) the nociception induced by acetic acid but less effective than the standard reference (84.06%). Tail-flick test showed that the extract at the doses 300 mg/kg showed efficient results when compared to the other two doses (100 and 200 mg/kg) with increase in the latency to response of tail to the thermal stimulation. **Conclusions:** The significant antinociceptive activity of the leaf extract is due to the presence of a single active constituent in higher levels or due to the combined effect of more than one phytoconstituent. This investigation supported the ethnomedicinal claims of *Delonix elata*.

1. Introduction

Natural products are the source of synthetic and traditional herbal medicine and are still the primary health care system for underdeveloped and developing countries. Pain is part of a defensive reaction against dysfunction of an organ or imbalance in its functions against potentially dangerous stimulus[1]. Many drugs are used to relieve the pain and a few drugs such as morphine[2] and aspirin[3] for centuries. Most of the pain relieving drugs produced pronounced side effects on the physiology of the body. In indigenous system of medicine, several plants possess antinociceptive property and many investigators screened the plant extracts for antinociceptive property *viz*., *Bowdichia virgilioides*[4], *Capparis ovata*[5], *Urtica circularis*[6], *Phlomis umbrosa*[7]. Reports also indicated that only a little information is available on the screening of antinociceptive activity of phytoconstituents such as, sesquiterpene dilactone from *Mikania cordata*, Burm.f.[8], divaricatol and hamaudol from *Saposhnikovia divaricata*, Turcz.[9], dioclenol and dioflorin from *Dioclea grandiflora*, Mart[10].

Delonix elata (Linn.) (*D. elata*) is a tree species belonging to the family Fabaceae (Leguminosae), sub family Caesalpinioideae. The traditional practioners residing in the villages of Chitradurga and Davanagere districts of Karnataka, India use the decoction of the leaves for relief from rheumatic problems such as pain and joint stiffness. The medical usefulness of the tree is acknowledged[11]. There are no reports available on antinociceptive property of leaf extract of *D. elata*. In the present investigation, we have made an attempt to investigate the antinociceptive property of leaf extract of *D. elata* by abdominal writhing and tail flicking methods.

2. Materials and methods

2.1. Plant material and extraction

The leaves of *D. elata* were collected from the villages near by Chitradurga district, Karnataka, India. The plant material was identified and authenticated by Dr. Manjunatha B.K., comparing with the voucher specimen deposited at Kuvempu University Herbarium specimen FDD-No.57, (Flora of Davanagere District, Karnataka, Manjunatha *et al* 2004). The leaves were cleaned with deionized water and were shade dried, grounded porously by using mechanical blender and passes through 40-mesh sieve. About 1 kg of powdered material was loaded into four Soxhlet timbles of 250 g each and extracted using ethanol for about 48 h. The extracts were filtered (Whatman No.1 filter paper) and concentrated in vacuum under reduced pressure using rotary flash evaporator (Buchi, Flawil, Switzerland) and then the extract was kept on water bath for complete evaporation

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of solvent. Finally the dried extract was preserved in air tight container until use.

2.2. Animals

Healthy Swiss albino mice weighing 20-25 g were procured from Central Animal House, National College of Pharmacy, Shimoga, Karnataka, India and were housed at (23 ± 2) ℃, humidity 55%-60% in the Department of Biotechnology, Kuvempu Univeristy, Shimoga, Karnataka, India. They were fed with standard commercial pellet diet (Durga Feeds and Foods, Bangalore) and water *ad libitum*. All the animals were acclimatized for one week before the experiments, and all experiments were carried out according to the institutional animal ethics committee guidelines (Permission No. NCP/IAEC/CLEAR/06/2007-08).

2.3. Acute toxicity study

The staircase method[12] was adopted for the determination of the acute toxicity. Healthy albino mice of either sex weighing 20-25 g were used to determine the safer dose. Water was used as a vehicle to dissolve the extract and was administered orally.

2.4. Antinociceptive activity

2.4.1. Abdominal writhing method

Antinociceptive activity of the crude ethanol extract was carried out using adult Swiss albino mice of either sex weighing 20-25 g, five groups with 6 animals per group were selected for abdominal writhing method[13]. Group I animals were treated with 0.6% acetic acid (dose 10 mL/kg) intraperitoneally. After 5 min of injection of acetic acid, number of writhes was counted for 20 min. This reading was taken as control. Group II, III and IV were administered orally with the water dissolved ethanol extract at the dose of 100, 200 and 300 mg/kg body weight, respectively. Group V was administered with acetyl salicylic acid (100 mg/kg) and used as standard drug for the comparison of antinociceptive activity. After one hour incubation all the groups except group I animals were administered with acetic acid. After 5 min, each group mice were observed for the number of writhes for the duration of 20 min. The mean value for each group was calculated. A reduction in the writhing number compared to the control group was considered as evidence of analgesia. The percentage inhibition of writhing was calculated as: % Inhibition = (C-T) / C × 100. Where C = mean number of writhes produced by the control group and T = mean number of writhes produced by the test groups.

2.4.2. Tail flick method

Swiss albino mice of either sex weighing between 20-25 g were divided into 4 groups of six mice in each group. Group I mice were treated with normal saline (10 mL/kg). Group II, III and IV were administered orally with the crude ethanol extract at the dose of 100, 200 and 300 mg/kg respectively. Antinociceptive effect of the test samples was determined by the tail-flick method described by Sewell and Spencer (1976). One to two centimeter of the tail of experimental mice was immersed in warm water kept constant at 50 ℃. The pain reaction time was the time taken by the mice to deflect their tails. The first reading is discarded and the reaction time was taken as a mean of the next two readings. The latent period of the tail-flick response was taken as the index of antinociceptive activity and was determined before and at 0.5, 1.0, 1.5, 2.0, 2.5 and 3.0 h after the administration of drugs. The maximum reaction time was fixed at 0.5 h (30 min). The maximum possible analgesia (MPA) was calculated according to the method of Idid *et al*(1998).

2.5. Statistical analysis

The data of antinociceptive activity was expressed as mean ± SEM of six animals in each group. The statistical analysis was carried out using one way ANOVA followed by Tukey's *t*-test. The difference in values at $P<0.01$ was considered as statistically significant.

3. Results

3.1. Acute toxicity study

After 72 h observation, a plot of mortality values *vs.* log dose showed that the ethanol extract at the dose of 3 000 mg/kg did not show any sign of mortality. One tenth of this dose (300 mg/kg) was considered as safer dose for administration.

3.2. Antinociceptive activity

3.2.1. Writhing method

The number of writhes observed during 20 min period in control group was 80.50 ± 0.76. The crude extract at the dose of 100, 200 and 300 mg/kg reduced the number of writhes to 38.07 ± 1.32 (with 52.70% protection), 27.00 ± 1.69 (with 66.45% protection) and 20.17 ± 1.49 (with 74.94% protection), respectively. These values indicated that the responses were dose dependent. But the effect of extract at different concentrations showed slightly less potent than standard drug acetyl salicylic acid which showed 5.23 ± 0.49 (with 84.06% protection) writhes. All the readings found to be significant ($P<0.01$) when compared to control.

3.2.2. Tail flick method

Throughout the 3 h observation, animals pretreated with

Table 1.
Antinociceptive activity of *D. elata* leaf extract by tail flick method(Mean ±SEM).

Group	Dose(*p.o.*)	0.5 h	1.0 h	1.5 h	2.0 h	2.5 h	3.0 h
Control (Normal saline)	10 mL/kg	3.03±0.05**	2.80±0.06**	2.90±0.04**	3.20±0.08**	3.10±0.02**	3.40±0.05**
Ethanol extract	100 mg/kg	8.40 ± 0.13**	7.80±0.08**	7.10±0.07**	6.90±0.05**	6.00±0.04**	5.60±0.05**
	200 mg/kg	10.40±0.08**	9.90±0.04**	9.70±0.04**	8.80±0.06**	7.20±0.04**	7.00±0.04**
	300 mg/kg	11.80±0.04**	11.10±0.02**	10.20±0.05**	9.60±0.04**	9.30±0.02**	7.70±0.13**

*$P < 0.05$, ** $P < 0.01$, ns: not significant, as compared to control group.

normal saline did not show significant effect on the latent period of tail-flick response. The antinociceptive effects of crude leaf extract in three different doses were evident within 0.5 h following oral administration and the effect remained significant ($P<0.01$) throughout the 3h observation period. At 100 mg/kg dosage, the MPA was increased from (5.60±0.05) to (8.40±0.13)%. Likewise, at 200 mg/kg dosage, the MPA increased to (10.40±0.08)%. At 300 mg/kg dosage the MPA value calculated was significantly ($P < 0.01$) increased to (11.80±0.04)%. The effects of crude extract on nociceptive responses induced by noxious heat (50 ℃) are shown in Table 1.

4. Discussion

In the present study, antinociceptive activity of leaf extract of *D. elata* was carried out using two different models namely tail flick method and acetic acid induced abdominal writhing using mice. The tail flick method was selected to investigate central antinociceptive activity[14]. In order to distinguish between the central and peripheral antinociceptive action, acetic acid induced abdominal writhing response in mice was conducted. The writhing induced by chemical substances is due to sensitization of nociceptors by prostaglandins[15,16]. The abdominal constriction response induced by acetic acid is a sensitive procedure to establish peripherally acting antinociceptives. This response is thought to involve local peritoneal receptors[17]. In this study tail flick and writhing methods were used to assess the antinociceptive activity of *D. elata*. The crude extract of leaf of *D. elata* in three different doses showed good peripheral antinociceptive activity by decreasing the number of writhes and exhibited central antinociceptive activity by showing significant effect on the latent period of tail-flick response throughout the 3 h observation. There was increase in the response when increases in the dose rate of 100 to 300 mg/kg. The responses were dose dependent. Among three doses, 300 mg/kg exhibited highly significant ($P< 0.01$) responses. Many investigators have evaluated the antinociceptive property of various herbal extracts using experimental mice. The significant effect of the phytoextracts is due to the presence of a single active constituent in higher levels or due to the combined effect of more than one phytoconstituent. The present study revealed that the ethanol extract of leaf of *D. elata* exhibited significant antinociceptive property but less effective than the standard reference acetyl salicylic acid.

This investigation revealed that the *D. elata* leaf extract produced significant and dose dependent antinociceptive effects in both tail flick and abdominal writhing models. Among the three different doses tested, the higher dose was found to be more potent. The present investigation supported the ethno medical claims of *D. elata*.

Conflict of interest statement

We declare that we have no conflict of interest.

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