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# PHCOG MAG.: Research Article
# Pharmacological effects of *Trigonella foenum graecum* Linn. seeds on various isolated perfused smooth muscle preparations

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**ABSTRACT -** In the present study, the pharmacological effects on different smooth muscles by the extracts like total alcoholic (TA), total aqueous (TQ), petroleum ether (PE), total alkaloidal (TK), total glycosidal (TG) and a phytochemical trigonelline (TR) from *Trigonella foenum graecum* Linn. seeds was investigated using isolated organ bath preparation method. 1 mg/ml dose of the extracts and the phytochemical showed significant anti-histaminic activity on guinea pig ileum, anti-cholinergic effect on rat colon and uterine stimulant activity on rat uterus. These results might be due the presence of some spasmolytic and spasmogenic constituents in the *Trigonella foenum graecum* Linn. (Fenugreek) seeds. These findings will help to invent some novel therapeutics from the fenugreek seeds.

**KEYWORDS:** *Trigonella foenum graecum*, total alkaloidal, total glycosidal, petroleum ether, ileum, spasmolytic, spasmogenic.

## INTRODUCTION

*Trigonella foenum graecum* Linn. belongs to the family Leguminosae and it is popularly known as Fenugreek (1). *Trigonella foenum graecum* Linn. is native to the area from the Eastern Mediterranean to Central Asia and Ethiopia, and much cultivated in India and China(2). The seeds of *Trigonella foenum graecum* Linn. is the main focus of this study. Fenugreek is one such plant whose seeds and leaves are used not only as food but also as an ingredient in traditional medicines (3). In India, the seeds of fenugreek were used in Ayurveda and Siddha to treat fever, dysentery and heart diseases, while in Unani system, this plant is used as a resolvent, aphrodisiac, diuretic, emmenagogue and tonic (4). In China, fenugreek seeds were used as a galactogogue to encourage lactation (5). The past phytochemical investigations on the seeds reveals the presence of Diosgenin, Trigonelline, Gitogenin, Vicenins 1 and 2, Vitexin, Quercetin, Luteolin, Kaempferol, β-Sitosterol etc., moreover the endosperm of the seeds is rich in galactomannan (6). In our previous study, for the first time we have proven that the seeds possess cardiotonic property (7). Also the seeds were shown to possess various pharmacological properties like gastric anti-ulcer activity (8), wound healing activity (9) and immuno modulatory activity (10).

Several synthetic spasmogens and spasmolytics have been used in the field of medicine, in spite of their side effects and minimum therapeutic index. Even though many drugs are available with spasmogenic and spasmolytic properties, search for a drug of plant origin with maximum potency and minimum side effects continues. Since no scientific report of the previous investigations detail are available on the effect of fenugreek on the isolated smooth muscles, the present study was undertaken to screen the extracts and a phytochemical of fenugreek on various smooth muscle preparations using isolated organ bath preparations.

## MATERIALS AND METHODS

### *Preparation of extracts*

Fenugreek seeds were obtained from the supermarket and authenticated by a botanist Prof. Dr. R. Rengasamy, C.A.S. Botany, University of Madras. A voucher specimen of the sample has been deposited in the department of Pharmacology and Environmental Toxicology, Dr. A.L.M. P.G.IBMS, University of Madras, Chennai.

### *Total alcoholic extract*

500 g of dried and coarsely powdered seeds of *Trigonella foenum graecum* Linn. was extracted with 95% ethanol for a period of one month. The filtrate

was taken and concentrated on a water bath using petridish. The temperature was maintained at 55°C. The concentrate obtained was weighed (25 g) and coded as TA.

***Total aqueous extract***

500 g of dried and coarsely powdered seeds of *Trigonella foenum graceum* Linn. was extracted with double distilled water for 24 hrs. The filtrate was taken and concentrated on a water bath using petridish. The temperature was maintained at 60°C. The concentrate obtained was weighed (16.4 g) and coded as TQ.

***Petroleum ether extract***

500 g of the dried and coarsely powdered seeds were extracted with petroleum ether and kept at room temperature, same as TA. The filtrate was obtained and evaporated to a concentrate using petridish on water bath. Concentrate obtained was weighed (25.6 g) and coded as PE.

***Total alkaloidal extraction***

10 g of total alcoholic extract concentrate was extracted with 0.1N HCl by allowing it to stand for 5 hrs. The aqueous acid extract was partitioned with 100 ml of chloroform in a separating funnel. This procedure was repeated for 2 more times and the combined chloroform layer was rejected. The aqueous layer was basified with ammonium hydroxide to pH 9.0 and was again partitioned with chloroform. The aqueous layer was rejected while the chloroform layer was collected and evaporated to obtain the concentrate (11). The total yield was 200 mg and it was coded as TK.

***Total glycosidal extraction***

500 g of shade dried and coarsely powdered seed of *Trigonella foenum graecum* Linn. was extracted with ethanol: water (1:2). The aqueous alcoholic extract thus obtained was treated with 5% neutral lead acetate to precipitate the tannins present. This procedure was repeated until no more precipitate of lead tannate was obtained which was filtered off. The clear filtrate was bubbled with $H_2S$ (Hydrogen sulphide gas) to remove the excess lead present in the solution as black lead sulphide. The black precipitate was filtered and the process was repeated until no more black precipitate of black lead sulphide was obtained or the filtrate smells strongly of $H_2S$. The clear filtrate was evaporated to a concentrate (12). The total yield was 22 g and it was coded as TG.

***Identification of Trigonelline in the alcoholic extract of fenugreek seeds***

Previous literature study reveals that fenugreek seeds contain Trigonelline. The separation and purification of Trigonelline would be a demanding, time consuming and expensive work. Instead of isolating the Trigonelline, it was obtained of pure grade from Sigma Chemical Co., USA and subjected to TLC for identification. The Trigonelline obtained was used for the pharmacological studies.

The alkaloid Trigonelline was identified in the total alcoholic extract using thin layer chromatography (TLC) by comparing it with the standard trigonelline of Sigma grade. TLC provides a chromatographic drug fingerprint. Silica gel 60 $F_{254}$ precoated TLC plates were used. The solvent system used for Trigonelline is Methanol:Ammonia (200:3) (13). Detection was done by placing the TLC plate in the ultraviolet chamber for Trigonelline. Fig.1 shows the presence of trigonelline in the total alcoholic extract when compared with the Trigonelline standard. All the experiments carried out in this study were approved by Institutional Animal Ethical Committee (IAEC).

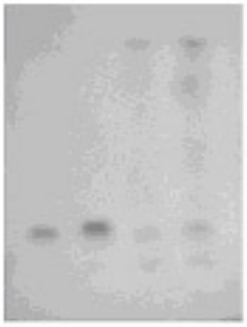


***Figure. 1: Identification of Trigonelline (TR) in the total alcoholic extract (TA) from Trigonella foenum graecum Linn. Seeds***

***Isolated perfused guinea pig ileum preparation***

Guinea pigs weighing about 200 - 300 g were used for the ileum experiment. This tissue proves to be a classical model for the bioassay of histamine. $H_1$ receptor mediated responses were studied in guinea pig longitudinal smooth muscle of the ileum (14). Ileum was removed from the guinea pig and placed in a petridish containing Tyrode solution (Composition in mM: NaCl – 136.89, KCl – 2.68, $MgCl_2$ – 1.05, $CaCl_2$ – 1.36, $NaH_2PO_4$ – 0.32, $NaHCO_3$ – 11.90 and Glucose – 5.55; made upto 1 litre of distilled water). Ileum was cut into small pieces and 2 pieces of threads were tied at the upper and lower ends of an ileal tissue.

The tissue was mounted in a micro organ bath of capacity of 10 ml, which was maintained at 37°C, aerated and perfused with Tyrode solution. The tied ileal tissue was attached through a thread to a frontal lever balanced at a tension of 0.5 g. The writing arm of the frontal lever was made to touch the kymograph drum. The drugs were administered in the organ bath containing the tissue and the response was recorded on the kymograph. The following cycle was adopted:

0 sec -the kymograph was switched on
15 sec -the standard drug was added
45 sec -the test compound was added
75 sec - the kymograph was switched off and the preparation was washed with a fresh change of perfusion fluid (15).

***Isolated perfused rat colon preparation***

Matured rats weighing about 200 - 250 g were used for this experiment. Rat colonic muscle is the best model for evaluating the muscarinic or anti-muscarinic effect of a test drug. Biochemical and Northern blot studies have shown that both muscarinic $M_2$ and $M_3$ receptors are present in mature rat colon (16). The first 4 cm of the descending colon was used in this experiment.

The colon was isolated from the rat and placed in a petridish containing Modified Ringer's solution (Composition in mM: NaCl – 171, KCl – 5.36, $NaHCO_3$ – 1.7, $CaCl_2$ – 0.270, Glucose – 5.55). The tissue was mounted in the micro organ bath perfused with Modified Ringer's solution that was maintained at 32°C, aerated and the contractions were recorded as described for the previous experiment.

***Isolated perfused rat uterus preparation***

The uteri were obtained from the virgin female albino rats pretreated with estradiol benzoate (0.5 mg/kg b.w, s.c) (17), 24 hrs prior to the experiment. The presensitized uterus of the rat has been used widely for evaluating the uterine stimulant/tocolytic activity of drug/extracts. The two horns of the uterus were placed in the petridish containing De Jalon's solution (composition in mM: NaCl – 154, KCl – 5.6, $NaHCO_3$ – 5.95, $CaCl_2$ – 2.2, Glucose – 2.8). A single horn was mounted in the micro organ bath of 10 ml capacity, which was maintained at 30°C, aerated and perfused with De Jalon's solution. The contractions were recorded as described for the previous experiment.

***Statistical analysis***

Six tissues from different animals were used for each series of experiment. The data were analyzed using the student's *t* - test. The values are expressed as mean ± S.E.M.

**RESULTS**

***Isolated perfused guinea pig ileum preparation***

The anti-histaminic activity of the extracts and the phytochemical of fenugreek seeds were observed. Fig. 2 reveals the response obtained from the extract and the phytochemical on guinea pig ileum. Histamine (1 μg) produced a contractile effect on the ileum. The alkaloidal extract (1 mg/ml) - TK and trigonelline (1 mg/ml) - TR produced significant blocking effects on the histamine induced contraction. The code TK showed 45.76% of antagonistic effect and the code TR showed 50.17% of antagonistic effect.

***Isolated perfused rat colon preparation***

The extracts and the phytochemical were analyzed for the anti-cholinergic effects. Fig. 3 reveals the anti-cholinergic activity of the extracts. Acetylcholine (1 μg) produced contraction on the rat colon muscle. The total aqueous (1 mg/ml) – TQ and the total glycosidal (1 mg/ml) – TG extracts showed significant antagonistic effect on the acetylcholine induced contraction. The code TQ showed 39.03% and code TG showed 43.91% blocking effect respectively.

***Isolated perfused rat uterus preparation***

The extracts produced significant contraction on the isolated rat uterus. Oxytocin (0.1 IU) and acetylcholine (1 μg) possessed significant contraction on the uterus muscle. Table 1. reveals the uterotonic effect of the alcoholic (1 mg/ml) – TA, aqueous (1 mg/ml) – TQ and petroleum ether (1 mg/ml) – PE extracts as same as the oxytocin, which was confirmed by the blocking effect of indomethacin (10 μg). Oxytocin produced 5.88 cm contraction, acetylcholine produced 3.13 cm contraction, while the codes TA produced 4.21 cm, TQ produced 3.96 cm and PE produced 3.98 cm respectively.

**DISCUSSION**

In various indigenous systems of medicine, the seeds of *Trigonella foenum graecum* Linn. were used. The extractions of seeds in previous research work were done in a rambling manner and some pharmacological investigations were done by using only alcoholic and aqueous extracts, moreover there was no evidence of isolation of alkaloids and glycosides from this seeds. So, it was decided to do the systematic extraction, which helped to compare the pharmacological potentials of different extracts on guinea pig ileum, rat colon and rat uterus muscles.

In guinea pig ileum, the longitudinal smooth muscle contains histamine receptors of both $H_1$ and $H_2$ types, with the former being responsible for the contractile response to histamine (18). The contractile response obtained with 1 μg of histamine was considered as

*Table 1: Effect of the extracts from the seeds of Trigonella foenum graecum Linn. on isolated perfused rat uterus preparation*

| Sl. No. | Drugs / Extract | De Jalon solution contraction | Atropine (10µg/ml) + De Jalon solution contraction | Indomethacin (10 µg/ml) + De Jalon solution contraction | Remarks |
|---|---|---|---|---|---|
| 1. | Oxytocin (0.1 I.U.) | 5.88 ± 0.33 | - | 4.51 ± 0.18 ** | Uterine Stimulant |
| 2. | Acetylcholine (1 µg/ml) | 3.13 ± 0.17 | 1.53 ± 0.08*** | - | Cholinergic activity |
| 3. | TA - Total alcoholic extract (1mg/ml) | 4.21 ± 0.20 | 4.10 ± 0.19 | 3.10 ± 0.15** | Uterine Stimulant |
| 4. | TQ - Total aqueous extract (1mg/ml) | 3.96 ± 0.14 | 3.86 ± 0.13 | 2.83 ± 0.08*** | Uterine Stimulant |
| 5. | PE - Petroleum ether extract (1 mg/ml) | 3.98 ± 0.31 | 3.93 ± 0.33 | 2.70 ± 0.09** | Uterine Stimulant |

*Values are expressed as cm - mean ± SEM of 6 tissues. (** $p < 0.01$; ***$p < 0.001$).*

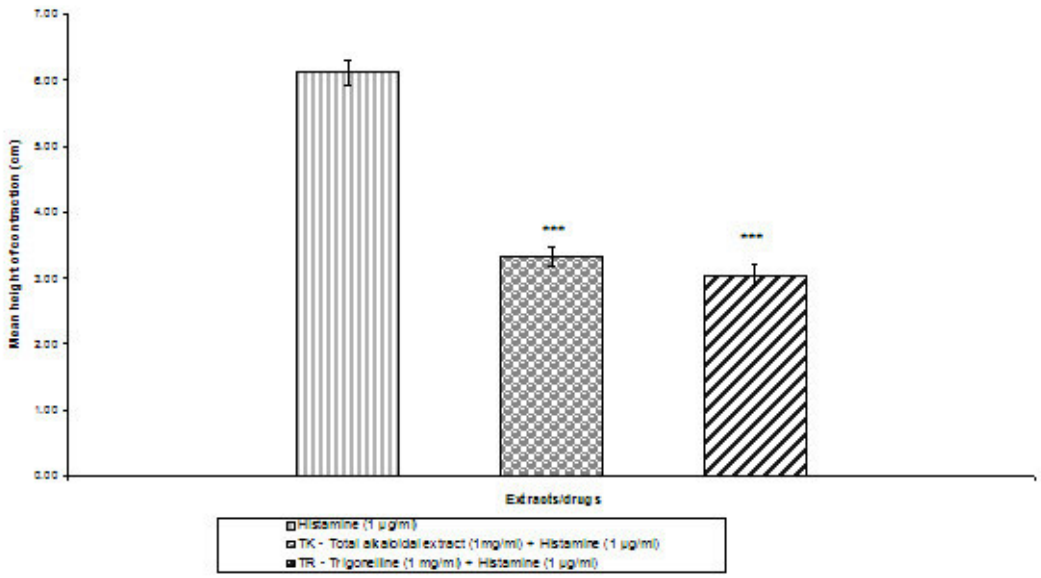


***Figure. 2: Effect of the total alkaloidal extract and trigonelline from the seeds of Trigonella foenum graecum Linn. on histamine induced contractions on isolated perfused guinea pig ileum preparation. Values are expressed as mean±S.E.M. (***p<0.001)***

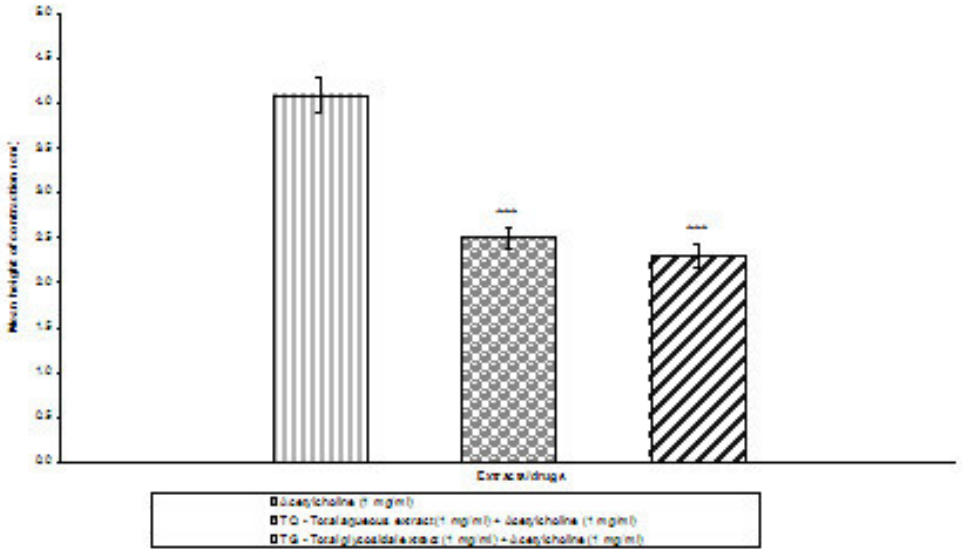

*Figure. 3: Effect of the extract from the seeds of Trigonella foenum graecum Linn. on acetylcholine induced contractions on isolated perfused rat colon preparation. Values are expressed as mean±S.E.M. (***p<0.001)*

100%. The extracts and the phytochemical of fenugreek seeds did not produce any contraction on the ileum individually. The contractile responses obtained with 1 μg of histamine were challenged against all the extracts of 1 mg/ml and trigonelline of 1 mg/ml. The histamine affinity and potency is decreased when the alkaloidal extract and trigonelline administered individually 10 min, before the administration of histamine. Thus the anti-histaminic activity was observed. Generally histamine activates $H_1$ receptors which leads to formation of inositol-1-4-5 triphosphate and diacyl glycerol, which inturn induces the calcium mediated contraction (19). The antagonistic effect of the codes TK and TR might be due to the prevention of binding of histamine to its receptors. Hence, this experimental result may help to invent new therapeutics from this seeds for the treatment of asthma, allergy and its related diseases.

In rat colon, the spasmogenic response obtained with 1 μg of acetylcholine was considered as 100% and none of the extracts and phytochemical elicited any contractile effects. The contractile response obtained with 1 μg of acetylcholine was challenged with all the extracts and the phytochemical. It has been reported that binding of an agonist to muscarinic receptors in the smooth muscle activates G-protein which induces $Ca^{2+}$ dependent contraction (20). The results of this study reveals that the aqueous (TQ) and glycosidal (TG) extracts when added individually to the bath containing the tissue for 10 min before the administration of acetylcholine, prevented contraction induced by acetylcholine. This indicates that the aqueous extract and glycosidal extract might contain some anti-cholinergic principles, which could have prevented the binding of acetylcholine to its receptors, through some non-specific way and blocked the contraction. This experimental result will lead a way for the use of this seeds in the treatment of diarrhoea and intestinal related diseases.

In rat uterus, the contractile response with 0.1 IU of oxytocin and 1 μg of acetylcholine was recorded on the kymograph. The codes TA, TQ and PE produced significant contraction on isolated estrogenised rat uterus preparation. This effect was not blocked by atropine (10 μg) a potent anti-cholinergic agent, but milder blockage was elicited by indomethacin (10 μg). The contractile effect of Ach was significantly blocked by atropine and the effect of oxytocin was significantly blocked by indomethacin. Normally in the sensitized uterus, the prostaglandin synthesis is increased and numbers of oxytocin receptors get increased in the endometrium, while in the myometrium, the sensitivity to the oxytocin is increased (21). In rat myometrium, as gestation progresses to term, there is a decline in muscarinic receptor-mediated phospho inositide hydrolysis, possibly because of the decrease in muscarinic receptor number to a certain extent (22). Oxytocin interacts not only with myometrial but also with endometrial receptors. It stimulates the synthesis of prostaglandins ($PGF_{2\alpha}$) in the uterus by interacting

with endometrium. Thus prostaglandin regulates the muscle contraction, which was significantly blocked by indomethacin (10 μg), a potent prostaglandin synthetase blocker. The possible mechanism of contraction by the codes TA, TQ and PE might be due to prostaglandin mediated contraction, because the response was significantly blocked by indomethacin. Further studies on this experiment will lead a way to find out the phytochemicals present in the extracts of *Trigonella foenum graecum* Linn. which possess uterine stimulant property.

From the above study we can conclude that, the extracts and the phytochemical of the fenugreek seeds possess different pharmacological effects on different types of smooth muscles. The codes TA, TQ and PE possess uterotonic activity, code TQ and TG possess anti-cholinergic activity and codes TK and TR possess anti-histaminic activity. These findings will be a useful tool for the future study in the herbal research using fenugreek seeds.

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