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Evaluation of anti-diarrheal activity of aqueous extract of *Trigonella foenum- Graecum*

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Abstract
Trigonella foenum-graecum (fabeceae) is an annual plant, has long history in Indian and Chinese medicine. The present study was to evaluate the anti diarrheal activity of aqueous extract of whole plant of *Trigonella foenum-graecum* by using castor induced diarrheal model. Animals were divided into four groups, control, standard, and two test groups 100mg/kg and 200mg/kg the aqueous extract have shown graded response with increase in the dose, decrease in the mean weight of feces with significant value $p < 0.05$.
Keywords: Castor induced diarrheal model, mean weight of feces, dose dependent

1. Introduction
Diarrhea is the characterized by an increase in frequency of bowel movement, wet stools, and abdominal pain[1]. It is a leading cause of malnutrition and deaths among children in the developing countries of world today[2]. According to the world health report, diarrhea is the cause of 3.3% of all the deaths worldwide. The worldwide distribution of diarrhea accounts for more than 5-8 millions deaths each year in children who were aged less than 5 years. The use of traditional medicine to combat the consequences of diarrhea has been emphasized by WHO in its diarrhea control program[3, 4, 5, and 6]. Many synthetic chemicals are available for treatment of diarrhea, but they have some side effects. The natural drugs are used as anti-diarrheal drugs, which are not always free from adverse effects[7].

Trigonella foenum-graecum is an annual plant in the family Fabaceae with leaves consisting of three small obovate to oblong leaflets. It is cultivated worldwide as a semi-arid crop, and its seeds are a common ingredient in dishes from the Indian Subcontinent.

Fenugreek has a long history of medical uses in Indian and Chinese medicine, and has been used for numerous indications, including labor induction, aiding digestion, and as a general tonic to improve metabolism and health.

Diarrhea is a tropical and subtropical disease caused by variety of infections allergy to emotional disturbances[8]. Treatment of Diarrhea with plants of plant preparations and medicaments has been mentioned in the ancient indigenous systems of medicine of many countries. Even today, rural folks and aboriginal tribes all over the world, including India, are using many plants in the treatment of diarrhea[9].

Trigonella foenum-graecum (Fabaceae) was used as ant diarrheal, antioxidant and ant platelet, etc. Traditionally this plant was used in various diseases. The present study was designed to evaluate the anti-diarrheal activity of aqueous extract of whole plant of *Trigonella foenum-graecum*..

2. Materials and Methods
2.1. Plant material collection and authentication
The whole plant of *Trigonella foenum-graceum* was collected and authentified by Prof. Mrs.V.Jaya M,sc;M.Phil., Botanist, Guntur.

2.2. Preparation of *Trigonella foenum-graceum* whole plant aqueous extract.
Fresh plants were collected, cleaned of extraneous matter, shade – dried and powdered mechanically. The powder was mixed with distilled water (3g of seed powder per 100ml of water). After mixing using a cyclomixer, the extract was centrifuged at 3000rpm for 10min. The supernatant was used as the aqueous extract for feeding the animals in the present study.

2.3. Phytochemical analysis
The aqueous extract is subjected to phytochemical analysis using conventional protocol like alkaloids, flavonoids,

carbohydrate and glycosides, steroids, tanins and proteins.

2.4. Experimental Animals

Albino rats of Wistar strain of either sex weighing between 150 and 200 g was obtained. They were housed in standard cages at room temperature (25 ± 2^{o}C) and relative humidity (55 ± 5 %) and 12/12 h light/ dark cycle. The animals were provided with food and water and deprived of food for 24 h before experimentation.

2.5. Castor oil induced diarrhea

Rats were divided into four groups and fasted for 18hrs before the experiment. First group 2ml(control) is treated with distilled water. Second group treated with standard drug loperamide (1mg/kg i.p.) as standard. For the 3rd and 4th groups are treated with 188mg/kg and 200 mg/kg aqueous extract 1ml and 2ml/kg. After 1hr of treatment, all the animals were challenged with 1ml of castor oil orally for inducing diarrhea. The animals were placed separately in metabolic cages over white clean Whatman filter paper, which was changed every hour. The severity of diarrhea was assessed each hour for 4 hours. The total number of diarrhea feces of the control group was considered 100%. For every one hour observe stools (figure.1)and remove the filter paper and weigh the feces and write observation every hour.

2.6. Experimental Design

Table 1. Methodology

Animals are divided into 4 groups of 6 animals each and acclimatized for 10 days

Groups	Drug treatment	Dose and route of drug administration
I	Distilled water	2 ml/kg, p.o.
II	Loperamide	1mg/kg, p.o.
III	Aqueous extract	1 ml/kg, p.o
IV	Aqueous extract	2 ml/kg, p.o.

2.6 Statistical analysis

The data's will be expressed as mean ± SD. The data of Antidiarrhoea activity will be analyzed by one way analysis of variance (ANOVA) followed by Dunnet's't' test. A *p* value less than 0.05 will be considered as statistically significant.

3. Results

3.1. Preliminary phytochemical analysis

Preliminary phytochemical analysis revealed the presence of carbohydrates, flavonoids, alkaloids, tannins and phenolic compounds, and glycosides in TFGE **(**Table 6.1).

Table -2 Preliminary phytochemical analysis of TFGE

Phytochemical test	Results
Carbohydrates	+
Alkaloids	+
Tannins and phenolic compounds	+
Flavonoids	+
Proteins,amino acids	-
Glycosides	+
Steroids	-

+: indicates the presence of compounds

- : indicates the absence of compounds

3.2. Anti Diarrheal Activity of TFGE by Castor Oil Induced Diarrhea

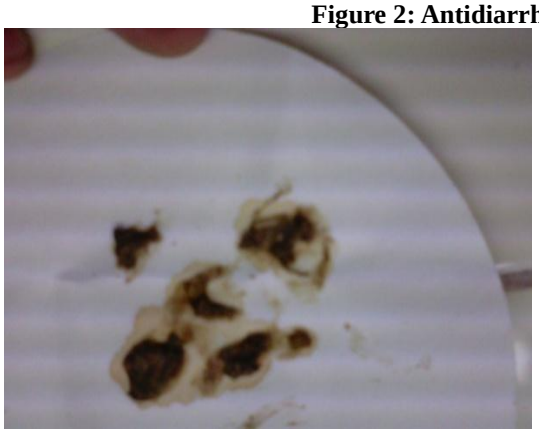
This study shows the mean weight of feces in animals were significantly reduced ($p<0.05$) by treatment with TFGE at two dose levels 100mg/kg and 200mg/kg when compared with control group 200mg/kg of TFGE group animals has shown very significant reduction compared with control group. There was significant difference in the mean values of 100mg/kg and 200mg/kg, shows the dose dependent activity of the extract. The results are shown in the table3, figure 2

Table 3 Anti diarrheal Activity of TGFE

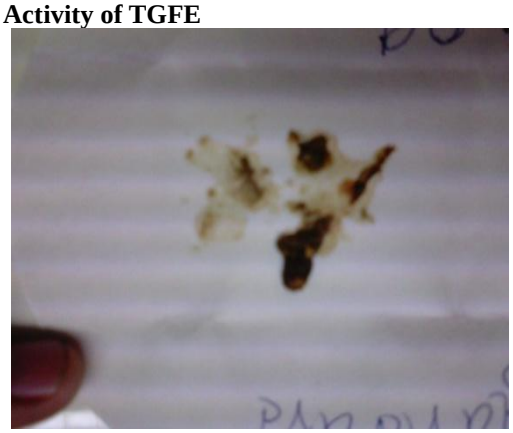
Group	Treatment	Dose (p.o)	Mean weight of faeces in 4 hours (mg)	% Protection
I	Distilled water	2ml/kg. p.o	3.333±0.003	-
II	Loperamide	1mg/kg. p.o	0.900±0.057***	72.21
III	Aqueous extract	100ml/kg. p.o	2.033±0.056*	40.15
IV	Aqueous extract	200ml/kg. p.o	1.232±1.875**	63.63

Values of aqueous extract expressed as mean ± SEM., at triglycerides level was compared with control. *p <0.05, **p<0.01 ***P < 0.001

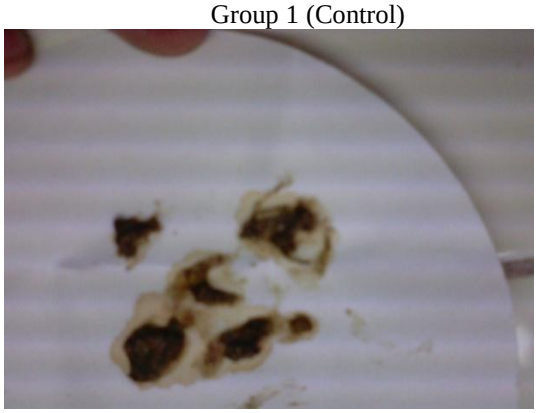
Figure 2: Antidiarrhoeal Activity of TGFE



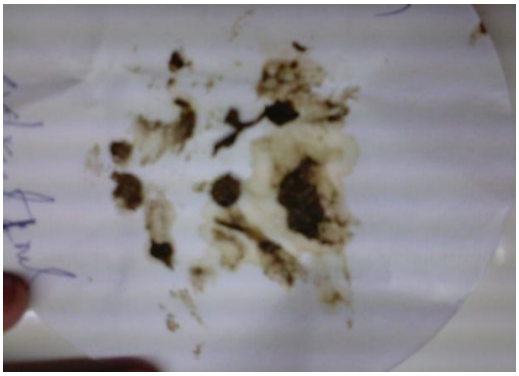
Group 1 (Control)



Group 2 (Standard)

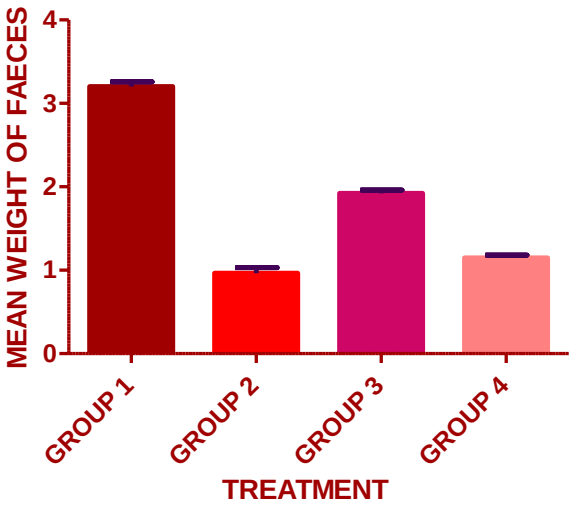


TFGE (100mg/kg)



TFGE (200mg/kg)

GRAPH .1 Anti diarrheal Activity of TGFE



5. Discussion

In the present study, aqueous extract of TFGE that have not been studied so far, was screened for its anti diarrheal potential against castor oil induced diarrhea. Aqueous extract of TFGE exhibited significant anti diarrheal activity against castor oil induced diarrhea in rats. The extracts had a less potency than loperamide. When tested at 100mg/kg, 200 mg/kg, there was statistically significant ($p<0.05$) reduction in the mean weight of stool when compared to untreated control rats.

Widely know that castor oil active component ricinoleic acid that results in a hypersecretory response and diarrhea. The liberation of ricinoleic acid from castor oil results in inflammation of the intestinal mucosa leading to release of prostaglandin biosynthesis which stimulates motility and secretions. Inhibitors of prostaglandin biosynthesis delayed castor oil induced diarrhea. These observations, tends to suggest that the anti diarrheal activity of aqueous extract may be due to the inhibition of prostaglandin biosynthesis. PGE2 also inhibits the absorption of glucose, a major stimulus to intestinal absorption of water and electrolytes. These observations tend to suggest that extract at a dose of 200mg/kg reduced diarrhea by inhibiting PGE2 induced intestinal accumulation of fluid.

Phytochemcal analysis of the extracts showed the presence alkaloids, saponins, flavonoids, sterols and/or terpenes and sugars. These constituents may be responsible for the anti diarrheal activity of TFGE extracts. The anti diarrheal activity of flavonoids has been ascribed to their ability to intestinal motility and hydro-electrolytic secretion, which are known to be altered in this intestinal condition[10]. The preliminary phytochemical analysis of extracts also revealed the presence of flavonoids. As a consequence, it is possible to suggest that the anti secretary and antioxidant properties of flavonoid could contribute to the observed anti diarrheal effect. The present study indicates that the aqueous extract of TFGE possess significant anti diarrheal activity due to its inhibitory effect both on GI propulsion and fluid secretion. The inhibitory effect of the extract justified the use of the plant as a non-specific anti diarrheal agent in folk medicine.

6. Conclusion

In the present study, aqueous extract of *Trigonella foenum-graecum* of whole plant showed significant anti diarrheal activity against castor oil induced diarrhea in dose dependent manner. Treatment of animals with the extract resulted in significant decrease in the mean weight of feces compared to control. The dose of 100mg/kg of the extract showed activity 40.15% anti diarrheal activity where as 200mg/kg of the extract showed 63.63 % of antidiarrhoeal activity.

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The results of the present study provide the evidence for the anti diarrheal activity of aqueous extract of *Trigonella foenum-graecum* whole plant as claimed in the traditional use. A further study on the exact mechanism of action and isolation of the active constituents is needed and different formulations of extract can be evaluated.

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