

EVALUATION OF SAPONINS FROM *TRIGONELLA FOENUM GRAECUM* SEEDS FOR ITS ANTIFERTILITY ACTIVITY

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ABSTRACT

Objective: To evaluate antifertility activity of saponins from *Trigonella foenum-graecum* seeds.

Study design: Saponin extract of *Trigonella foenum-graecum* (SE-TFG) was prepared. Preliminary phytochemical studies were performed followed by oral acute toxicity studies as per OECD 423 guideline. The saponin extract of *Trigonella foenum-graecum* (SE-TFG) was administered at two doses. Antifertility activity was evaluated in different experimental model such as anti-implantation study, Abortifacient activity, estrogenic and anti-estrogenic activity.

Results: Preliminary phytochemical studies revealed the presence of saponins, steroids, flavonoids, amino acids in saponin extract of *Trigonella foenum-graecum* (SE-TFG). Toxicity study showed mortality with saponin extract to the dose of 2000mg/kg p.o. Hence, 1/10th of toxic dose was selected for carrying out antifertility evaluation. The saponin extract was found to be more effective in anti-implantation and abortifacient activity. The extract also exhibited weak estrogenic activity in immature ovariectomized rats.

Conclusion: All observations suggest that the saponin extract of *Trigonella foenum-graecum* (SE-TFG) has antifertility activity.

Keywords: Saponin, *Trigonella foenum-graecum*, Antifertility, Anti-implantation, Abortifacient, Estrogenic and anti-estrogenic.

INTRODUCTION

Trigonella foenum-graecum commonly known as Methika (Sanskrit), Greek hay, Fenugreek (English), Kasuri methi, Methi, Sag methi (Hindi), Methi (Marathi) consist of dried seeds of the plant *Trigonella foenum graecum* Linn. Seeds are oblong compressed, smooth, dark yellow to brown when dried, 4-5 cm long, 2-3 mm wide. *Trigonella foenum graecum* Linn. is cultivated all over the world and generally distributed in India, North Africa, and Eastern Mediterranean.¹

The plant has been scientifically used for wounds, inflammation, gastrointestinal ailments, cholesterol lowering agent¹, diabetes² bronchitis, inflammation, chronic cough, liver disorders³, As an antifertility agent⁴. *Trigonella foenum graecum* is reported to contain several possible active chemical constituents such as alkaloids, saponins, steroids, tannins, flavonoids, amino acids and trigonilline.⁴

Literature review suggested the mechanism of saponin from *Camellia oleifera*, *Gleditschia horrid*⁵ showing antifertility action. Various studies have revealed the potential activity of fenugreek seeds seen in male and female rats and rabbit models and suggested that it may be due to several possible active chemical constituents like saponins, steroids, alkaloids, tannins, flavonoids, amino acids, trigonilline.^{4,6} However the role of saponins from *trigonella foenum graecum* in female fertility is still not evaluated. The study is undertaken to evaluate the role of saponins in female fertility in rat.

MATERIAL AND METHODS

Drugs and Chemicals

Ethinyl estradiol tablet (Lynoral), distilled water, pet ether, methanol, chloroform, ethyl acetate, N-butanol, acetone was used for study. All chemicals & solvents used were of AR grade.

Plant collection

Seeds of the *Trigonella foenum graecum* was bought from the local Shirpur market, Maharashtra and was authenticated from Agharkar Research Institute, Pune.

Plant extraction

Extraction of Saponin

500 gm of fenugreek seeds was taken and ground to obtain Coarse was subjected to successive soxhlet extraction first with pet ether and subsequently by methanol. The extract was vacuum dried using rotary vacuum flash evaporator to yield solid residue. Weight of the solid obtained after drying was 60 gm.

Solid residue was refluxed with 80%w/v ethyl acetate for half an hour. Solvent was decanted off and discarded. The residue was then dissolved in 90%w/v methanol. It was further filtered, concentrated and was precipitated in acetone to yield saponin glycosides. The solvent was decanted off, precipitate was filtered, dried and weighed to estimate total saponin content of the plant drug.⁷

Phytochemical investigation

The saponin extract of *trigonella foenum graecum* (SE-TFG) was subjected to preliminary phytochemical screening to evaluate the presence of chemical groups according to standard methods.⁸

Residual solvent analysis:⁹

Residual solvent analysis carried on gas chromatography (PerkinElmer Clarus400) for class 1, class 2, and class 3 solvents. Mixture of pet. Ether, acetone, methanol, butanol prepared and used for comparison with 1000 ppm solution of extract.

Column: Fused Silica Capillary Column: 2 m long; 18" internal diameter.

Method:

Oven temperature 1	: 80°C
Time 1	: 1 min.
Rate 1	: 3°C / min.
Oven temperature 2	: 95°C
Time 2	: 1 min.
Rate 2	: 5°C / min.
Oven temperature 3	: 115°C
Time 3	: 1 min.
Rate 3	: 45°C / min.
Oven temperature 4	: 140°C
Time 4	: 1 min.
Injector temperature	: 180°C
FID temperature	: 200°C
Carrier gas (N ₂) flow	: 10 ± 0.05 mL/min.
FID Range	: 1
Run time	: 13 Min.

Experimental animals:¹⁰

Wistar albino adult male and female rats were used for antiimplantation and abortifacient activity. Wistar albino immature female rats were used for estrogenic and anti-estrogenic activity. All animals were housed in a polypropylene cage with stainless steel top containing husk at a temperature 25±2°C having a 12hr light-

dark cycle. A free excess of food and water were provided ad libitum. Animals were segregated into four groups of six rats in each and stored in the animal house facility of SPTM, NMIMS, Shirpur campus. The experimental protocol was approved by the Institutional Animal Ethics Committee (IAEC) of NMIMS, SPTM, Dhule, Maharashtra and conducted according to the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA).

Acute toxicity studies

Acute toxicity study of saponin extract of *Trigonella foenum graecum* Linn were carried out in rats according to OECD guidelines 423. Extract at different doses up to 2000 mg/kg, p.o. was administered and animals were observed for behavioural changes, any toxicity and mortality up to 48 h. Thereafter, they were kept under observation up to 14 days after drug administration to find out the mortality if any.¹¹

Anti-implantation study

Female rats of proestrus phase were kept with male rats of proven fertility in the ratio of 2:1. The female rats were examined in the following morning for evidence of copulation. The animal which showed thick clumps of spermatozoa in vaginal smear were separated from the male partner and divided into 3 groups (n=6). The day when spermatozoa was detected in vaginal smear was considered as day 1 of pregnancy. The selected rats were treated with (i.e. group 1 served as control, group 2 and 3 were administered with test extract of saponin at a dose of 100mg/kg, 200mg/kg respectively). The dosing was done from 1st day to 7th day of pregnancy. The animals were laparotomised on day 10 of the pregnancy under anesthesia and uteri were examined to determine the number of implantation sites. The abdominal wound was sutured layer by layer and animals were allowed for wound healing.¹²

Abortifacient activity

The same groups were followed to evaluate the abortifacient activity. The dosing was continued from 8th day to 14th day of pregnancy. After delivery, numbers of litters were observed and percentage of resorption was calculated.¹²

Estrogenic and Anti-estrogenic activity

Immature female wistar rats weighing 55-60 g were ovariectomized. They were maintained for 1 week on standard laboratory diet and water ad libitum. The animals were divided into four groups containing six animals per group.

Group 1 - Served as control and did not receive any treatment.

Group 2 - Ethinyl estradiol (0.02 mg/kg, p.o.) and served as standard.

Group 3 - Test + Standard (200 mg/kg + 0.02 mg ethinyl estradiol)

Group 4 - Test saponins extract (200 mg/kg, p.o.)

The dosing was continued for 7 days after ovariectomy. Animals were evaluated for significant increase in uterine weigh and diameter.^{11,12}

Statistical evaluation

Data obtained was presented as mean \pm standard error of mean (S.E.M.) for the number of animals in each group (n = 6). The groups were compared using one-way analysis of variance (ANOVA) followed by Dunnett's test and P<0.05 was considered significant.

RESULTS

Extractive value

Saponin extract of *Trigonella foenum-graecum* was found to be 8 gm i.e. 13.33% w/w on dried weigh bases.

Preliminary phytochemical screening:

It was found that saponin extract of *Trigonella foenum-graecum* (SE-TFG) shows presence of saponins, steroids, flavonoids and amino acids.(Table 1)

Residual solvent analysis

Gas chromatography data revealed that there were no residual solvent found in saponin extract which is evident from the results shown in Fig.1 & 2.

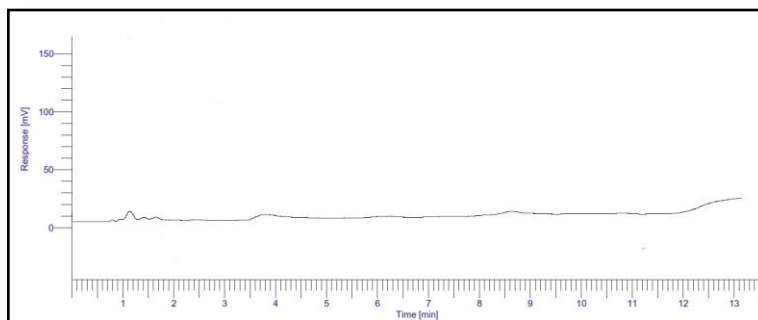


Fig 1: Chromatogram of sample (Saponin extract of *Trigonella foenum graecum*)

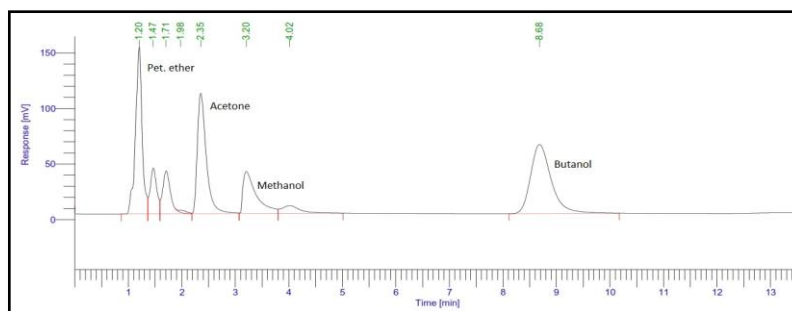


Fig 2: Chromatogram of mixture of solvents (Standard)

Table 1: Results of preliminary phytochemical screening of saponin extract.

S. No.	Constituent	Inference
1	Alkaloids	-
2	Flavonoids	+
3	Carbohydrate	-
4	Saponin	+++
5	Steroids	++
6	Amino acids	+
7	Tannins	-

Table 2: Anti-implantation study of saponin extract of *Trigonella foenum graecum*

Treatment	Dose	Number of implantation sites (Mean \pm SEM)	No. of rats having no implantation sites on day 10/no.	Anti-implantation activity (%)
Control	--	7.67 \pm 0.42	0/6	0
SE TFG	100 mg/kg	3.33 \pm 0.76***	1/6	16.66
SE TFG	200 mg/kg	2.17 \pm 0.79**	2/6	33.33

N= 6 animals in each group. ***P < 0.0001; **P < 0.01; *P < 0.05 vs. control. Values are expressed as mean \pm S.E.M.

Table 3: Abortifacient activity of saponin extract of *Trigonella foenum graecum*

Treatment	Dose	Abortifacient activity		
		Number of implantation sites	Number of litters born	% resorption
Control	--	7.67 \pm 0.42	7.67 \pm 0.42	0
SE TFG	100 mg/kg	3.33 \pm 0.76***	2.00 \pm 0.58***	40
SE TFG	200 mg/kg	2.17 \pm 0.79***	0.83 \pm 0.40***	62

N= 6 animals in each group. ***P < 0.0001; **P < 0.01; *P < 0.05 vs. control. Values are expressed as mean \pm S.E.M.

Table 4: Estrogenic and anti-estrogenic activity of saponin extract of *Trigonella foenum graecum*

Treatment	Uterine Weight (mg/100gm) (Mean \pm SEM)	Diameter of uterus (Mean \pm SEM)
Control	90.12 \pm 2.34	0.35 \pm 0.014
Standard (0.02)	172.22 \pm 3.48***	0.80 \pm 0.017***
Test + Std (200+0.02)	136.05 \pm 4.41**	0.44 \pm 0.013**
SE TFG (200 mg/kg)	161.22 \pm 3.20***	0.54 \pm 0.015***

N= 6 animals in each group. ***P < 0.0001; **P < 0.01; *P < 0.05 vs. control. Values are expressed as mean \pm S.E.M.

Acute toxicity studies

No signs of toxicity were noted in rats treated with a single oral dose of saponin extract upto 1500 mg/kg. However behavioural changes and mortality in rats was observed at a dose of 2000 mg/kg po. Based on acute toxicity result, 100mg/kg and 200mg/kg doses were selected as experimental dose to evaluate antifertility action of drug doses for antifertility evaluation.

Antiimplantation and abortifacient activity

A dose dependent antiimplantation response was evident (Table 2). With an increase in the dose of saponin extract, the percentage of implantation failure increased and was significant at dose 200 mg/kg (P < 0.0001; P < 0.05). saponin extract produced dose dependent abortifacient activity which was evident from the number of implants versus number of litter with 62% resorption at 200 mg/kg body weight dose level (Table 3).

Estrogenic and antiestrogenic activity

The effect of the saponin extract on the immature rat uterus is shown in (Table 4). Oral administration of the extract 200 mg/kg, p.o. Caused significant increase in uterine and diameter in immature ovariectomised rats. When standard and test were given simultaneously, the weight of uterus increased and was found to be more as compared to test alone, but not more than standard alone due to competitive antagonism between ethinyl estradiol and saponin extract.

DISCUSSION

Preliminary phytochemical screening indicated the major chemical constituent was steroidal saponin in the crude saponin extract of *Trigonella foenum graecum* (SE TFG). Literature review suggested the saponin compound is known to exhibit antifertility activity⁵. According to results saponin extract of *Trigonella foenum graecum* (SE TFG) shows antifertility activity.

The experimental groups were treated with standard Ethinyl estradiol 0.02 mg/kg and test drug i.e. saponin extract of *Trigonella foenum graecum*. The experiment was conducted in accordance with parallel design where two doses of test drug i.e. 100 and 200 mg/kg was administered to two separate group of animal at a single time. The effects of different doses of saponin extract was examined on the female reproductive system .

The treated animals showed anti-implantation and abortifacient activity in antifertility study. The number of litters born due to this treatment was significantly less than that of controls. This indicates the anti-implantation and abortifacient nature of saponin extract. This study clearly reveals that the extract is effective before and after the implantations occurred.

In another set of experiment on immature ovariectomized rat it has been observed that extract exhibited estrogenic activity by increase in uterine weight, diameter of uterus as compared to control. While, in case of standard alone there was significant increase in a weight of uterus. When standard and test were given simultaneously weight of uterus was more as compared to test alone, but not more than standard alone due to competitive antagonism between ethinyl estradiol and saponin extract. Anti-fertility activity of extract is mainly due to presence of saponin. It produces anti-fertility effect by negative feedback mechanism.

In the experiment like this, estrogen is known to stimulate these changes in the uterus. It is well known that for implantation the exact equilibrium of estrogen and progesterone is essential, and any disturbance in the level of these hormones may cause infertility. The compound with hormonal activity usually disturbs the hormonal milieu in the uterus and provokes an infertility effect in experimental animals. In this study, the anti-implantation activity may be due to estrogenic activity, causing the expulsion of the ova from the tube, disrupting the luteotrophic activity of the blastocyst.

Diosgenin is an important precursor for the synthesis of steroidal hormone like testosterone and progesterone.¹⁵ It is an important steroidal saponin present in fenugreek seeds.³ The estrogenic effect shown by the saponin extract of fenugreek may be due to the presence of diosgenin, yamogenin³ and tigogenin.

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