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Evaluation of analgesic, neuropharmacological and cytotoxic activity of *Trigonella foenum-graecum* Linn.

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Abstract

The present study was carried out to investigate the possible analgesic, neuropharmacological and cytotoxic activities of the methanolic extract of *Trigonella foenum-graecum* Linn. leaves. The analgesic and neuropharmacological activities of *Trigonella foenum-graecum* Linn. were investigated at the doses of 100mg/kg, 200mg/kg and 400mg/kg of body weight in mice. Analgesic potential of the extract was evaluated for centrally acting analgesic property using tail immersion method and peripheral analgesic actions using acetic acid-induced writhing test. In acetic acid-induced writhing test, extract produced a significant ($p < 0.001$) inhibition of writhing response in a dose dependent manner but maximum inhibition (93.46%) of writhing was found at 400mg/kg dose. In tail immersion method, extract caused a significant ($p < 0.001$) increase in latency time and the results were comparable to the standard drug Diclofenac-Sodium. In addition, neuropharmacological property of crude extract was carried out by Hole cross and Open field test. The extract significantly ($p < 0.05$-0.001) displayed a dose dependent suppression of motor activity, exploratory behaviour. Furthermore, the extract was subjected to Brine Shrimp lethality bioassay for primary evaluation of cytotoxicity, where the extract was found to be highly toxic to Brine Shrimp nauplii, having LC_{50} values of 10µg/ml while the LC_{50} of the reference anticancer drug vincristine sulphate was 0.66µg/ml. The results of this present study suggest that the extract possesses analgesic, cytotoxic and CNS depressant activities.

***Key Words*:** *Trigonella foenum-graecum* Linn., cytotoxicity, neuropharmacological, analgesic activity.

INTRODUCTION

Trigonella foenum-graecum Linn. (Fabaceae) is one of the traditionally used medicinal plants with nearly smooth erect annual which commonly known as Fenugreek. Leaflets are oblanceolate-oblong, toothed. Flowers are axillary, sessile. Calyx is teeth linear. Pods are long. Its seeds and leaves are used not only as food but also as an ingredient in many medical formulations in traditional medicine. The petroleum ether, chloroform, ethyl acetate and methanolic extracts of leaves and seeds of *Trigonella foenum-graecum* Linn. (Fabaceae) possess antinociceptive activity (Bhalke *et al.*, 2009). The *Trigonella foenum-graecum* leaves extract also possess anti-inflammatory and antipyretic effects (Toppo *et al.*, 2009). The aqueous and alcoholic extracts of *Trigonella foenum-graecum* leaf were tested for hypoglycaemic activity in normal and alloxan-diabetic rats; a significant reduction of blood glucose concentration was noticed. On the other hand ethanolic extract of *Tigonella foenum graecum* leaf produced no reduction in blood glucose concentration in normal rats but intra-peritoneal administration of the ethanolic leaf extract to diabetic rats produced a significant reduction of blood glucose concentration (Ahmadiani *et al.*, 2001). Modulatory effect of *Trigonella foenum-graecum* L. extract on deltamethrin induced low dose immunosuppression in mice has shown to possess several medicinal properties. In clinical studies, it has shown hypoglycemic and anti-diabetic properties (Toppo *et al.*, 2009).In addition, Flavonoids of fenugreek extract have been observed to possess anti-oxidant activity (Moskaug *et al.*, 2005; Myhrstad *et al.*, 2002; Ozcan et al 2005). Other different activities such as anticancer, antiseptic, aphrodisiac, astringent, demulcent, emollient,

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expectorant, anthelmintic, wound healing and gastro protective effects were found in the extract (Toppo *et al.*, 2009). Present study reports analgesic, neuropharmacological and cytotoxic activity methanolic extracts of *T. foenum-graecum* leaves.

MATERIALS AND METHODS

Chemicals and drugs

Methanol used as Solvent for the extraction of the plant and Acetic Acid used in writhing test were purchased from Merck, Germany. Tween 80 was also collected from Merck, Germany; Diclofenac-sodium and Diazepam were collected from Square Pharmaceuticals Ltd. Bangladesh.

Plant material

The plant of *Trigonella foenum-graecum* Linn. was collected from the Sham Bazar of old town, Dhaka in February 2010 and was identified by the experts of Bangladesh National Herbarium, Mirpur, Dhaka (Accession no 34488). Date of investigation by the herbarium was February 8, 2010 when a voucher specimen is deposited for future reference.

Preparation of the Extract

After shade drying for 5 days, the leaves part of *Trigonella foenum-graecum* Linn. were extracted by Soxhlet extraction method using methanol at 40°C. 140gm powder was obtained from the dried leaves. The extract was concentrated by evaporation and dried to in an oven.

Test animal

Swiss Albino mice of either sex, 3-4 weeks of age, weighing between 20-28g, were collected from the Animal Research Branch of the International Center for Diarrhoeal Diseases Research, Bangladesh (ICDDR, B). They were housed in plastic cages having dimension of (28×22×13cm). Soft wood shavings were used as bedding of cages. Animals were maintained under standard environmental conditions (temperature: (24.0±1.0°C), relative humidity: 55-65% and 12 hrs light/12 hrs dark cycle). Pellets of mice foods, provided by ICDDR, B were given to the mice with fresh water *ad libitum*. The newly brought mice were given a resting period of one week to get over the food and water restrictions incurred during transit and to get themselves adapted with the new environment of the laboratory, before being employed in any experiment (Hasan *et al.*, 2009).

Analgesic Activity

Acetic acid induced writhing test

The acetic acid induced writhing method is an analgesic behavioral observation assessment method that demonstrates a noxious stimulation in mice. The method described by (Howlader *et al.*, 2006). Test samples and control were given orally by means of a feeding needle. A thirty (30) minutes interval was given to ensure proper absorption of the administered substances. Then the writhing inducing chemical, acetic acid solution (0.7%) 0.2ml was administered intraperitoneally to each of the animals of a group. After an interval of 15 minutes, this was given for absorption and no of squirms (writhing) was counted for 5 minutes. Diclofenac-sodium was used as reference standard drug.

Tail immersion 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 at 55°C-55.5°C which is monitored. If a sample contains any analgesic principle it increases the ability of the mice to retain its tail in the hot water which is reflected in the increase in the tail flicking time (Ahmad *et al.*, 1992). Diclofenac-sodium was used as reference standard drug.

Neuropharmacological activity

Hole cross test

The most consistent behavioral change is a hyperemotional response to novel environmental. The experiment was carried out as described by Takagi *et al.* (1971). The aim of this study was to characterize the emotional behavior of mice using the hole-board test. The number of head-dips in the hole-board test in single-housed mice was significantly greater. Spontaneous movement of the animals through the hole from one chamber to the other was counted for three minutes in this test. The observations are made on 0, 30, 60, 90, and 120 minutes after oral administration of the leaves extract of the *T. foenum-graecum*.

Table 1: Effect of Methanolic Extract of *Trigonella foenum-graecum* on acetic acid-induced writhing in mice

Groups	Dose	Route of Adminis-tration	Number of writhing	% Inhibition
Control	0.50ml	Oral	41.3±1.32	0
Positive control	50mg/kg	Oral	11.0±0.42*	73.36
Group-1	100mg/kg	Oral	32.0±1.22*	22.51
Group-2	200mg/kg	Oral	4.8±1.62*	88.37
Group-3	400mg/kg	Oral	2.7±0.58*	93.46

Values are expressed as Mean ±SEM (n=5); * donates $p < 0.001$. Dunnett test as compared to control. Control: Tween-80 + Water, Positive Control: Diclofenac-Na

Open field test

The Open Field Test (OFT) is clearly the most frequently used of all behavioural tests in pharmacology and neuroscience. The method described by Gupta *et al.* (1971) was adopted for this test. Despite the simplicity of the apparatus, however, open field behaviour is complex. Consequently, it has been used to study a variety of behavioural traits, including general motor function, exploratory activity and anxiety-related behaviours in rodents. The floor of an open field of half square meter was divided into a series of squares each alternatively colored black and white. The apparatus had a wall of 40cm height. The number of squares visited by the animals was counted for three min at 0, 30, 60, 90 and 120 min after oral administration of the test drugs.

Brine shrimp lethality bioassay

For Brine Shrimp lethality bioassay the eggs of Brine Shrimp (*Artemia salina* Leach) were collected and hatched in a tank at a temperature around 37°C equipped with constant oxygen supply for 24 hours. 5mg of each of the extracts was measured and dissolved in DMSO. Finally the concentration was adjusted to 320µg/ml that served as the mother solution. A series of solutions of lower concentrations were prepared by serial dilution with DMSO. From each of these test solutions 30µl were added to pre-marked test tubes containing 5ml of seawater and 10 shrimp nauplii. So, the final concentrations of samples in the test tubes were 320µg/ml, 160µg/ml, 80µg/ml, 40µg/ml, 20µg/ml, 10µg/ml, 5µg/ml, 2.5µg/ml, and 1.25µg/ml. After 24 hours, the test tubes were observed and the numbers of survived nauplii in each test tube were counted and the results were noted. From this observation, the percentage of lethality of Brine Shrimp nauplii was calculated for each concentration of the extract.

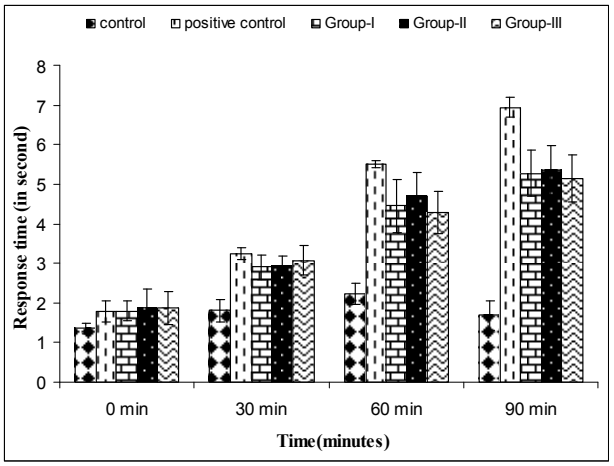

Figure 1: Effect of Methanolic Extract of *Trigonella foenum-graecum* on Tail Withdrawal Reflex in Mice. Values are expressed as Mean ±SEM (n=5); ** donates $p < 0.001$. Dunnett test as compared to control. Control: Tween-80 + Water, Positive Control: Diclofenac-Na (50 mg/kg); Group-I: Extract (100mg/kg), Group-II: Extract (200mg/kg) and Group-III: Extract (400mg/kg).

Statistical analysis

Statistical analysis for animal experiment was carried out using one-way ANOVA followed by Dunnett's multiple comparisons. The results obtained were compared with the vehicle control group; $p < 0.05$, 0.001 were considered to be statistically significant.

RESULTS AND DISCUSSION

Acetic acid induced writhing test

In acetic acid-induced writhing test, methanolic extract of *Trigonella foenum-graecum* produced a significant ($p < 0.001$) inhibition of writhing response (table 1) in a dose dependent manner but maximum inhibition (93.46%) of writhing was found at 400mg/kg dose and the results were comparable to the standard drug Diclofenac-Sodium. It has been postulated that acetic acid acts indirectly by inducing the release of endogenous mediators, such as PGE2 (prostaglandin E2) and PGE2α in peritoneal fluids, as well as lipooxygenase products, which stimulate the nociceptive neurons sensitive to NSAIDs (Derardt *et al.*, 1980; Collier *et*

Table 2: Effect of Methanolic Extract of *Trigonella foenum-graecum* on Hole Cross Test.

Group	Route of Administration	Number of hole crossed				
		0 min	30 min	60 min	90 min	120 min
Control	Oral	15.0±1.34	7.0±0.85	4.6±0.93	3.6±0.87	1.4±0.75
Positive control	Oral	22.4±1.63	11.8±0.66*	11.4±0.75**	7.8±0.86*	10.2±0.37**
Group-1	Oral	9.4±1.32	7.2±0.58*	6.2±1.01*	4.4±0.97*	2.2±0.48**
Group-2	Oral	8.0±0.83	6.4±0.67*	5.0±0.77**	3.6±1.02*	1.2±0.58**
Group-3	Oral	10.6±1.4	6.4±1.36*	3.0±0.89**	1.2±0.73**	0.6±0.4**

Values are expressed as Mean ±SEM (n=5); ** and * donate $p < 0.001$ and $p < 0.05$, respectively. Dunnett test as compared to control. Control: Tween-80 + Water, Positive Control: Diazepam (1mg/kg); Group-1: Extract (100mg/kg), Group-2: Extract (200mg/kg) and Group-3: Extract (400mg/kg)

al., 1968). Therefore, the results of the acetic acid-induced writhing strongly suggests that the mechanism of this extract may be linked partly to the inhibition of lipooxygenase and/or cyclooxygenase in the peripheral tissues, thereby reducing PGE2 synthesis and interfering with the mechanism of transduction in the primary afferent nociceptor.

Tail immersion test

The central analgesic effect of the methanolic extract of *Trigonella foenum-graecum* could be supported by the results recorded in the tail immersion test, which is a selective method able to screen centrally acting opiate analgesic drugs (Abbott and Melzack, 1982). It was demonstrated that oral administration of methanolic extract of *Trigonella foenum-graecum* (100, 200 and 400mg/kg) exerts significant ($p < 0.001$) prolongation in the response latency time to the heat stimulus (Figure 1). The results were comparable to the standard drug Diclofenac-Sodium at 50mg/kg.

Hole cross test

Neuropharmacological property of crude extract was carried out by Hole cross test. The extract significantly ($p < 0.05$-0.001) displayed a dose dependent suppression of motor activity and exploratory behaviour in this test. The locomotor activity lowering effect was evident at the 3rd observation (60 min) and continued up to 5th observation period (120 min); results are shown in table 2 and figure 2.

Open field test

Historically, open-field defecation and activity have been used to assess the "fearfulness" or "emotional reactivity" of rodents (Hall, 1934, 1936; Broadhurst, 1957; DeFries *et al.*, 1970, 1974, 1978; Blizard, 1981; reviewed by Boissy, 1995; Weiss and Greenberg,

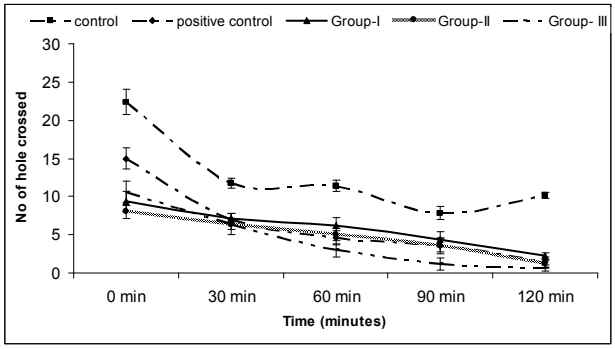

Figure 2: Graphical representation of effect of Methanolic Extract of *Trigonella foenum-graecum* on Hole Cross Test. Values are expressed as Mean ±SEM (n=5); ** and * donate $p < 0.001$ and $p < 0.05$ respectively. Dunnett test as compared to control. Control: Tween-80 + Water, Positive Control: Diazepum (3 mg/kg); Group-l: Extract (100mg/kg), Group-ll: Extract (200mg/kg) and Group-lll: Extract (400mg/kg)

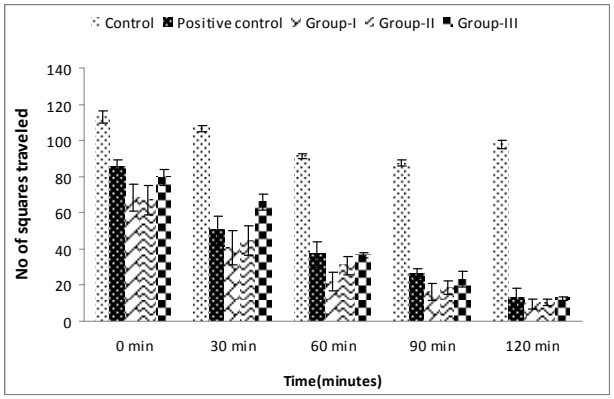

Figure 3: Effect of Methanolic Extract of *Trigonella foenum-graecum* on Open Field Test. Values are expressed as Mean ±SEM (n=5); ** and * donate $p < 0.001$ and $p < 0.05$ respectively. Dunnett test as compared to control. Control: Tween-80 + Water, Positive Control: Diazepum (3 mg/kg); Group-l: Extract (100mg/kg), Group-ll: Extract (200mg/kg) and Group-lll: Extract (400mg/kg)

1998). Open field test showed that the depressing action of the extracts was evident from the second observation period in the test animals at the dose of 100mg/kg body weight. Maximum depressant effect was observed from 2nd (60 min) to 5th (120 min). The results were dose dependent and statistically significant ($p < 0.05$-0.001).

Brine shrimp lethality bioassay

The extract of *Trigonella foenum-graecum* was subjected to Brine Shrimp lethality bioassay for primary evaluation of cytotoxicity. The extract showed different mortality rate at different concentrations. The mortality rate of brine shrimp was found to be increased in concentration of the sample and plot of percent mortality versus Log concentration on the graph paper produced an approximate linear correlation between them. From the graph the concentration at which 50% mortality (LC_{50}) of brine shrimp nauplii occurred were obtained by extrapolation. The extract was found to be highly toxic to Brine Shrimp nauplii, having LC_{50} value for the extract was found to be 10µg/ml.

CONCLUSION

Phytochemical screening of the extract showed that the *Trigonella foenum-graecum* possess fibers, flavonoids, polysaccharides, saponins, flavonoids and polysaccharides fixed oils and some identified alkaloids viz., trigonelline and choline (Jayaweera, 1981; Yoshikawa *et al.*, 1997). The analgesic, CNS depressant and cytotoxic properties of *Trigonella foenum-graecum* observed in animal model might, in part, be due to the presence of such compounds. The results also suggest a rationale for the traditional uses of this plant. However, studies are required on higher animal model and subsequently on human subjects to prove its clinical efficacy as an analgesic, CNS depressant and cytotoxic agent.

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