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Laxative Activity of *Trigonella Foenum- Graecum* Seed on Loperamide Induced Constipation in Rats

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The main objective of the study was to extract phytoconstituents from fenugreek seeds. The present study will be helpful in determining the quality and purity of a crude drug and laying down pharmacopoeial standards for *Trigonella foenum-graecum*. Fenugreek seed was extracted with water using hot continuous percolation method and the aqueous extract was used for determining the laxative activity.

Keywords: *Fenugreek Seeds, Laxative activity, Constipa- tion, Trigonella foenum-graecum.*

GJMR-G Classification: *NLMC Code: WA 360*



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Laxative Activity of *Trigonella Foenum-Graecum* Seed on Loperamide Induced Constipation in Rats

R. Chandrasekar [α], G.G.P. Madhuri [σ], T. Bhavya [ρ], K. Keerthi [Ω], S. Aishwarya [¥], Y. Saipavan [§] & M. Niranjan Babu [χ]

Abstract- ***Trigonella foenum-graecum*** L. (fenugreek) is widely used for its medicinal properties all over the world and it is a very important spice in Indian culture. The genus name Trigonella means 'tri-angled', maybe because of triangular shape of its flowers, whereas the species name foenum-graecum means 'Greek hay'. It is an annual crop and dicotyledonous plant belonging to the subfamily Papilionaceae, family Fabaceae. It is used as a functional food, traditional food and as a nutraceutical, as well as its physiological utilization such as antibacterial, anticancer, antiulcer, anthelmintic, hypocholesterolemic, hypoglycemic, antioxidant and anti-diabetic agent.

The main objective of the study was to extract phytoconstituents from fenugreek seeds. The present study will be helpful in determining the quality and purity of a crude drug and laying down pharmacopoeial standards for *Trigonella foenum-graecum*. Fenugreek seed was extracted with water using hot continuous percolation method and the aqueous extract was used for determining the laxative activity.

Phytochemical screening of the extract revealed the presence of alkaloids, amino acids, carbohydrates, proteins, saponins, tannins, terpenoids, and vitamins. The present study was carried out to investigate the laxative activity of fenugreek seeds in Albino Wistar rats. Rats were divided into four groups of 5 animals each, the first group as a control; the second group served as standard (sodium picosulfate) while group 3 and four were treated with aqueous seed extract of *Trigonella foenum-graecum* at doses of 250 and 500 mg/kg body weight (b.w.), *p.o.* Respectively. The laxative activity was determined based on the weight of the fecal matter.

The results revealed that the aqueous extract of fenugreek seed has shown significant laxative activity and supports its traditional claim in herbal medicine. From the available literature, it's evident that there is a need for the development of proper medication and dosage form for the treatment of constipation.

Keywords: Fenugreek Seeds, Laxative activity, Constipation, Trigonella foenum-graecum.

I. Introduction

Constipation affects all ages; it affects elderly people at the age of 60 and above, adults and children. Constipation can be classified into different categories which include common constipation, occasional constipation, chronic constipation, travel-related constipation, age-related constipation, pregnancy-related constipation, chronic idiopathic constipation and functional constipation. This condition may be due to consumption of low fiber-rich food, improper time in consuming food, lack of exercise, life style habits, less intake of fluids, etc. Constipation may end in restlessness, tired, indigestion, discomfort, vomiting, and accumulation of fecal matter in the intestine. Laxatives commonly bulk laxatives can relieve constipation in smoothening the walls of the intestine, colon and rectum and ease the fecal matter out of the rectum.

Fenugreek belonging to *Fabaceae* family is one of the well-known spices in food. Its seeds and green leaves are used in food as well as its medicinal value in the treatment of various diseases. It provides natural food fiber and other nutrients required to the human body. [1] Fenugreek has a strong spicy and a sweet flavor. [2] Aromatic and flavouring fenugreek is a popular spice and is widely used for well recognized culinary and medicinal properties. [3] "*Kasuri Methi*" is very famous for its appetizing fragrance and it is used for culinary preparations. [4] In a recent trend, fenugreek is also used as spice adjunct. [5] India is a major producer of fenugreek and also a major consumer of it for its culinary uses and medicinal application. It is used in functional food, traditional food, nutraceuticals as well as its pharmacological activities such as an antibacterial, anticancer, antiulcer, anthelmintic, hypocholesterolemic, hypoglycemic, antioxidant, and anti-diabetic agent. It has a beneficial influence on digestion and also can modify food texture.

The mechanisms of action of laxatives include enhancement of fluid retention by hydrophilic or osmotic mechanisms, decreasing net absorption of fluid through effects on the fluid and electrolyte transport in the small or large intestines, and finally an alteration of motility by inhibiting nonpropulsive contractions or stimulating propulsive contractions. Laxatives are often classified into four categories that include: bulk-forming laxatives, osmotic laxatives, stimulant laxatives, and stool softeners or surfactant laxatives. [6]

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Fenugreek is a mild bulk-forming laxative that's best suited for long-term use in people with constipation. The laxatives most frequently used world-wide come from plants. Herbal laxatives are either bulk-forming or stimulating. Bulk-forming laxatives come from plants with a high fiber and mucilage content that expand when they come in contact with water; examples include psyllium, flaxseed, and fenugreek. As the volume in the bowel increases, a reflex muscular contraction occurs, stimulating a bowel movement. These mild laxatives are best suited for long-term use in people with constipation. [7] Fenugreek fiber could be useful for treating constipation and hinder the development of diverticulosis and diverticulitis. Fenugreek fiber promotes the normal location due to imperfect fermentation in the large intestine. It can make the waste bulky, soften the stool by holding water and minimize the transit time through the intestine; hence, it helps to keep constant and steady stool time. Fenugreek is a mild bulk-forming laxative that's best suited for long-term use in people with constipation. [1]

II. Materials and Methods

a) Collection of plant material

The seeds of *Trigonella foenum-graecum* were collected from departmental stores, Tirupati. They were identified and verified taxonomically and authenticated in the Department of Botany, S. V. University, Tirupati. The seeds were coarsely powdered by using a mixer grinder and the powder was stored in airtight plastic containers. The preserved powder was used for physicochemical analysis.



Figure 1: Fenugreek Seeds

b) Determination of physicochemical parameters

Determination of physicochemical parameters such as total ash, acid insoluble ash, water-soluble ash, extractive values such as water soluble extractive value, ethanol soluble extractive value and ether soluble extractive value of the crude drug was determined according to WHO guidelines on quality control methods for medicinal plant materials (WHO, 1992). [8]

c) Preparation of extracts

The collected plant material was washed and dried at room temperature for seven days and was subjected to size reduction. The prepared powder was used for extract preparation. The aqueous extract was prepared by the Soxhlet extraction method.



Figure 2: Soxhlet Extraction Method

d) Phytochemical Evaluation

The freshly prepared aqueous extract of *Trigonella foenum-graecum* was qualitatively analyzed for the presence of major phytochemical constituents. [9]

e) Pharmacological studies

i. *Experimental animals*

The animals were acclimatized to standard laboratory conditions (temperature: 25 ± 5°C), humidity (55 ± 5%) and maintained on a 12-h light: 12-h dark cycle. They were provided with regular rat chow and drinking water and libitum. The experimental protocols were approved by the Institutional Animal Ethics Committee CPCSEA Reg. No. (1995/PO/RE/S/17/ CPCSEA)

f) Laxative Screening

i. *Animals*

Wistar rats of either sex with an average weight of 150-200 g were obtained from Bangalore. The animals were housed in clean cages placed in a well ventilated house. They were acclimatized to the animal house condition for seven days during which they were allowed free access to commercial pelleted rat chow. All experimental procedures were performed in compliance with international policies governing the Institutional Animal Ethical Committee for the treatment of experimental animals.

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g) Acute toxicity studies

Acute oral toxicity study for the test extract of the *Trigonella foenum-graecum* was carried out as per the guidelines set by Organization for Economic Co-operation and, revised draft (OECD) 425 and by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Social Justice and Empowerment, Government of India. The study revealed that the administration of aqueous seed extract of *Trigonella foenum-graecum* was safe up to a dose of 2000 mg/kg. No death was observed up to this dose, and the experimental animals were physically active. Hence 1/4th (250 mg/kg) and 1/8th (500 mg/kg), were selected as working doses for the present study. [10]

h) Experimental design

Animals were divided into four groups, each group containing five animals.

- Group I - Normal control, control rats - received normal saline (negative control)
- Group II - Received standard drug sodium picosulfate (positive control) 5mg/kg p.o.
- Group III - Aqueous extract of *Trigonella foenum-graecum* seed at a dose of 250 mg/kg body weight p. o. (Test –I)
- Group IV - Aqueous extract of *Trigonella foenum-graecum* seed at a dose of 500 mg/kg body weight p. o. (Test –II)

i) Evaluation of laxative activity

i. *Laxative activity of aqueous extract of Trigonella foenum-graecum in rats*

The animals were fasted for 12 hours before the experiment, but provided with water ad libitium. The animals were divided into four groups of five in each and were placed individually in cages lined with clean filter paper. Group I treated as control, (2 ml p.o. Normal Saline), group II received sodium picosulfate (1ml/kg p.o) served as standard and Group III received *Trigonella foenum-graecum* seed aqueous extract (250 mg/kg p.o.) and Group IV received *Trigonella foenum-graecum* seed aqueous extract (500 mg/kg p.o.) respectively. Immediately after dosing, the animals were separately placed in cages suitable for collection of faces. After 8 hours of drug administration, the feces were collected and weighed. Food and water were given to all rats and fecal outputs were weighed after 16 hours. After 8-16 hrs test drug exhibited an increase in fecal output. The extract showed a dose dependant increase in fecal output of rats when compared to the control group (Table 4). The effects of *Trigonella foenum-graecum* increased significantly fecal output at doses of 250 and 500 mg/kg (p.o.) of rats compared to control group ($p < 0.05$ and $p<-0.01$ respectively). The method of Capasso *et al*. [11] was followed for this activity. All oral administration was done using metal oropharyngeal cannula. The water and feed intake and the number of fecal pellets of all the rats were recorded during the experimental period.

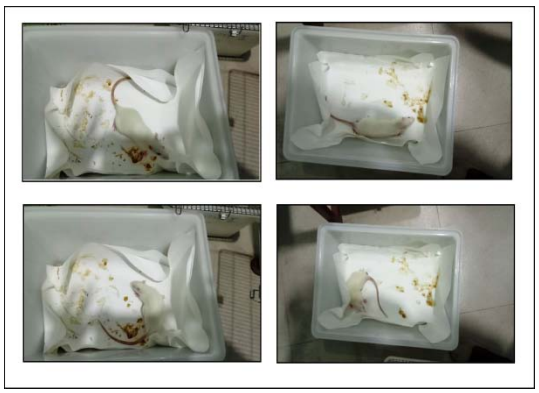


Figure 3: Pictorial representation of fecal output

ii. *Determination of total number, dry weight, and water content of fecal pellets*

The excreted fecal pellets of individual rats were collected at 10.00 h throughout the experiment. The total number, weight and water content of the pellets were determined. The water content was calculated as the difference between the wet and dry weights of the pellet. [12] The water content of feces was calculated as: fecal water content (%) = (feces weight before dried - feces weight after dried)/feces weight before dried × 100. [13, 14]

The laxative activity of the *Trigonella foenum-graecum* seed aqueous extract was evaluated by observing the stool consistency parameters like normal pellet stool, soft-formed stool, watery stool and mucus stool. The onset and duration of feces was recorded. *Trigonella foenum-graecum* seed aqueous extract at a dose 500 mg/kg P.O. showed a significant Laxative Activity $p< 0.05$.

iii. *Effect of Trigonella foenum-graecum seed aqueous extract on Loperamide induced constipation in rat*

The laxative activity of *Trigonella foenum-graecum* seed aqueous extract was evaluated by observing the fecal output. Rats were allowed to fast for 18 hours and divided into four groups of five animals each. Rats were placed individually in cages lined with clean filter paper. Group I received *Trigonella foenum-graecum* seed aqueous extract (250 mg/kg p.o.) and Group II received *Trigonella foenum-graecum* seed aqueous extract (500 mg/kg p.o.) respectively. Group II treated as control, (2 ml p.o. Normal Saline), group IV received sodium picosulfate (5mg/kg p.o) served as standard. After one h, all the animals received Loperamide (5 mg/kg, *p.o.*) by gavage. It was observed that after 8 hours of treatment. Extract effect at the higher dose of *Trigonella foenum-graecum* seed aqueous extract 500 mg/kg (p.o.) was similar to that of the standard drug sodium picosulfate (5 mg/kg, p.o.).

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The reduction of Loperamide-induced constipation at 500 mg/kg (p.o.) of *Trigonella foenum-graecum* seed aqueous extract treatment was found to be almost comparable with that of treatment by 5 mg/kg of sodium picosulfate. Hence *Trigonella foenum-graecum* seed aqueous extract showed significant laxative activity ($P<0.05$) at 500 mg/kg dose level when compared to standard. The feces production (total number) in all groups was monitored for eight h. This study was carried out, as described by Takahara *et al*. [15, 16]

j) Data analysis

The data obtained by the various parameters were statistically evaluated by one-way analysis of variance (ANOVA). The mean values ± SEM were calculated for each parameter. $P< 0.05$ was considered significant.

III. Results and Discussion

a) Pharmacognostical and Phytochemical Evaluation

To establish the quality and purity of the raw material used for the various physiochemical parameters such as ash values and extractive values were evaluated and reported in table 1 & 2.

The results revealed that the plant *Trigonella foenum-graecum* shows the percentage of total ash as well as extractive values.

Table 1: Different Ash values of *Trigonella foenum-graecum*

S. No.	Ash Values	Ash Values in Percentage
		Trigonella foenum-graecum
1	Total ash	4.76
2	Water soluble ash	1.2
3	Acid insoluble ash	2.1

Table 2: Different Extractive Values of *Trigonella foenum-graecum* seeds

S. No.	Extractive Values	Extractive values in Percentage
		Trigonella foenum-graecum
1	Water soluble extractive value	6.3
2	Alcohol soluble extractive value	6.1
3	Ether soluble extractive value	5.15

b) Phytochemical Evaluation

The extracts are subjected to various qualitative phytochemical tests and reports are shown in table no.3. The results reveal the presence of alkaloids, carbohydrates, flavonoids, proteins and saponins, etc.

Table 3: Phytochemical analysis of *Trigonella foenum-graecum*

S. No.	Phytochemical analysis	*Trigonella Foenum-Graecum* AE
1.	Alkaloids	+
2.	Carbohydrates	+
3.	Proteins	+
4.	Amino acids	+
5.	Glycosides	-
6.	Steroids & sterols	-
7.	Flavonoids	+
8.	Tannins	+
9.	Phenolic compounds	-
10.	Terpenoids	+
11.	Saponins	+
12.	Fats and oils	-
13.	Gum and Mucilage	+
14.	Vitamins	+

(+) Present (-) Not Present

Table 4: Laxative activity of aqueous extract of *Trigonella Foenum-Graecum* in rats

Groups	Treatment	Dose	Faeces output (g)	
			0-8 hrs	8-16 hrs
GP I	Control	(5 ml/kg)	0.4975±0.04975	0.56±0.056
GP II	Sodium Picosulfate	(5 mg/kg)	3.4875±1.4874**	3.85±0.45**
GP III	Test I TFG AQ Ex p. o.	(250 mg / kg)	2.49875±0.74875*	2.66125±0.91125*
GP IV	Test II TFG AQ Ex p. o.	(500 mg / kg)	3.05±0.55**	3.4875±0.4875**

Values are expressed as mean ± S.E.M (n = 5); * $p < 0.05$ compared to control group; and ** $p < 0.01$ compared to control group.

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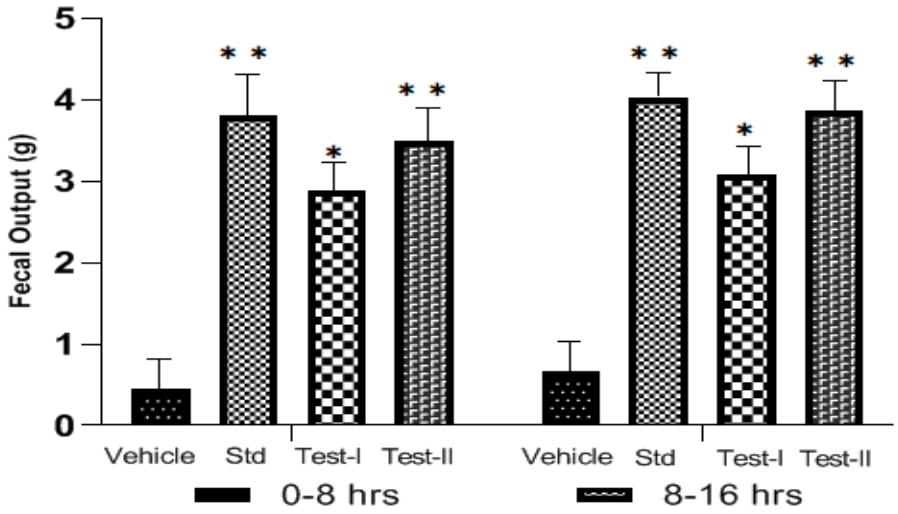


Figure 5: Time interval of fecal output of aqueous extract of *Trigonella Foenum-Graecum* in rats

Table 5: Loperamide induced constipation on aqueous extract of *Trigonella Foenum-Graecum* in rats

Groups	Treatment	Dose	Weight of faeces (g)
GP I	Control	(5 ml/kg)	0.4375±0.1685
GP II	Sodium Picosulfate	(5 mg/kg)	4.45±0.6141**
GP III	TFG AQ Ex	(250 mg / kg)	2.4±0.5043*
GP IV	TFG AQ Ex	(500 mg / kg)	4.07±0.49**

Values are expressed as mean ± S.E.M (n = 5); * p < 0.05 compared to control group; and **p < 0.01 compared to control group.

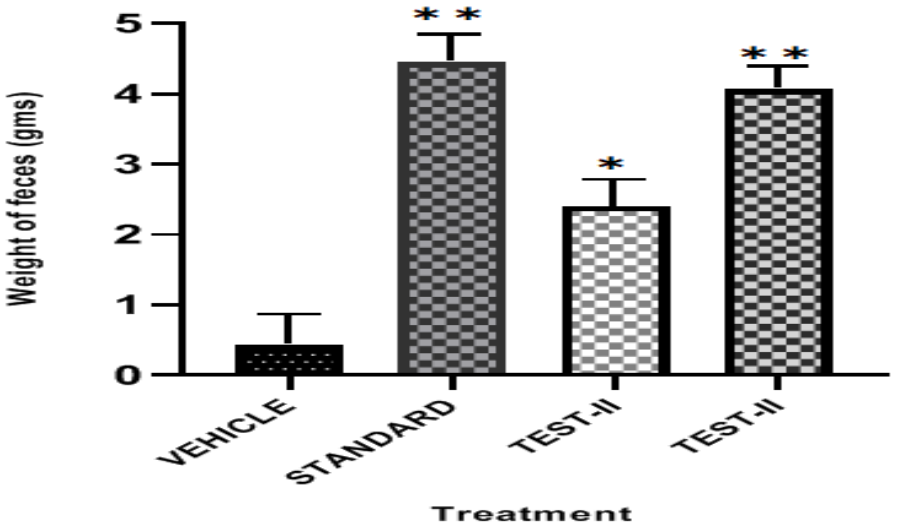


Figure 6: Loperamide induced constipation of *Trigonella Foenum-Graecum* seed aqueous extract

IV. Discussion

Trigonella foenum-graecum was subjected to systematic physicochemical evaluation and phytochemical screening by extracting with aqueous solvent to determine the soluble constituents present in a given amount of the plant material. The present work was helpful in determining the quality and purity of a crude drug. In this study the parameters used for the evaluation of *Trigonella foenum-graecum* were, Extractive values by different solvents (includes water, ethanol and petroleum ether) ash values (total ash, water soluble and acid insoluble ash). On incineration, drugs leave an ash which consists of carbonates, phosphates and silicates of sodium, potassium, calcium and magnesium. The determination of ash value is useful for detecting the adulterants, exhausted drugs,

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low-grade products and excess of sandy matter which is especially applicable to powdered drugs. [17]

Phytochemical analysis was performed on the aqueous extracts of *Trigonella foenum-graecum*. The aqueous extract contains carbohydrates, proteins and amino acids, glycosides, alkaloids, flavonoids, phenolic compounds, phytosterols, and tannins. The present study was carried out to investigate the laxative activity of fenugreek seeds in albino Wistar rats. Rats were divided in 4 groups of 5 animals each, first group as control, second group served as standard (sodium picosulfate) while group 3 and 4 were treated with aqueous seed extract of *Trigonella foenum-graecum* at doses of 250 mg/kg and 500 mg/kg body weight (b.w.), *per oral* respectively. The laxative activity was determined based on the weight of the fecal matter. The results showed that the aqueous extract of *fenugreek* has a significant laxative activity and supports its traditional claim in herbal medicine.

Fecal output depends on the dietary fiber, water-electrolyte balance, the rate of absorption and secretion from the lumen. Many laxatives have common mechanism of action in increasing water electrolyte secretion, decreasing its absorption in the colon. The presence of terpenoids, flavonoids, sterols, phenolic compounds can be responsible for the laxative activity of the plant. Although the Phytochemical screening revealed the presence of terpenoids, flavonoids like components. The laxative activity of *Trigonella foenum-graecum* seed aqueous *extract was* studied in rats. Oral administration of extract showed the significant and dose-dependent increase in fecal output of rats in regards to the accumulation of water in the intestine.

V. Conclusion

The plant *Trigonella foenum-graecum* has shown a higher percentage of total ash as well as alcohol- soluble extractive values. Qualitative Phytochemical screening of the plant extract *Trigonella foenum-graecum* reveals the presence of alkaloids, amino acids, carbohydrates, proteins and saponins. *Trigonella foenum-graecum* extract was prepared by Soxhlet extraction. Form the available literature it was found that *Trigonella foenum-graecum* contains more number of proteins, amino acids, vitamins, minerals, and flavonoids. The *Trigonella foenum-graecum* seed aqueous extract has shown better laxative activity indicating the additive property of the herbs. *Trigonella foenum-graecum* seed has a tremendous scope on further studies mainly as a Nutraceuticals, and dietary supplements; because it contains many amino acids, carbohydrates, fatty acids, vitamins and minerals, etc., therefore further research work to be carried out on this plant towards enhancing the medicinal claims. More research work is recommended on the plant for isolation and characterization of bioactive compounds that may be active against many diseases.

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Conflict of Interest

The authors do not have any conflict of interest.

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