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EVALUATION OF ANALGESIC, ANTIDIARRHEAL AND CNS DEPRESSANT ACTIVITY OF SEED EXTRACTS OF *Trigonella foenum graecum L.*

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ABSTRACT

The methanolic extract of *Trigonella foenum graecum L.* showed significant peripheral analgesic activity at a dose of 400 mg/kg and 200 mg/kg body weight with percentage of inhibition of acetic acid induced writhing 68.10% ($P<0.001$) and 52.80% ($P<0.001$), respectively compared to the standard diclofenac (62.50%) ($P<0.001$) group. The crude methanolic extract also showed significant antidiarrheal activity by castor oil induced diarrhea test with percentage of inhibition of defecation 45.16% (400mg/kg does, $p<0.001$) and 38.71% (200mg/mg dose, $p<0.001$) compared to the standard loperamide (61.29%). In case of CNS depressant activity by phenobarbitone induced sleeping time test, the crude methanolic extract significantly reduced sleep latency by 84.16% and increased the duration of sleep by 39.03% and 36.47% compared to the standard diazepam (89% and 44.58%, respectively). In light of available literature, these findings provided some scientific justification for significant folkloric use of the seeds of the plant for the treatment of algesia, CNS depression and diarrhea.

Key words: *Trigonella foenum graecum L*, ethno-pharmacological, analgesic, antidiarrheal and CNS depressant.

INTRODUCTION

In recent times in developed countries interest in using complementary and natural medicine to alleviate and improve health conditions is increasing (Marshall *et al.*, 2000). Herbal drugs have been used since ancient times as medicines for the treatment of a range of diseases (Proma *et al.*, 2018). Medicinal plants have been used as dietary adjunct and in the treatment of numerous diseases without proper knowledge of their function (Chaudhari *et al.*, 2013). The bioactive compounds of medicinal plants are also used for antimicrobial (Sharafati *et al.*, 2011), anti-cancer (Shirzad *et al.*, 2012), anti-diabetic (Kazemi *et al.*, 2010), anti-atherosclerosis (Khosravi *et al.*, 2012), immunomodulatory (Shirzad *et al.*, 2009), and even Reno-protection or Hepato-protective effects (Rafieian *et al.*, 2013; Baradaran *et al.*, 2013). Therefore, the quest for plants is in need of plants, particularly of ethno-botanical significance for a complete range of biological activities, which ranges from antibiotic to anti-cancerous.

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Trigonella foenum graecum Linn. (Fenugreek) is belonging to the family fabaceae and it is an annual crop. This plant has been described around 1500 BC in Egyptian literature for various medical and also for dietary uses. People all over the world used different parts of the plants like stem, root, leaves and fruits for curing diseases. This plant used as anti-fertility, anti-microbial, anti-diabetic, anti-parasitic and antiepileptic, hypocholestrolaemic, antibronchitis, carminative, antipyretic, aphrodisiac, analgesic, anti-cancer, anti-oxidant, immunomodulator and currently reported in the balancing blood sugar concentration (Chauhan *et al.*, 2011).

After observing significant Hypoglycemic activity of *Swietenia Mahagoni* in animal models at our Phytochemistry research laboratory (Naima *et al.*, 2017), *Trigonella foenum graecum Linn.* has been taken in consideration for analyzing various properties of it in Swiss Albino mice model. The main objectives of our research were to evaluate the analgesic, antidiarrheal and CNS depressant effect of *Trigonella foenum graecum*.

MATERIALS AND METHODS

Solvents and Chemicals:

Analytical and laboratory grade (e.g. SIGMA, E. Merck or BDH) solvents and chemicals were used in most of the experiments. Acetic acid, Methanol, Tween-80, Loperamide (Square pharmaceuticals Ltd., Bangladesh), Diclofenac sodium (Incepta pharmaceuticals Ltd., Bangladesh), Diazepam (Opsonin pharma Ltd), Sodium phenobarbitone and normal saline were used. Highly purified castor oil was purchased from local market.

Plant Collection and Authentication:

Plant sample of *Trigonella foenum graecum* was collected from Ishwardi Upazila of Pabna district in December 2016. The plant was identified and authenticated by Dr. Shaikh Bokhtear Uddin, Taxonomist and Associate Professor of the Department of Botany, University of Chittagong, Bangladesh.

Preparation of Plant Extract:

After washing the seeds were cut into small pieces and then air dried for several days. The pieces were then oven dried for 24 hours at considerably low temperature to effect grinding. Then the pieces were ground into coarse powder in the Phytochemical Research Laboratory, Biological Faculty, University of Chittagong. About 800 gm of the powdered material was taken in a clean, round bottomed flask (5 Liters) and soaked in 3.5 liters of methanol for 15 days. The extract was filtered through Whatman filter paper number 1 and concentrated on a rotary evaporator (RE 200, Bibby Sterling Ltd., England) at 40°C under reduced pressure. The concentrated extract was finally evaporated to dryness on a water bath which afforded 28 g of crude extract.

Experimental Animals:

Swiss-albino mice of either sex, aged 4-5 weeks, weighing 20-25 gm each obtained from the BCSIR laboratories, Chittagong were used for the experiment.

Acute Toxicity Studies:

Acute toxicity study was conducted and the LD_{50} for each of the extract was determined and one tenth of the extract dose (LD_{50}) was selected as maximum dose for the evaluation of different pharmacological activity.

Assessment of In-vivo Pharmacological Activities:

Peripheral Analgesic Activity:

Analgesic activity was evaluated by acetic acid-induced writhing method (Aoki *et al.*, 2006). The acetic acid induced writhing method is an analgesic behavioral observation assessment method that demonstrates a noxious stimulation in mice (Naima *et al.*, 2019). The test consists of injecting 0.7% (v/v) acetic acid solution intraperitoneally and then observing the animal for writhing (specific contraction of body) (Suresha *et al.*, 2014). The animals were pretreated with standard drug Diclofenac (50 mg/kg, i.p) and samples (200 mg/kg and 400 mg/kg, p.o) 40 minutes before induction of writhing. The number of writhes per animal was counted for the next 15 minutes. The percentage protection against acetic acid was calculated using the following formula (Anafi *et al.*, 2017).

$$\% \text{ inhibition} = \frac{\{(\text{No. of writhes in control group} - \text{No. of writhes in test group}) \times 100\}}{\text{No. of writhes in control group}}$$

Antidiarrheal activity:

The antidiarrheal activity of the methanolic extract of *Trigonella foenum graecum L.* was evaluated by using castor oil-induced diarrhea in mice (Awouter *et al.*, 1978). Test samples (200 mg/kg and 400 mg/kg), control and standard drug Loperamide (50 mg/kg) were given orally by means of a feeding needle to the mice at zero hour. 30 minutes later, 1ml of castor oil was given to each mouse for inducing diarrhea. Then each of the mice was observed for four hours. Each time a mouse had given stool was recorded. The average of total number of defecation given by the test group and the average of the total number of defecation given by the control group was compared. Calculation of defecation was measured by the following equation:

$$\% \text{ of inhibition of defecation} = (1 - B/A) \times 100$$

A = Mean number of defecation by castor oil
B = Mean number of defecation by drug or extract

CNS depressant activity:

CNS depressant activity was measured by Phenobarbitone induced Sleeping Time Test. The test groups received 200 mg/kg and 400 mg/kg doses of methanolic extract of

Trigonella foenum graecum while positive control was treated with diazepam (1 mg/kg i.p.) and negative control with vehicle (1% Tween 80 in normal saline). Thirty minutes later, phenobarbitone (40 mg/kg, i.p) was administered to each mouse to induce sleep. The animals were observed for the latent period (time between phenobarbitone administration to loss of righting reflex) and duration of sleep (time between the loss and recovery of righting reflex) (Ghosh *et al.*, 2007).

Statistical Analysis:

Results of the study were represented by mean ± SEM (Standard Error Mean). Data were analyzed by one-way ANOVA followed by Dunnett's t test and P values <0.05 were considered statistically significant.

RESULTS AND DISCUSSION:

Peripheral Analgesic Activity:

The test was performed by taking methanolic extract at doses of 200 mg/kg and 400 mg/kg body weight. The result was found statistically significant. The methanolic extract of *Trigonella foenum graecum L.* dose dependently induced a significant (P<0.001) decrease in the number of writhens with 52.80% and 68.10 % of inhibition at the dose of 200 and 400 mg/kg body weight, respectively when compared to the control untreated group which was comparable to that of the standard drug diclofenac sodium (60.68 % inhibition, P<0.001) (Table 1).

TABLE 1: ANALGESIC ACTIVITY OF METHANOLIC EXTRACT OF *TRIGONELLA FOENUM GRAECUM L.*

Animal Group	Number of writhing (Mean±SEM)	%of inhibition of writhing
Control	43.20±2.94	
Standard	16.20±0.917***	62.5
ME200	20.40±2.21***	52.8
ME400	13.80±.80**	68.1

Note: Each value represents the mean ± SEM. (n= 5). One- way ANOVA followed by Dunnett's t test. *P<0.05, **P<0.01, ***P<0.001 compared to control. ME = Methanolic Extract.

Antidiarrheal activity:

The data showed that the methanolic extract induced a significant decrease in the total number of defecation in 4 hours (69.05 % of inhibition, P<0.001) when compared to the control which is almost similar to the standard drug loperamide (71.43% inhibition, P<0.001) (Table 2).

TABLE 2: EVALUATION OF ANTIDIARRHEAL ACTIVITY OF CRUDE EXTRACT OF *TRIGONELLA FOENUM GRAECUM L.*

Animal Group	Dose Mg/kg	Mean ± SEM				
		1st hr (% of inhibition)	2nd hr (% of inhibition)	3rd hr (% of inhibition)	4th hr (% of inhibition)	Total (% of inhibition)
Control	-	0.80 ± 0.37	4.00 ± 0.55	4.20 ± 0.37	3.00 ± 0.84	12.40 ± 0.51
Standard	10	0.40 ± 0.24 50.00***	1.20 ± 0.20 70.00***	1.60 ± 0.24 61.29***	1.60 ± 0.51 46.67***	4.80 ± 0.31 61.29***
ME	400	1.00 ± 0.32 20.00***	1.40 ± 0.40 65.00***	3.60 ± 0.51 14.29***	1.60 ± 0.89 46.67***	7.60 ± 0.68 38.71***
ME	200	0.80 ± 0.20 25.89***	2.20 ± 0.58 45.00***	2.00 ± 0.71 52.38***	1.80 ± 0.58 40.00***	6.80 ± 0.37 45.16***

Note: Each value represents the mean ± SEM. (n= 5). One- way ANOVA followed by Dunnett t test. *P<0.05, **P<0.01, ***P<0.001 compared to control. ME = Methanolic Extract.

CNS depressant activity:

The two doses of methanolic extract of plant significantly reduced sleep latency by 98.20% and 84.16% and increased the duration of sleep by 36.47% and 39.03% respectively compared to control which were comparable to that of standard diazepam (89.28% and 44.58% respectively) (Table 3).

TABLE 3: CNS DEPRESSANT ACTIVITY OF METHANOLIC EXTRACT OF *TRIGONELLA FOENUM GRAECUM L.*

Group	Mean ± SEM	
	Onset of sleep (% of reduction time)	Duration of sleep (% of elongation time)
Control	44.20 ± 4.18	70.20 ± 2.18
Standard	4.8 ± 0.37* (89%)	101.7 ± 3.11 (44.58%)
ME 200	87.60 ± 13.72* (98.20%)	44.60 ± 14.37 (36.47%)
ME 400	81.40 ± 7.68* (84.16%)	97.60 ± 19.00 (39.03%)

Note: Each value represents the mean ± SEM. (n= 5). One- way ANOVA followed by Dunnett t test. *P<0.05, **P<0.01, ***P<0.001 compared to control. ME = Methanolic Extract.

CONCLUSION

The whole seed powder extracts of *T. foenum-graecum Linn.* revealed that it has significant analgesic, CNS depressant, and antidiarrheal activities. However, further research is needed to confirm the precise mechanisms of actions involved as well as the chemical compounds responsible for the pharmacological actions.

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