

**EVALUATION OF ANTI-DIARRHOEAL ACTIVITY OF
FENUGREEK SEED EXTRACTS IN RATS**

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Summary

Fenugreek (*Trigonella foenum-graecum*) seeds are used as spice, vegetable and medicinal plant. It is therefore important and useful to identify plants with anti-diarrhoeal activity. Fenugreek seeds are quoted by many traditional healers as a plant with this activity. Therefore, the present study was undertaken to evaluate the effect of methanol and aqueous extracts of *T. foenum-graecum* for its anti-diarrhoeal activity against several experimental models of diarrhoea in Albino Wistar rats. The anti-diarrhoeal activity of methanol and aqueous extracts of was evaluated using castor oil-induced diarrhoea model in rats. Further, we screened the effect of methanol and aqueous extracts on gastrointestinal tract motility after charcoal meal administration and PGE₂ induced intestinal fluid accumulation (enteropooling). The plant extracts showed significant (P<0.01) inhibitor activity against castor oil induced diarrhoea and PGE₂ induced enteropooling in rats when tested at 250 mg/kg, 1ml/rat. The methanolic extract showed P<0.01 and aqueous extract (P<0.05) reduction in gastrointestinal motility in charcoal meal test in rats. Our observations suggest that methanolic extract of TFG seed has significant anti-diarrhoeal activity compared to aqueous extract.

Key Words: *Trigonella foenum-graecum* (TFG), antidiarrhoeal, methanolic extract

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Introduction

Diarrhoeal diseases are one of the potential cause of morbidity and mortality especially in children and younger in the developing countries. Medicinal plants are promising source of anti-diarrhoeal drugs, (1) the important advantages claimed therapeutic uses of medicinal plants in various ailments are their safety besides being economical, effective and their easy availability (2). For this reason international organizations such as WHO have encouraged studies for treatment and prevention of diarrhoeal diseases depending on traditional medicinal practices. *Trigonella foenum-graecum* (TFG) commonly known as Fenugreek (Methi in Hindi) is an aromatic, bitter, erect annual herb, 30 to 60 cm in height. The plant is widely cultivated in many parts of India, and known for its various medicinal benefits for more than 2500 years. TFG seeds have been reported to possess immunomodulatory (3), antibacterial (4), anti-inflammatory (5) antioxidant (6), antinociceptive (7), gastroprotective (8) and anxiolytic (9). However, there is no scientific proof justifying the traditional use of TFG in the treatment of diarrhoea. Hence, the present work was undertaken to investigate its potential anti-diarrhoeal efficacy in various experimental models of diarrhoea in albino rats.

Material and methods

Plant Material

Seeds of TFG were purchased from local market in the month of April 2005 and cleaned for extraneous matter. The authentication was done by Prof. K. Prabhu, Department of Pharmacognosy, S.C.S. College of Pharmacy, Harapanahalli. A voucher specimen (No. SCSCP/PG/32/2005) has been deposited at the herbarium.

Preparation of Extracts

The dried seeds were coarse powdered and defatted with petroleum ether (60–80°C) using Soxhlet extractor. The marc obtained was subjected to extraction with methanol. The extract was concentrated using rotary flash evaporator. The dried extract was stored in airtight container in refrigerator below 10°C.

Preparation aqueous extract (8)

The seeds were cleaned of extraneous matter, dried and were ground in to a fine powder. The powder was mixed with distilled water (3 g of seed powder per 100 ml of water). After through mixing using a cyclomixer the extract was centrifuged at 3000 rpm for 10 min. The supernatant was used as the aqueous extract 1 ml/rat for feeding the animals in the present study.

Preliminary phytochemical screening

Preliminary chemical investigation was carried out for the methanolic and aqueous extracts of seeds of TFG (10).

Animals Used

Albino Rats (Wistar strain) of either sex weighing 150-200 g and female albino mice weighing 20-25 g were used in the present study. The animals were allowed for acclimatization for ten days under laboratory conditions. They were housed in polypropylene cages and maintained at 27°C ± 2°C, relative humidity 65 ± 10% under 12 hrs light / dark cycle. The animals were fed with rodent pellet diet (Gold Mohur, Lipton India Ltd.) and water *ad libitum*. The study protocol was approved from the Institutional Animal Ethics Committee (IAEC).

Determination of acute toxicity (LD₅₀)

The acute toxicity of methanolic extract of seeds of TFG was determined in female albino mice. The animals were fasted overnight prior to the experiment. Fixed dose OECD guideline No. 420; (Annexure-2d) method of CPCSEA was adopted for toxicity studies (11). Group of three mice were used for each test dose and 1/10 of LD₅₀ cutoff value of the extract was selected as screening dose.

Castor oil induced diarrhea

Rats were divided into four groups (n = 6) and, fasted for 18 hrs and water was provided *ad libitum*. The methanolic and aqueous extracts of TFG (250 mg/kg, 1ml/rat) were administered orally to the first two groups of rats. One group received 0.2ml of 2% w/v of gum acacia as control. Another group received the standard drug loperamide (1 mg/kg, i.p.) as standard. After 1 hr of treatment, all the animals were challenged with 1 ml of castor oil orally by gavage and observed for consistency of faecal material (12). The frequency of defecation and mean weight of stool was noted in transparent plastic dishes placed beneath the individual rat cages up to six hrs (13).

Gastrointestinal motility test:

Rats were divided into four groups (n = 6) and fasted for 18 hrs before the experiment. Each animal was orally administered with 1 ml of charcoal meal (5% deactivated charcoal in 2% aqueous tragacanth) followed by oral administration of methanolic and aqueous extracts to the first two groups of animals in the dose of 250 mg/kg and 1ml/rat. The third group was treated with 0.2 ml of 2% w/v aqueous gum acacia served as a control. The fourth group received atropine (5 mg/kg, i.p.) as standard. Thirty minutes later, each animal was killed and the intestinal distance moved by the charcoal meal from the pylorus to caecum was measured and expressed as percentage of distance moved (14).

PGE₂ -induced enteropooling

In this method rats were deprived of food and water for 18 hrs and placed in four cages, with six animals per cage. The first two groups were treated with 250 mg/kg, 1ml/rat dose of methanolic and aqueous extracts of TFG respectively. The third group was treated with 1 ml of a 5% v/v ethanol in normal saline (i.p.) and then it was treated with 0.2 ml of 2% w/v gum acacia suspension, which served as negative control. Immediately after the extract administration PGE₂ (Astra Zeneca, India) was administered orally to each rat (100 µg/kg) in the first three groups. The fourth group was treated with PGE₂ (100µg/kg in 5% v/v ethanol in normal saline orally) as well as 0.2 ml of 2% w/v gum acacia and served as the PGE₂ control group. After 30 min following administration of PGE₂, each rat was sacrificed and the whole length of the intestine from the pylorus to the caecum was dissected out, its content collected in a test tube, and the volume measured (14).

Statistical analysis

The data were analysed statistically using one-way analysis of variance followed by ANOVA test. The data are expressed as mean ± S.E.M.

Results

The results of the preliminary phytochemical screening of methanolic and aqueous extracts of TFG seeds are presented in Table 1.

Table 1: Phytochemical screening of *Trigonella foenum-graecum*

Test	Methanolic Extract	Aqueous Extract
Alkaloids	+	+
Carbohydrates	+	+
Flavonoids	+	+
Phenolic compounds & Tannins	+	+
Steroids	+	-

(- Absence; + Presence)

Castor oil induced diarrhea

Administration of castor oil produced characteristic semi-solid diarrhoea dropping in 18 h starved rats of the control group during the 4 h observation period (Table 2). The test drug vehicle were given p.o. and loperamide i.p. Results are expressed as mean \pm S.E., n=6. Statistically significance test will control was one by ANOVA test, when compared to control.

Both extracts of TFG seeds exhibited marked anti-diarrhoeal activity. The extracts significantly ($p < 0.001$) inhibited both the frequency of defecation and as well as mean weight of stool in rats, when compare to control group. Anti-diarrhoeal potency of the test extracts was found less than that of the loperamide.

Table 2: Effect of Methanolic and aqueous extracts of TFG on castor oil induced diarrhoea in rats.

Treatment	Dose mg/kg	Mean weight of faeces \pm S.E.M. after 6 hrs (in g)	Percentage of inhibition
Control	–	2.65 \pm 0.15	–
Lopermide	1	0.41 \pm 0.03*	84.52
Methanolic extract	250	0.92 \pm 0.04*	65.28
Aqueous extract	1 ml/rat	1.61 \pm 0.07*	39.24

Values are Mean \pm S.E.M. (n=6); Significance Vs Control group: *P < 0.001,

Effect on Small intestinal transit

The methanolic and aqueous extracts decreased propulsion of the charcoal meal through the gastrointestinal tract as compared with control group (2% gum acacia). A similar reduction in the gastrointestinal transit of charcoal meal in rat was achieved with the intraperitoneal injection of atropine sulphate (5 mg/kg) (Table 3). The test drug vehicle were given p.o. and Atropine was given i.p. Results are expressed \pm S.E.M., n=6. Statistical significance test with control was done by ANOVA test. (* P<0.01, ** P<0.05) when compared to control. This observation was significantly (P<0.001, P<0.05) different from what was seen in control group. Anti-motility effect of test extracts was seen less than the atropine, the standard anti-muscarinic durg.

PGE₂ -induced enteropooling

Both extracts significantly ($P < 0.01$) inhibited PGE_2 induced enteropooling in rats at an oral dose of 250 mg/kg and 1ml/rat (Table 4). The test drug and vehicle were given p.o. Results are expressed as mean \pm S.E.M., $n = 6$. Statistical significance test with control was done by ANOVA test. PGE_2 induced a significant increase in the fluid volume of the rat intestine when compared with control animals received ethanol in normal saline.

Table 3: Effect of methanolic and aqueous extracts of TFG on gastro intestinal transit in rats.

Treatment	Mean distance travelled by charcoal \pm S.E.M (cm)	Mean % Movement of charcoal (cm)	Percentage of inhibition
Control	70.21 \pm 2.50	74.10	–
Atropine	18.53 \pm 1.67**	24.57	75.42
Methanolic extract	35.08 \pm 1.17**	56.25	50.83
Aqueous extract	61.81 \pm 2.48*	65.90	34.09

Values are Mean \pm S.E.M. ($n=6$); (* $P < 0.01$, ** $P < 0.05$) when compared to control.

Table 4: Anti-enteropooling effect of methanolic and aqueous extracts of TFG in rats.

Treatment	Mean volume of intestinal fluid (ml) \pm S.E.M
Control	0.75 \pm 0.03
PGE_2 in ethanol	2.75 \pm 0.06*
Methanolic extract	1.30 \pm 0.06*
Aqueous extract	1.73 \pm 0.07*

Values are the Mean \pm S.E.M. ($n=6$)

Discussion

In developing countries, a quarter of infant and childhood mortality is related to the diarrhea (15). The highest mortality rates have been reported to be in children less than five years of age. During the past decade oral dehydration therapy has reduced mortality from acute diarrhoeal disease, whereas chronic diarrhoea remains a life-threatening problem in those regions, in which malnutrition is a common co-existing and complication factor. Number of factors, such as infective, immunological and nutritional has been involved in the perpetuation of the diarrhoeal syndrome (16). Many plants conveniently available in India are used in traditional folklore medicine for the treatment of diarrhoea and dysentery. Of the indigenous plants used, *Andrographis paniculata*, *Asparagus racemosus*, *Butea monosperma*, *Cassia auriculata*, and others are mentioned (17). Several studies have shown that prior administration with some plant extracts had a protective effect on the intestinal tract (18-20). In the present study, methanolic and aqueous extracts of TFG that have not been studied so far, was screened for its anti-diarrhoeal potential against castor oil induced diarrhoea, gastrointestinal motility in charcoal meal test and PGE₂ induced enteropooling in Albino Wistar rats.

Both extract of TFG seeds exhibited significant antidiarrhoeal activity against castor oil induced diarrhoea in rats. The extracts had a less potency than loperamide, when tested at 250 mg/kg, 1ml/rat, and statistically significant reduction in the frequency of defecation and the mean weight of stool when compared to untreated control rats.

It is widely known that castor oil or its active component ricinoleic acid induces permeability changes in mucosal fluid and electrolyte transport that results in a hypersecretory response and diarrhoea (21, 22). The experimental studies in rats demonstrated a significant increase in the portal venous PGE₂ concentration following oral administration of castor oil (23). Ricinoleic acid markedly increased the PGE₂ content in the gut lumen and also caused on increase of the net secretion of the water and electrolytes into the small intestine (24). The liberation of ricinoleic acid from castor oil results in irritation and inflammation of the intestinal mucosa, leading to release of prostaglandins, which stimulate motility and secretion (25). Inhibitors of prostaglandin biosynthesis delayed castor oil induced diarrhoea (12). Earlier reports indicate seeds of TFG exhibited significant anti-inflammatory activity in the carrageenan-induced rat paw oedema (5). These observations, tends to suggest that the anti-diarrhoeal effect of methanolic and aqueous extracts may be due to the inhibition of prostaglandin biosynthesis.

The extract appears to act on all parts of the intestine. Thus, it reduced the intestinal propulsive movement in the charcoal meal treated model; at 250 mg/kg, 1ml/rat both extracts showed activity less than that of atropine. Previous study shows that activated charcoal avidly absorbs drugs and chemicals on the surface of the charcoal particles thereby preventing absorption (26). Thus, gastrointestinal motility test with activates charcoal was carried out to find out the effect of methanolic and aqueous extracts on

peristaltic movement. The results also show that the methanolic and aqueous extracts suppressed the propulsion of charcoal meal thereby increased the absorption water and electrolytes.

The extracts also significantly inhibited the PGE₂ induced intestinal fluid accumulation (entero pooling). It has been shown that E type of prostaglandins cause diarrhoea in experimental animals as well as human beings (27). Their mechanism has been associated with dual effects on gastrointestinal motility as well as on water and electrolyte transport (28). PGE₂ also inhibit the absorption of glucose, a major stimulus to intestinal absorption of water and electrolytes (29). These observations tend to suggest that both extracts at a dose of 250 mg/kg reduced diarrhoea by inhibiting PGE₂ induced intestinal accumulation of fluid.

Previous experimental studies demonstrated that, the plant extract containing of tannin, flavonoids, alkaloids, saponins, reducing sugars and sterols and/or terpenes (30-33) exhibited anti-diarrhoeal activity. Phytochemical analysis of the extracts showed the presence of alkaloids, saponins, flavonoids, sterols and /or terpenes and sugars. These constituents may responsible for the anti-diarrhoeal activity of TFG extracts.

The antidiarrhoeal activity of flavonoids has been ascribed to their ability to inhibit intestinal motility and hydro-electrolytic secretion (34-36), which are known to be altered in this intestinal condition. In vitro and in vivo experiments have shown that flavonoids are able to inhibit the intestinal secretory response, induced by prostaglandins E₂ (37). In addition, flavonoids present antioxidant properties (38) which are presumed to be responsible for the inhibitory effects exerted upon several enzymes including those involved in the arachidonic acid metabolism (39). Adamska M. Lutomski J. (40) reported isoflavone from the seeds of TFG. The preliminary phytochemical analysis of extracts also revealed the presence of flavonoids. As a consequence, it is possible to suggest that the antisecretory and antioxidant properties of flavonoid could contribute to the observed anti-diarrhoeal effect. In some cases, it has been found that anti-diarrhoeal activity is associated with the antibacterial (41). To confirm this We have also carried out antibacterial activity of crude methanolic extract of TFG seeds and found significant antibacterial activity (unpublished data).

The present study indicates that the methanolic and aqueous extract of TFG possesses significant anti-diarrhoeal activity due to its inhibitory effect both on gastrointestinal propulsion and fluid secretion. The inhibitory effect of the extract justified the use of the seeds as a non-specific antidiarrhoeal agent in folk medicine. The phytochemical studies are also progress to isolate and characterize the active constituents of fenugreek seeds. The isolated compounds may serve as useful prototype of antidiarrhoeal drugs of natural origin possessing the desired pharmacological activities while lacking certain untoward effects.

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