ANTINOCICEPTIVE ACTIVITY OF METHANOLIC EXTRACT OF THE LEAVES OF *FERONIA LIMONIA* LINN

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ABSTRACT

The antinociceptive activity of the methanolic extract of the leaves of Feronia limonia Linn. (Family Rutaceae) was investigated using acetic acid-induced writhing model and tail flick test in swiss albino mice. The extract produced about 26.01% (p< 0.01), 39.88% (p< 0.01) and 57.07% (p< 0.01) writhing inhibition at the dose of 100, 200 and 400 mg/kg of body weight respectively, which was comparable to the standard drug diclofenac sodium where the inhibition was about 63.58% (p< 0.01) at the dose of 25 mg/kg of body weight. All doses of extracts significantly (p<0.05) increased latency of flick tail in tail immersion method. The methanolic extract showed dose dependent antinociceptive activity in both type of test in swiss albino mice. Phytochemical investigations of the leaves extract indicate the presence of tannins, saponins, steroids, alkaloids and flavonoids. The preliminary study of the methanolic extract showed antinociceptive activity in both writhing and tail flick test in mice.

Key words: *Feronia limonia, antinociceptive activity, acetic acid-induced writhing, tail flick, phytochemical test.*

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INTRODUCTION

Feronia limonia Linn. (Family Rutaceae, Bengali Kathbel, English Wood apple) is a moderate-sized tree with straight sharp strong spines, 1.2-3.7 cm long, which is widely distributed throughout Bangladesh, India, Ceylon and Java[1,2,3]. The fruits of the plant are edible and considered to be a stomachie, astringent, diuretic, cardiotonic and tonic to liver and lungs[3]. Its fruit is antiscorbutic, carminative, stimulant to the digestive system. Leaves are astringent; used for indigestion, flatulence, diarrhoea, dysentery and haemorrhoids. The leaves and stem bark contain the coumarins, luvangetin, xanthotoxin and limonin and the steroids, sitosterol and sitosterol-O-beta-D-glucoside. Antifungal compounds, psoralene from stembark; xanthotoxin and osthenol from root bark and 2,6-dimeth-oxybenzo-quinone from the fruit shell are reported. Leaves are astringent, carminative and given in indigestion, flatulence, diarrhea, dysentery, vomiting, hiccup and haemorrhoids[1]. The plant is also used in diabetes[3] and snake bite[4]. Antitumor acidic polysaccharides has been isolated from the fruit part of this plant species[5]. The stem bark contains several flavanone having antimicrobial properties[6]. Acetone extract of dried leaves showed potent mosquito larvicidal activity[7]. The present report describes the antinociceptive activity of the leaves and exploration of the justification of its use as folk medicine.

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MATERIALS AND METHODS

Plant materials and extractions:

Leaves of *Feronia limonia* L. were collected from Ramkantopur at Pabna district, Rajshahi division on January 2010 and taxonomically identified by Bangladesh National Herbarium, Dhaka (accession number 31105). The leaves were dried by shade drying for 15 days and then again dried in a hot air oven at 40^0C for 1 hour to remove moisture. Fine powder was obtained after grinding. The leaves were extracted by hot extraction method where100gm powder was macerated with 250ml methanol. The container with its contents was sealed and kept for a period of 7 days accompanying occasional shaking and stirring. The whole mixture then underwent

a coarse filtration by a piece of clean, white cotton material. Then it was filtered through filter paper. The filtrate (methanol extract) thus obtained was evaporated by using a suitable rotary evaporator to get a viscous mass. The viscous mass was then kept at room temperature under a ceiling fan to get a dried extract (about 10% yields). The extract (MFL) thus obtained was used for antinociceptive activity.

Phytochemical Tests

Phytochemical investigations of the leaves extract indicate the presence of tannins, saponins, steroids, alkaloids and flavonoids. The crude methanolic extract of the leaves of *Feronia limonia* was tested for its different chemical groups as tannins, saponins, steroids, alkaloids and flavonoids by the phytochemical analysis processes described by Evans[8].

Animals

Young Swiss-albino mice of either sex, aged 3-4 weeks, average weight 20-25 gm were purchased from the Animal Research Branch of the International Centre for Diarrhoeal Disease and Research, Bangladesh (ICDDR, B). The animals were kept at animal house for adaptation after their purchase under standard laboratory conditions (relative humidity 55- 65%, room temperature 25.0±2.0^{o}C and 12 hours light: dark cycle) and fed with standard diets (ICDDR, B formulated) and had free access to tap water.

Antinociceptive activity study using acetic acid-induced writhing assay:

Antinociceptive activity of the methanolic extract of *Feronia limonia* was tested using the model of acetic acid induced writhing in mice[9]. The experimental animals were randomly divided into five groups, each consisting of five animals. Group I was treated as 'control group' which received 1% (v/v) Tween-80 solution in water by p.o. route; group II was treated as 'positive control' and was given the standard drug Diclofenac sodium at dose of 25 mg/kg of body weight; group III, IV and V were test groups and were treated with methanolic extracts of *Feronia limonia* at the doses of 100, 200 and 400 mg per kg of body weight respectively. Standard drug and extracts were administered orally, 30 min prior to acetic acid (0.7 %) injection. Then after an interval of 15 min, the number of writhes (squirms) was counted for 5 min.

Antinociceptive activity study using tail-flick test:

Immersion of an animal's tail in hot water provokes an abrupt movement of the tail and sometimes the recoiling of the whole body. Again, it is the reaction time that is the time to flick the tail from hot water which is monitored[10]. The extract was administered orally at three doses (100, 200 and 400 mg/kg body weight) using Diclofenac Sodium (25mg/kg) as standard. The distal 2-3 cm portion of mouse-tail was immersed in hot water maintained at 55 ± 0.5°C. The screening cut-off time was 5 sec, while the test cut-off time was 10sec. The post drug reaction times were measured at 0, 30, 60 and 90 minutes later. The time to flick the tail from water (reaction time) was recorded. A maximum immersion time of 10 seconds was maintained to prevent thermal injury to the animals. A significant increase in reaction time compared with control animals was considered a positive analgesic response.

Statistical analysis

Statistical analysis was done with SPSS statistics 17.0 using one way ANOVA followed by Dunett t test. Significance level of <0.05 was considered as significant.

RESULTS AND DISCUSSION

In the statistical analysis all groups show significant ($p<0.05$) antinociceptive activity compared to the control group (Table-1 and Table-2). The preliminary phytochemical screening of leaves extract of *Feronia limonia* showed the presence of tannins, saponins, steroids, alkaloids and flavonoids in our laboratory. These compounds have well known anti-inflammatory effects[11]. In writhing assay the extract produced about 26.01% (P< 0.01), 39.88% (P< 0.01) and 56.07% (P< 0.01) writhing inhibition at the kose of 100, 200 and 400 mg/kg of body weight respectively, which was comparable to the standard drug diclofenac sodium where the inhibition was about 63.58% (P< 0.01) at the dose of 25 mg/kg of body weight. Acetic acid induced writhing is a sensitive method for screening peripheral analgesic effect of compounds. Increased levels of PGE2 and PGF2α in the peritoneal fluid have been reported to be responsible for pain sensation caused by intraperitoneal administration of acetic acid[12,13]. Prostaglandins elicit

Table-I

Effect of methanolic leaves extract of F. limonia on acetic acid induced writhing in mice:

Treatment (mg/kg)	Number of writhings	% inhibition
Control	34.60 ± 3.85	-
Diclofenac (50)	12.60 ± 1.81*	63.58
MFL (100)	25.60 ± 2.30*	26.01
MFL (200)	20.80 ± 5.81*	39.88
MFL (400)	15.20 ± 1.92*	56.07

n = 5. The observation are mean S.E.M. *p<0.05, as compared to control. (ANOVA followed by Dunnett t test). MFL = Metahnolic leaves extract of *Feronia limoina*.

Table-2

Effect of MFL (100, 200 AND 400 mg/kg) on Tail immersion method in mice.

Treatment (mg/kg)	Latency to flick tail (second)			
	0 min	30 min	60 min	90 min
Control	1.90 ± 0.41	1.94 ± 0.18	2.19 ± 0.14	1.85 ± 0.16
Diclofenac (25)	1.79 ± 0.15	5.74 ± 0.30	7.26 ± 0.26*	6.88 ± 0.16*
MFL (100)	1.83 ± 0.60	2.63 ± 0.38	3.16 ± 0.15*	2.98 ± 0.12*
MFL (200)	1.72 ± 0.12	2.48 ± 0.09	3.50 ± 0.24*	4.10 ± 0.38*
MFL (400)	1.85 ± 0.12	2.51 ±0.16	4.15 ± 0.23*	4.66 ± 0.28*

n = 5. The observation are mean S.E.M. *p<0.05, as compared to control. (ANOVA followed by Dunnett t test). MFL = Metahnolic leaves extract of *Feronia limoina*.

pain by direct stimulation of sensory nerve endings and also sensitize sensory nerve endings to other pain provoking stimuli[14]. Moreover, prostaglandins especially PGE1 was reported to act on cell membrane during inflammatory conditions leading to changes in lipoprotein structure of cell membrane. This causes destabilization of cell membrane furthering to degenerative cellular changes[15]. The abdominal constriction is related to the sensitization of nociceptive receptors to prostaglandins. It is therefore possible that extract produced analgesic effect may be probably due to the inhibition of synthesis or action of prostaglandin. Therefore, it is likely that *Feronia limonia* leaves extracts might suppress the formation of these substances or antagonize the action of these substances and thus exerts its peripheral analgesic activity in acetic acid-induced writhing test.

The tail-flick method originally described by Woolfe and Mac Donald[16] has been found to be suitable for the evaluation of centrally acting but not peripherally acting analgesics. The nociceptors seem to be sensitized by

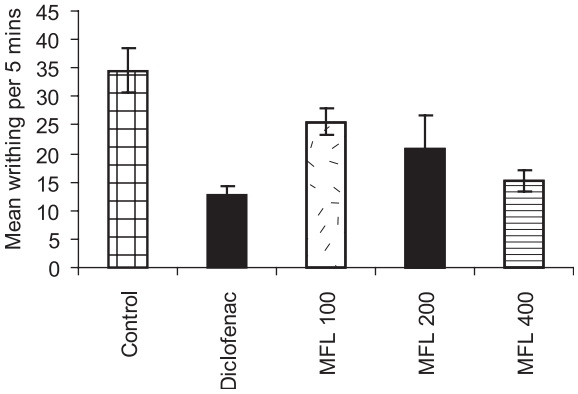

Fig.-1: *Effect of methanolic leaves extract of F.limonia on acetic acid induced writhing in mice. MFL = Methanolic extract of Feronia limonia. Data are expressed as mean ± SEM.*

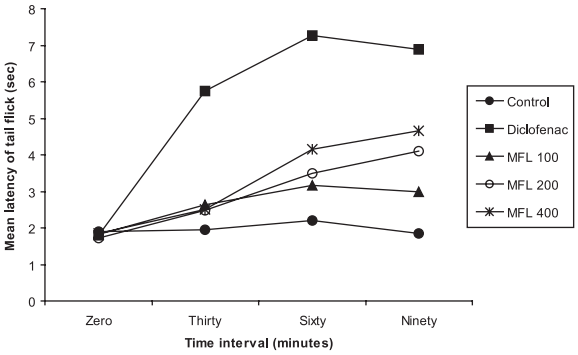

Fig.-2: *Effect of methanolic leaves extract of F.limonia in tail flick assay. MFL = Methanolic extract of F.limonia. Data are expressed as mean SEM.*

sensory nerves. A significant increase in the reaction time for tail flick method indicated the analgesic effect by methanolic extract of *F.limonia*. This elucidates the involvement of central mechanism in analgesic action. On the basis of the result of acetic acid induced writhing test and tail-flick test, it can be concluded that the methanolic extract of the leaves of *Feronia limonia* might possess an antinociceptive activity. The study also provides a strong evidence for the use of the leaves *F. limonia* in folkloric treatment as analgesic agent. The activity may be due to the presence of one or more phytochemical constituents present in the extract further study is warranted, for isolation of the constituents responsible for the activity and also to explore the exact mechanism of action of the activity.

CONCLUSION

In conclusion, it can be suggested that the crude methanolic extract of *Feronia limonia* Linn. may possess antinociceptive activity, which correlate well with the traditional use of the plant. Therefore, further pharmacological investigations of bioactivity guided phytochemical studies are required to find out the actual

constituents responsible for antinociceptive action of the extract of *Feronia limonia* Linn.

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