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ARTICLE

Analgesic Activity of the Methanol Leaf Extract of *Delonix Regia*

EZEJA, M. I.*, EZEIGBO, I. I., MADUBUIKE, K. G. and EKPE, I. J.

Department of Veterinary Physiology, Pharmacology and Biochemistry, College of Veterinary Medicine, Michael Okpara University of Agriculture Umudike Abia State, Nigeria. *Corresponding author: E- mail: ezejamaxwell@yahoo.com; Tel.: +2348033238975.

SUMMARY

The analgesic activity of the methanolic leaf extract of *Delonix regia* in albino Wistar mice using acetic acid –induced writhing reflex, tail immersion and hot plate experimental models was evaluated. Three test doses (200, 400 and 800 mg/kg body weight) of the extract were used while 400 mg/kg of acetylsalicyclic acid (aspirin) was used as reference drug all administered by gastric gavage. In acetic acid-induced writhing reflex model, the extract and acetylsalicyclic acid dose dependently and significantly ($p< 0.0001$) decreased the abdominal constriction or writhing. The extract also increased the percentage inhibition of abdominal constriction from 0% in the negative group to 80% in the highest dose of the extract (800 mg/kg) treated group. In the tail immersion model, the extract at the doses of 400 and 800 mg/kg significantly ($p< 0.03$) increased the pain reaction time (PRT) while in the hot plate model there was no significant difference in the pre-drug pain reaction time (PRT) but the extract at the dose of 800 mg/kg significantly ($p< 0.05$) increased the post- drug PRT. In the tail immersion and the hot plate models, the extract at the dose of 200 mg/kg had no analgesic effect. In conclusion, the methanolic leaf extract of *Delonix regia* demonstrated significant analgesic activity that may be mediated through peripheral and central mechanisms.

KEY WORDS: *Delonix regia*, analgesic, aspirin, writhing, hot plate.

INTRODUCTION

Pain can be defined as an unpleasant sensory and emotional experience associated with actual or potential tissue damage. It is a disabling accompaniment of many medical conditions and pain control is one of the most important therapeutic priorities (Rang *et al.,* 2003). It is always a warning signal and primarily protective in nature but often causes a lot of discomfort and may lead to many adverse effects (Keay, 1989).

Analgesics are drugs used to treat or reduce pain. Non-steroidal anti-inflammatory drugs have their origin in natural products and many synthetic compounds with similar mechanisms of action have been developed but are associated with serious adverse effects such as ulceration, gastro intestinal bleeding, addictive potential, respiratory distress, drowsiness, nausea etc. (Laurence *et al.,* 1997; Mate *et al.,* 2008). There is therefore the need for the search for bioactive compounds from medicinal plants that have analgesic activity with little or no side effects.

Delonix regia belongs to the family of *Caesalpinoideae*. It is a tree that grows up to 12-15m in height with a trunk of about 1m in diameter and has smooth bark. The leaves are alternate, about 30-60cm long, bipinnate with 10-25 pairs of opposite pinnate. The flowers are red to orange in

colour with 5 oblong thick sepals. The fruits are woody, long, flat pod that may be straight or somewhat curved and about 30-40cm long and dark brown or black when ripe (Arbonnier, 2004).

It is also known as flamboyant tree in English and 'Otroshi' in Enugu –Ezike local dialect.

The leaf extract has been reported to have antibacterial, anti-malarial, antifungal and anti-diabetic properties (Rahman *et al.*, 2011). Preliminary phytochemical analysis shows that the leaves contain flavonoids, phenolic compounds, triterpenes and sterols (Vaishali *et al.*, 2012). In eastern Nigeria the leaves are used for treating pains in traditional medicine practice.

Literature search revealed no previous report on the analgesic activity of *D. regia*. The present study was therefore designed to evaluate the analgesic potentials of D*elonix regia* with the aim of establishing the pharmacological basis for its use in treating pains in folkloric medicine.

MATERIALS AND METHODS

Plant collection and identification.

Fresh leaves of *Delonix regia* were collected from the premises of Michael Okpara University of Agricukture, Umudike, Abia State, Nigeria. The plant specimen was identified by Dr. D.O. Dike of the forestry department of the same university and a voucher specimen number MOUAU/CVM/VPP/0023/11 was deposited in department of Veterinary Physiology, Pharmacology and Biochemistry herbarium.

Preparation of plant materials

The leaves of *D. regia* were cut into pieces, dried under mild sunlight and later pulverised into a coarse powder of about 1mm in diameter. The pulverised plant material was extracted by cold maceration method in 80% methanol with intermittent shaking at 2 hr interval for 48 hr. The extract was then filtered with Whatman no 1 filter papers and then the filtrate was concentrated to dryness in an oven at 40°C. The percentage yield was calculated using the formula below:

$$\frac{\text{Weight of extract}}{(\text{Weight of plant material used})} \times \frac{100}{1}$$

Animals

Adult albino Wistar mice of mixed sexes weighing between 23-31g obtained from the laboratory facilities of the faculty of Veterinary medicine, University of Nigeria Nsukka were used for the experiment. The animals were kept in stainless steel cages with temperatures varying between 25-30° C and lighting period of about 12 hr. Clean drinking water and commercial pelleted feed (Vital feed®, Jos, Nigeria) were provided *ad libitum*. Ethical conditions governing the conducts of experiments with live animals as stipulated by Zimmerman, (1983) was strictly followed.

Acute toxicity test

Acute toxicity study was done using the method of Lorke (1983).

Twenty five adult mice of mixed sexes were randomly divided into 5 groups of 5 mice per group. *Delonix regia* extract at the doses of 100,500. 1000. 2000 and 3000 mg/kg were given to mice in groups 1, 2, 3, 4 and 5 respectively. All the mice were given feed and water and were observed for 48 hr for signs of toxicity and death.

Acetic acid induced- writhing reflex test in mice

This study was carried out using the method of Koster (1959) as modified by Dambisya and Lee (1999).

Twenty five adult mice randomly grouped into 5 equal groups and fasted for 12 hr were treated as follows: Group 1 (negative control group) mice received 10 ml/kg of

distilled water, group 2 mice (positive control group), received 400 mg/kg acetylsalicylic acid while groups 3, 4 and 5 mice received 200, 400 and 800 mg/kg of *D. regia* extract respectively all by gastric gavage. One hour after drug and extract administration , 0.6% glacial acetic acid was given intraperitonealy at the dose of 10 ml/kg to all the mice to induce pain characterized by abdominal constrictions or writhing reflex. The number of writhes observed in each mouse was counted for 30 min and recorded. The percentage pain inhibition was also calculated according to the formula of Dambisya and Lee (1999) thus:

$$\% \text{ Analgesia} = \left\{ \begin{array}{l} (\text{Mean of negative control group} - \\ \text{Mean of treated group}) \div (\text{Mean} \\ \text{of negative group}) \end{array} \right\} \text{X} \frac{100}{1}$$

Tail immersion or flick test

This was carried out using the method described by Uma-Devi *et al.,* (1999).

Twenty five adult mice were randomly grouped into 5 equal groups and fasted for 12 hr. The mice were pretreated 1 hr before tail immersion with 10 ml/kg distilled water for group 1 (negative control group), 400 mg/kg of aspirin for group 2 (positive control group) and 200, 400 and 800 mg/kg of *D. regia* extract for groups 3, 4 and 5 respectively all by gastric gavage. About 2-3 cm of the tail of each mouse was dipped into a water bath containing warm water maintained at the temperature of 50 ± 1° C and the time taken for the mouse to flick the tail known as the pain reaction time (PRT) was recorded.

Hot plate test

This was carried out using the effect of hot plate-induced pain in the paws of mice as described by Shethy and Anika (1982):

Twenty five adult albino Wistar mice were randomly grouped into 5 equal groups. The animals were fasted for 12 hr with clean drinking water provided. Each of the mice was placed on a hot plate maintained at the temperature of 55 ± 1° C and the PRT or latency period determined with a stop watch and recorded. This was regarded as the pre –drug PRT. The response to pain stimulus considered included: jumping, raising and licking the hind feet and the cut off time was put at 20 sec to avoid damage to the paws. The mice were then treated with 10 ml/kg distilled water for group 1 (negative control group), 400 mg/kg of aspirin for group 2 (positive control group) and 200, 400 and 800 mg/kg of the extract for groups 3, 4 and 5 respectively all by gastric gavage.

DATA ANALYSIS

The results were presented as mean ±SEM and analyzed using one way analysis of variance (ANOVA). The differences between the means were tested using Post Hoc Dunnett and T-test and values of $P < 0.05$ were considered statistically significant.

RESULTS

Plant extraction

The yield of the *Delonix regia* extract was 5.32% w/w dry matter and was dark green in colour

Acute toxicity test

No death or signs of toxicity was observed even at the dose of 3000mg/kg after 48 hours.

Acetic acid-induced writhing reflex

The effect of *Delonix regia* crude extract on the acetic acid-induced abdominal constriction in mice is presented in Table 1. The results show that the extract at the doses of 200, 400 and 800 mg/kg and the reference drug (aspirin, 400 mg/kg) dose dependently and significantly ($p < 0.0001$) decreased the abdominal writhes in mice when compared to the negative control

group, reducing the mean number of abdominal constrictions from 129.8 ± 5.99 in the negative control group of mice to 49. 6 ± 16.74 in the reference drug treated group of mice and to 26.0 ± 4.90 at the dose of 800 mg/kg of the extract. The percentage abdominal constriction was dose dependently increased from 0% in the negative control group to 80% at the dose of 800 mg/kg of *D. regia* extract.

Tail immersion or flick test

The effect of *D. regia* on the tail immersion or flick test is presented in Table 2. The results show that the extract at the dose of 400 and 800 mg/kg and the reference drug significantly p< 0.03 increased the pain reaction time (PRT) or the latency period in the mice when compared to the negative control group. At the dose of 200 mg/kg, the extract did not increase the PRT, though there was a marginal increase in the PRT.

TABLE I: Effect of *D. regia* on acetic acid-induced writhing reflex in mice

Group	Treatment	Mean no of writing in 30mins ± SEM	% Protection
1	Distilledwater (10mg/kg)	129.8± 15.99	0
2	ASA (400mg/kg)	49.6 ± 16.74*	62
3	DRE (200mg/kg)	69.6 ± 15.60*	46
4	DRE (400mg/kg)	47.6 ± 7.52*	63
5	DRE (800mg/kg)	26.0 ± 4.90*	80

* P < 0.0001 when compared to group 1

TABLE II: Effect of *D. regia* on tail immersion response in mice.

Group	Treatment	Mean PRT (Sec)
1	Distilledwater (10mg/kg)	2.6 ±0.10
2	ASA (400mg/kg)	3.60 ± 0.24*
3	DRE (200mg/kg)	3.00 ± 0.77
4	DRE (400mg/kg)	4.00 ± 0.45*
5	DRE (800mg/kg)	7.40 ± 2.23*

*P < 0.03 when compared to group 1

Hot plate test

Table 3 shows the effect of *D. regia* on hot plate- induced pain in mice. The results show that there was no significant difference in the mean pre-drug PRT among the groups. After drug and extract administration, the reference drug and the extract at the doses of 400 and 800 mg/kg significantly ($p < 0.05$ and $p < 0.01$ respectively) increased the PRT when compared to the negative control group. The extract at the dose of 200 mg/kg did not produce any significant increase in the PRT.

In all the experiments, the highest dose of the extract (800 mg/kg) produced a better effect than the reference drug (acetylsalicylate, 400 mg/kg).

TABLE III: Effect of *D. regia* on hot plate induced pain in mice

Group	Treatment	Mean pre-drug PRT ± SEM	Mean post -drug PRT ± SEM
1	Distilled water 10mg/kg)	4.02 ± 0.82	3.98 ± 0.55
2	ASA (400mg/kg)	5.05 ± 0.64	6.54 ± 1.00*
3	DRE (200mg/kg)	4.92 ± 0.49	5.15 ± 0.64
4	DRE (400mg/kg)	5.48 ± 0.29	7.95 ± 0.66*
5	DRE (800mg/kg)	3.95 ± 0.46	6.19 ± 1.20**

*P < 0.05, **P <0.01 when compared to group 1

DISCUSSION

Animal tests of analgesic drugs commonly involve testing the reactions of an animal to painful stimuli (Rang *et al.,* 2003). The stimulus may be thermal (tail immersion and hot plate), electrical (tail, paw or dental pulp stimulation), mechanical (tail and paw pressure tests) or chemical (acetic acid-induced writhing or formalin test) (George *et al.,* 2009), thus justifying the use of the three analgesic models for this study.

The methanolic leaf extract of *D. regia* produced no death or signs of toxicity even at the dose of 3000 mg/kg which suggests that the extract was well tolerated by the mice and the doses used were safe.

The acetic acid-induced abdominal constrictions or writhing reflex model is a sensitive method for screening analgesic effect of compounds (Bentley *et al.,* 1983). Pain sensation in acetic acid-induced writhing reflex test is elicited by triggering localized inflammatory response resulting in the release of arachidonic acid from tissue phospholipid (Ahmed *et al.*, 2006) via cycloogense (COX) and prostaglandin biosynthesis (Duarte *et al.*, 1988). Pain is indirectly generated via endogenous mediators like prostaglandins (Onansanwo and Elegbe, 2006).

In this experiment, the extract and the standard reference drug significantly (P< 0.0001) and dose dependently reduced the number of abdominal constrictions in the mice. Also the percentage inhibition of constriction was increased from 0% in the negative control group to 80% at the dose of 800mg/kg as against the 62% in the standard drug treated group.

Agents that reduce the number of writhing will render analgesic effect preferably by inhibition of prostaglandin synthesis, a peripheral mechanism of pain inhibition (Ferdous *et al.*, 2008). Machioro *et al.,* (2005) also observed that the percent reduction in the number of abdominal constrictions indicate the level of analgesia in the acetic acid-induced model.

All these point to the potent analgesic activity of *D. regia* and also suggest that the analgesic effect may be mediated through peripheral pain mechanism and or may be through inhibition of the activities or synthesis of prostaglandins.

In the tail immersion method, the procedure is based on the observation that morphine like drugs selectively prolongs the reaction time of the typical tail withdrawal reflex in mice (Ramadran and Basinath, 1986) while in hot plate, the paws of mice are very sensitive to temperatures

of between 50-60° C (Raquibul, *et al*., 2010).

In tail immersion and hot plate tests, the extract also significantly (P<0.003 and P < 0.05 respectively) increased the PRT of the mice at the dose of 400 and 800mg/kg.
In both models, increase in PRT indicates the level of analgesia of a drug or extract (Ramadram and Basinath, 1986) and both models are used for the study of centrally acting analgesics (Wolfe and MacDonald, 1994). Also in these models, increase in stress tolerance capacity of animals indicates the involvement of higher center (Vogel and Vogel, 1997).

The results obtained using these models suggest that *D. regia* has analgesic activity and may suggest that the analgesic effects of *D. regia* at higher doses involves central activity since the lowest dose of the extract produced no analgesic effect in these models.
Phytochemical analysis of *Delonix regia* showed that it contains flavonoids, phenolic compounds, triterpenes and sterols (Vaishali *et al*., 2012). Flavonoids and triterpenes have been shown to have analgesic activities (Gokhale *et al*., 2002). Flavonoids being a powerful antioxidant are reported to play a role in analgesic activity primarily by targeting prostaglandins synthesis or action (Raquibull *et al*., 2010). This therefore suggests that the analgesic activity of *Delonix regia* may be due to its phytochemical constituents.

CONCLUSION

The methanolic leaf extract of *Delomix regi*a showed a significant analgesic potentials in the models used which may be due to its phytochemical content, thus establishing a pharmacologic basis for its use in folkloric medicine and its action may be mediated through peripheral pain mechanism and central pain mechanism at higher doses.
However, more work is required to isolate and characterize the active principle and to establish the exact mechanism of action.

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