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ANALGESIC AND ANTI-INFLAMMATORY ACTIVITIES OF *TRIGONELLA FOENUM-GRAECUM* (SEED) EXTRACT

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Abstract: Analgesic and anti-inflammatory effects were examined in a partially purified fraction (MTH) of the *Trigonella foenum-graecum* seed extract. The analgesic effects of graded doses of fraction (MTH in 10-40 mg/kg *p.o.*) were evaluated in mice against acetic acid induced writhing (chemically induced pain) and hot-plate method (thermally induced pain). The analgesia produced by MTH was compared with the standard analgesics pentazocine (PTZ, 5 mg/kg *p.o.*) and diclofenac sodium (DIS, 5 mg/kg *p.o.*). Acute anti-inflammatory activity of fraction (MTH) was also evaluated in carrageenan-induced rat paw edema model at the doses 10 and 20 mg/kg *i.p.* and compared with diclofenac sodium (5 mg/kg *i.p.*). In comparison to control group MTH showed highly significant, dose dependent analgesic activity against thermally as well as chemically induced pain ($p < 0.001$). MTH at the dose of 40 mg/kg has shown significant analgesic activity ($p < 0.001$) as compared to diclofenac sodium and pentazocine at the doses employed. In comparison to control, MTH at the employed doses produced marked acute anti-inflammatory activity in rats ($p < 0.001$). The results suggest that the water soluble fraction (MTH) of herbal origin has significant analgesic and anti-inflammatory potential as reflected by the parameters investigated. Further investigations are, however, necessary to explore mechanism(s) of action involved in these pharmacological activities.

Keywords: *Trigonella foenum-graecum*, analgesic activity, anti-inflammatory activity, fenugreek seeds

The clinically useful drugs against pain and inflammation exhibit many adverse effects; this leads to considerable interest in search of safer drug for these conditions. *Trigonella foenum-graecum* L. (Leguminoseae) is an erect, strongly scented annual herb that is extensively cultivated in warm temperate and tropical regions in India, the Mediterranean region, North Africa, and Yemen (1). Seeds has been documented for its multiple pharmacological activities including hypoglycemic (2, 3) gastro-protective and anti-ulcer effects (4). The leaves were also evaluated for potent antinociceptive (5), anti-inflammatory and antipyretic activities in the rat (6). Literature revealed that saponins, glycoside-D and trigofoenoside-A are the major components in the seeds (7), while alkaloids, cardiac glycosides, and phenols are found in the leaves extract (6). In traditional medicine the plant has also been recommend for the treatment of rheumatism (8). The present study was planned to explore any possible analgesic and anti-inflammatory potential of the partially separated fraction (MTH) obtained from the *Trigonella foenum-graecum* seed extract.

EXPERIMENTAL

Materials

The dried seeds of *Trigonella foenum-graecum* were purchased from local market and identified by pharmacognosist, Pentazocine (PTZ) and diclofenac sodium (DIS) injections (Novartis and Ranbaxy, India, respectively) were purchased. Solvents and chemicals used for experimental work were of AR grade.

Animals

Swiss albino mice (20-25 g) and Wistar rats (150-200 g) of either sex were used for analgesic and anti-inflammatory studies, respectively. They were housed at the temperature 24 ± 2°C with 12 h light/dark cycles in polypropylene cages in groups of six animals each. The animals were fasted overnight before the experiment and given water *ad libitum*. The study conformed to the guiding principles of Institutional Animal Ethics Committee (IAEC), M.G.M. Medical College, Indore, India.

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Preparation of MTH

Dried coarsely powdered seeds (500 g) were extracted with methanol using Soxhlet apparatus at 80 ± 2°C for 10 h. The solvent was removed by distillation and under vacuum. The crude sticky extract was subsequently treated with chloroform and then with acetone several times. The solid hygroscopic fraction thus separated was preserved and coded as MTH (yield 2%). The fraction MTH was subjected to physicochemical characterization using TLC (thin layer chromatography) on pre-coated silica gel-G plates using acetone : water : acetic acid (3 : 0.5 : 0.1, v/v/v) as mobile phase and iodine as visualizing agent. MTH was found to be soluble in water. Phytochemical screening was done for detection of alkaloids, glycosides, saponins and steroids in MTH. For pharmacological screening MTH was dissolved in distilled water to prepare desired concentration just before use.

Preliminary screening

Different doses 5, 10, 20 and 40 mg/kg body weight of MTH were arbitrarily selected and administered by oral and intra-peritoneal route to albino mice. The animals were observed for any gross behavioral changes, sedation, morbidity and mortality. Based on preliminary studies, doses 10, 20 and 40 mg/ kg were selected for further experiments.

Analgesic studies

Analgesic activity in mice was assessed in chemically as well as thermally induced pain using acetic acid induced writhing model (9) and hot plate assay (10), respectively.

Acetic acid induced writhing method

Five groups of mice (n = 6) were randomly formed. The groups were treated as control (distilled water, *p. o.*) and standard (DIS 5 mg/kg *p.o.*) while test groups received fraction (MTH) 10, 20 and 40 mg/kg *p.o.* (MTH-10, MTH-20 and MTH-30), respectively. Acetic acid solution 0.6% v/v (10 mL/ kg) was injected by intraperitoneal route one hour after treatment and number of writhes (i.e. index of pain reaction against chemical stimuli characterized by abdominal muscle contraction together with turning of trunk and extension of hind limbs) was counted over a period of 20 min. Analgesic activity was expressed as percentage of inhibition of writhes with respect to the control group (Table 1).

Hot plate method

Hot plate was maintained at 55 ± 1°C. Albino mice were divided in five groups. The animals were placed on the hot plate and the basal reaction time taken to cause a discomfort (licking of paw or jumping response whichever appeared first) was recorded at 0 min. Cut-off period 15 sec. was established to prevent damage to the paws. The treatment and groupings of mice was done in the same manner as has been documented in acetic acid induced writhing model except that standard group received pentazocine (5 mg/kg *p.o.*). The reaction time in seconds was reinvestigated at 30, 60, and 120 min after the treatment. Changes in reaction time were noted (Table 2). Anti-inflammatory potential of MTH was also investigated at its minimal dose producing analgesia (i.e. 10 and 20 mg/ kg, *i. p.*) against carrageenan induced rat paw edema.

Anti-inflammatory studies

Acute anti-inflammatory activity of MTH at 10 and 20 mg/kg was evaluated using carrageenan induced edema in rats described by Winter et al. (11). Four groups of albino rats (n = 6) were randomly distributed in control, standard and test (MTH-10 and MTH-20) groups. The initial paw volumes of each animal were measured by means of a mercury plethysmometer. The standard group was treated with DIS injection (5 mg/kg, *i.p.*) while MTH aqueous solution (10 and 20 mg/kg, *i.p.*) and distilled water (10 mL/kg, *i.p.*) were given to the test and control groups, respectively. Thirty minutes after treatment 0.1 mL of 1% carrageenan solution was injected in the plantar region of the left hind paw of rats. Paw volumes were again measured 3 h after carrageenan injection. The acute difference in edema volume was calculated in each control, test and standard group and compared with the control group for determination of the percentage of inhibition of the paw edema (Table 3).

Statistical analysis

A SPSS-11 statistical computer program was used to evaluate the results. One-way analysis of variance (ANOVA) test followed by multiple Tukey's comparison test was applied. $p < 0.05$ was considered statistically significant.

RESULTS

Phytochemical screening of the separated fraction (MTH) from methanolic extract obtained from *Trigonella foenum-graecum* seeds was found to be water-soluble and contains glycoside and steroid principals. The thin layer chromatography (TLC) showed four spots with Rf values 0.06, 0.14, 0.49 and 0.87. Preliminary screening was carried out in mice for four graded doses viz. 5, 10, 20, and 40 mg/kg administered orally as well as intraperi-

Table 1. Analgesic effect of different doses of MTH fraction in acetic acid induced writhings in mice

Treatment	Dose, *p.o.* (mg/kg)	No. of writhes in 20 min The mean ± SE	% Reduction
Control (DW)	10 mL/kg	51.50 ± 0.99	—
DIS	5	18.66 ± 0.88*	63.77
MTH-10	10	36.66 ± 0.95*	28.82
MTH-20	20	26.50 ± 0.84*	48.58
MTH-40	40	12.50 ± 0.76*†	75.73
One way ANOVA	F	309.45	
	p	< 0.001	

DIS, diclofenac sodium; DW, distilled water; MTH, Fraction MTH. One way ANOVA followed by multiple Tukey's comparison test. Values are presented as the mean ± SE (standard error); n = 6 for all groups, df = = 4, 25. * $p < 0.05$ as compared to control; † $p < 0.05$ as compared to diclofenac group.

Table 2. Analgesic effect of different doses of MTH fraction on hot plate test in mice.

Treatment	Dose, *p.o.* (mg/kg)	Mean reaction time in seconds			
		0 min.	30 min.	60 min.	120 min.
Control (DW)	10 mL/kg	3.93 ± 0.04	4.01 ± 0.45	3.96 ± 0.04	3.96 ± 0.04
DIS	5	3.91 ± 0.05	7.46 ± 0.17*	8.06 ± 0.15*	7.61 ± 0.14*
MTH-10	10	3.88 ± 0.05	4.32 ± 0.06	4.91 ± 0.07	4.76 ± 0.11
MTH-20	20	3.91 ± 0.07	4.70 ± 0.09*	5.45 ± 0.08*	5.2 ± 0.06*
MTH-40	40	3.92 ± 0.05	5.32 ± 0.07*	6.12 ± 0.08*	5.75 ± 0.14*
One way ANOVA	F	0.088	178.89	249.7	155.87
	p	> 0.05	< 0.01	< 0.01	< 0.01

DIS, diclofenac sodium; DW, distilled water; MTH, fraction MTH. One way ANOVA followed by multiple Tukey s comparison test. Values are the mean ± SE, n = 6 in each group, df = 4, 25.
* $p < 0.05$ when compared to control group.

Table 3. Anti-inflammatory effect of different doses of MTH fraction on carrageenan induced rat paw edema in rats.

Treatment	Dose, *i.p.* (mg/kg)	The increase in paw volume (mL)	% Inhibition from control	*p* value *vs.* control
Control (DW)	10 mL/kg	0.61 ± 0.01	—	—
DIS	5	0.14 ± 0.01	75.90	< 0.001
MTH-10	10	0.42 ± 0.02	29.84	< 0.001
MTH-20	20	0.25 ± 0.01	59.02	< 0.001
One way ANOVA	F	155.76		
	p	< 0.001		
	df	3, 20		

DIS, diclofenac sodium; DW, distilled water; MTH, fraction MTH. One way ANOVA followed by multiple Tukey's comparison test. Values are presented as the mean ± SE (standard error); n = 6 for all groups, df = 4, 25.

toneally and indicated no gross behavioral changes, sedation, morbidity and mortality at these doses.

Fraction MTH indicated highly significant and dose dependent analgesic activity against both thermally and chemically induced pain. In acetic acid induced writhing method MTH (10, 20, and 40 mg/kg *p.o.*) and standard (DIS 5 mg/kg *p.o.*) treated animals showed significantly reduced number of writhing in 20 min at the rate of 28.82%, 48.58%, 75.73% and 63.77%, respectively, when compared to that of control group ($p < 0.001$). Analgesia produced by MTH 40 mg/kg was also found to be higher than that of observed for standard diclofenac sodium at the employed doses (Table 1).

On hot-plate test, MTH showed significant elevation in pain threshold in comparison to control, as

represented in Table 2 and indicated significant analgesic activity ($p < 0.05$) as compared to control at all tested doses. However, in this model, analgesic activity of MTH 40 mg/kg was observed to be lower as compared to PTZ at employed doses.

Acute anti-inflammatory potential of MTH was investigated at its minimal dose producing analgesia (i.e. 10 and 20 mg/ kg, *i.p.*) against carrageenan induced rat paw edema. It was noted that the standard drug DIS (5 mg/ kg *i.p.*) showed 75.9 % inhibition of edema whereas MTH showed 29.84 and 59.02 % inhibition, respectively, exhibiting significantly anti-inflammatory activity with respect to control ($p < 0.001$). In addition, the study also demonstrated lower anti-inflammatory response of MTH at the used doses in comparison to DIS at 5 mg/ kg *i.p.* The results are summarized in Table 3.

DISCUSSION AND CONCLUSION

In the present investigation, MTH was studied for its analgesic potential in both peripheral (non-narcotic) and central (narcotic) type pain models. Diclofenac sodium (5 mg/kg, *p.o.*) and pentazocine (5 mg/kg, *p.o.*) were used as standard drugs for comparing analgesic effects at peripheral and central levels, respectively. MTH pretreatment markedly reduces the painful response produced by acetic acid, manifested as writhing at the employed doses. Pain is a complex process mediated by many physiological mediators e.g. prostaglandins, bradykinins, substance-p etc. In the acetic acid induced writhing model the constrictions induced by acetic acid in mice results from an acute inflammatory reaction with production of PGE_2 and $PGF_2\alpha$ in the peritoneal fluid (12, 13). Therefore, it is likely that MTH might suppress the formation of these substances or antagonize their action for exerting analgesic activity. The hot-plate test is commonly used to assess narcotic analgesics or other centrally acting drugs (14) and the present results showed that MTH also significantly elevates the response latency period suggesting centrally mediated analgesic effect. It also showed moderate anti-inflammatory potential at the employed doses. It has been proposed that inflammatory reaction occurs in two phases viz, release of histamine, serotonin and bradykinin in the early or first phase, followed by the release of prostaglandin in the late or second phase (12). A significant anti-inflammatory activity against carrageenan induced inflammation was also observed in MTH, evaluated by carrageenan induced rat paw edema method, suggesting influence of MTH on release, synthesis or action of the inflammatory mediators. Further, the studies on leaf extract of *Trigonella foenum-graecum* (TFG) done by Parvizpur et al. showed that the central action and spinal 5-HT system is partially involved in the analgesia induced by it in the second phase of formalin test and also indicates a co-existence of other analgesic mechanisms (15). Other studies indicated that the blockade of spinal purinoceptors may contribute in the analgesic effect of *Trigonella foenum-graecum* (TFG) leaves extract (16). MTH contains glycoside and steroidal moieties that may be contributing to the observed pharmacological activities. We conclude, the aqueous solubility of MTH indicated that active herbal principles are polar in nature and possess potential analgesic and anti-inflammatory activities. Further investigations are required to understand its influence on various pain and inflammatory mediators.

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