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RESEARCH ARTICLE

Antinociceptive Activity of *Trigonella foenum-graecum* Leaves and Seeds (Fabaceae)

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ABSTRACT

Present study reports analgesic activity of petroleum ether, chloroform, ethyl acetate and methanolic extracts of leaves and seeds of *Trigonella foenum-graecum* Linn. (Fabaceae) using hot plate method for evaluation of central analgesic activity and acetic acid induced writhing test for evaluation of peripheral analgesic activity in mice. All the extracts of *Trigonella foenum-graecum* showed significant central and peripheral analgesic activity at the dose of 50 mg/kg intraperitoneally. Amongst all the extract methanolic extract of leaves of *Trigonella foenum-graecum* showed highest increase in reaction time in hot plate method and more inhibitory effect on writhing induced by acetic acid. Pentazocin and paracetamol was taken as standard drug for hot plate and writhing model respectively.

Keywords: *Trigonella foenum-graecum, Analgesic activity, Hot plate model, Acetic acid-induced writhing model*

Trigonella foenum-graecum L. (Fabaceae) is nearly smooth erect annual. Leaflets are oblanceolate-oblong, toothed. Flowers are axillary, sessile. Calyx is teeth linear. Pods are long. Seeds are traditionally used as antipyretic, antinociceptive, cure leprosy [1, 2]. Seeds also posses antifertility activity [3], antineoplastic activity [4], hypolipedemic activity [5, 6] and antioxidant effect [7, 8]. One of its constituent alkaloids, called 'trigonelline', has shown potential for use in cancer therapy. Seeds contained alkaloids as triginelline. Furostanol glycosides as trigofoenosides A-1, trigofoenosides D-1, saponin as trigofoenoside E-1, diosgenin, gitogenin, sarsapogenin were isolated from seeds [1, 2]. The seed contains the saponin, diosgenin an important substance in the synthesis of oral contraceptives and sex hormones [9]. Present study reports antinociceptive activity various extracts of *T. foenum-graecum* leaves and seeds.

MATERIAL AND METHODS

Plant material

Leaves and seeds of *Trigonella foenum-graecum* was collected from Ahmednagar district of Maharashtra in October 2006 and gets authenticated by Botanical Survey of India, Pune (Voucher specimen No. TF1).

Preparation of the extract

Leaves and seeds were shade dried, powdered and subjected to successive solvent extraction in Soxhlet extractor using petroleum ether (60-80^{o}C), chloroform, ethyl acetate and methanol as solvents. The extracts were concentrated by vacuum distillation and then dried in open air [10].

Animals

Animals were procured from National Toxicological Center, Pune. Male Swiss Albino mice, weighing between 25-30 g were used for all the experimental protocols. The animals were housed at least one week in the laboratory animal room prior to testing. Food and water were given *ad libitum*. All procedures described were reviewed and approved by Institutional Animal Ethical Committee.

Analgesic activity
Hot plate test

Central analgesic activity was evaluated using hot plate method as per described by Woolfe and MacDonald [11]. Mice were divided into ten groups of six animals each which are adequate. The first group served as control and received only vehicle, second group was administered standard drug pentazocine (50 mg/kg, i.p.). The animals of third to sixth group were

Table 1. Antinociceptive activity of various extracts of leaves and seeds of *T. foenum-graecum*

Treatments	Latency to lick the paw (sec ± SEM)						
	Predrug reaction time(min)	30 min	60 min	90 min	120 min	150 min	180 min
Vehicle	3.6±0.5	3.8±0.88	4.0±0.44	3.8±0.37	3.8±0.20	3.57±0.58	4.45±0.37
Pentazocin	10.5±0.49	12.09±0.81#	13.0±0.67*	18.0±0.58*	17.5±0.74	13.5±0.65	10.54±0.47
PEL	11.87±0.81	9.42±0.67*	12.27±0.81	12.99±0.62#	13.17±0.29	5.13±0.84	1.81±0.62
CHL	5.69±0.82	13.01±0.25*	13.12±0.49	15.9±1.25	10.84±0.83#	6.25±1.09	6.45±0.94
EAL	4.49±0.84	11.9±0.64*	18.00±2.01	16.48±0.87	16.80±0.26	10.09±.83	12.81±0.34
MEL	2.86±0.56	13.89±0.76	18.83±0.85*	19.39±0.58#	20±1.42	19.46±0.99	16.43±0.65
PES	5.88±0.87	13.33±0.72	7.29±0.67	8.90±0.93	12.31±0.74#	10.31±0.59	15.78±0.29
CHS	10.24±0.78	13.38±0.47#	12.21±0.68	13.7±0.59	18.36±0.47	15.28±0.56	10.52±0.83
EAS	6.02±0.49*	10.25±0.39	13.11±0.78	11.20±0.71	10.11±0.43*	10.52±0.28#	8.72±0.86
MES	5.15±0.68	13.03±0.45	9.22±0.70	11.21±0.97	16.43±1.21	17.21±0.76	14.24±0.23*

All the values are expressed as mean ± SEM; n=6, #$p<0.05$, *$p<0.0001$ significant compared to control. All the extracts and pentazocine were given intraperitoneally at 50 mg/kg dose. PEL-petroleum ether extract, CHL-chloroform extract, EAL- ethyl acetate extract, MEL-methanol extract of *T. foenum-graecum* leaves and PES-petroleum ether extract, CHS-chloroform extract, EAS- ethyl acetate extract, MES-methanol extract of *T. foenum-graecum* seeds respectively.

treated with petroleum ether, chloroform, ethyl acetate and methanol extracts (50 mg/kg, i.p.) of leaves and animals of seventh to tenth group were treated with petroleum ether, chloroform, ethyl acetate and methanol extracts (50 mg/kg, i.p.) of seeds of *Trigonella foenum-graecum* respectively. Mice were placed individually on the hot plate maintained at 55 ^{0}C ± 1 ^{0}C and latency of nociceptive response such as licking, flicking of a hind limb or jumping was noted. The readings were taken at 0, 30, 60, 90, 120, 150 and 180 min after administration of extracts. The experiment was terminated 20 second after their placement on the hot plate to avoid damage to the paws.

Writhing test

Peripheral analgesic activity was evaluated using acetic acid-induced writhing test [12]. Mice were divided into ten groups of six animals each. The animals received petroleum ether extract or chloroform extract or ethyl acetate extract or methanol extracts (50 mg/kg, i.p.) of leaves or petroleum ether extract or chloroform extract or ethyl acetate extract or methanol extracts (50 mg/kg, i.p.) of seeds of *Trigonella foenum-graecum* or standard drug paracetamol (50 mg/kg, i.p.) or vehicle, 30 min before intraperitoneal injection of 0.1 ml of 0.6 % solution of acetic acid. Mice were placed individually into glass beakers after administration of acetic acid and five minutes were allowed to elapse. The mice were then observed for the period of 30 minutes and then number of writhes recorded for each animal.

Chemicals

The following drugs were used: pentazocine lactate injection (Ranbaxy, Ahmedabad), paracetamol injection (Heilenlab, Goa), acetic acid (AR Grade, PCL, Pune), petroleum ether (60-80^{o}C), chloroform, ethyl acetate and methanol (AR Grade, PCL, Pune). Petroleum ether (60-80^{o}C), chloroform, ethyl acetate and methanol extracts of *Trigonella foenum-graecum* were suspended into minimum volume of DMF and then volume is adjusted with water for injection, and administered intraperitoneally in a constant volume (8 ml/kg). All drug solutions were prepared immediately before starting the experiment.

Statistical significance

The results were analyzed for statistical significance using students't' test. $p<0.05$ and $p<0.0001$ were considered as significant.

RESULTS AND DISCUSSION

Hot plate test

All the extracts of *Trigonella foenum-graecum* showed significant analgesic activity at 50 mg/kg, i.p. dose (Table 1). Analgesic activity was comparable with standard drug pentazocine. Among all the extracts, methanolic extract of leaf of *Trigonella foenum-graecum* showed highest increase in reaction time.

In the present study, all the extracts showed significant ($p<0.05$ and $p<0.0001$) analgesic activity but among all the extracts, methanolic extract of leaves of *Trigonella foenum-graecum* showed highest increase in reaction time. Thermic painful stimuli are known to be selective to centrally active drugs [13]. Prostaglandins and bradykinins were suggested to play an important role in analgesia [14, 15]. Flavonoids and tannins are reported to inhibit prostaglandin synthesis [16]. A number of flavonoids and tannins have been reported to produce analgesic activity [17]. As phytochemical tests showed presence of flavonoids and tannins in methanolic extract of leaf of *Trigonella foenum-graecum*, they might suppress the formation of prostaglandin and bradykinins or antagonize their action and exert its activity.

Writhing test

All the extracts of *Trigonella foenum-graecum* at dose of 50 mg/kg, i.p., significantly attenuated the number of writhing and stretching induced by intraperitoneal 0.6% acetic acid (Table 2). Methanolic extract of leaf of *Trigonella foenum-graecum* showed more inhibitory effect on writhing induced by acetic

Table 2. Effect of various extracts of *T. foenum-graecum*.L. on acetic acid-induced writhing in mice

Treatment	Number of writhing
Vehicle	63.0±0.94
Paracetamol	9.95±0.75*
PEL	15.23±0.78
CHL	32.49±1.02*
EAL	41.83±0.97*
MEL	8.12±0.37#
PES	48.35±1.23#
CHS	51.34±0.69#
EAS	57±1.76#
MES	45.09±0.91*

All the values are expressed as mean ± SEM; n=6, #p<0.05, *p<0.0001 significant compared to control. All the extracts and paracetamol were given intraperitoneally at 50 mg/kg dose. PEL-petroleum ether extract, CHL-chloroform extract, EAL- ethyl acetate extract, MEL-methanol extract of *T. foenum-graecum* leaves and PES-petroleum ether extract, CHS-chloroform extract, EAS- ethyl acetate extract, MES-methanol extract of *T. foenum-graecum* seeds respectively.

acid as compared to other extracts as well as standard drug paracetamol.

Peripheral analgesic activity was assessed by acetic acid-induced writhing test, which showed significant (p<0.05 and p<0.0001) suppression of writhing by all the extracts, but methanolic extract of leaf of *Trigonella foenum-graecum* showed more inhibitory effect on writhing induced by acetic acid as compared to other extracts and standard drug paracetamol (Table 2). It was observed that onset of writhing was delayed and duration of writhing was shortened. Acetic acid is known to trigger the production of noxious substances within the peritoneum, which induces the writhing response [18]. The effect of the extracts against the noxious stimulus may be an indication that it depressed the production of irritants and thereby reduction in number of writhes in the animals.

Overall we can say that leaves of *Trigonella foenum-graecum* posses better antinociceptive activity.

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