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Evaluation of antifertility activity of *Trigonella foenum graecum* seeds

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ABSTRACT

Petroleum ether, ethanol and water extracts of Trigonella foenum graecum seeds were investigated separately to determine their antiimplantation (antifertility) and estrogenic/antiestrogenic activity in female albino rats and Swiss mice. Among these, ethanol extract was found to be the most effective as antiimplantation and estrogenic agent. In preliminary phytochemical screening, ethanol extract showed positive test for alkaloids, saponins and flavonoids.

Key words: *Trigonella foenum graecum*, Antiimplantation, Estrogen, Phytoestrogen.

INTRODUCTION

World population is increasing at an alarming rate despite a number of synthetic contraceptive agents available. This is because these cannot be used continuously due to their severe side effects. Hence in the twenty first century a search was made for plants having potential as antifertility agents. It is commonly considered that herbal drugs are cheaper and safer as compared to synthetic drugs and may be used without or minimum side effects [1]. *Trigonella foenum graecum* (Leguminoceae) seed is well known for its various medicinal properties viz its galactogogue and aphrodisiac properties [2]. It contains phyto-estrogen which is a term applied to non-steroidal plant materials displaying estrogenic activity [3]. Phyto-estrogens encompass several classes of compounds including flavonoids, isoflavonoids and coumestans [4]. *Trigonella foenum-graecum* (TFG) is assumed to have a stimulating effect on digestive process [5]. In Ayurveda and Siddha it is used as bitter tonic, antipyretic and as an anthelmintic. Its use has been reported in inflammatory conditions and in certain heart disease and for curing leprosy, bronchitis and piles [6]. Recently it has been reported that TFG seeds possess antiulcer [7], immunomodulatory [8], hypocholesteramic [9], hypoglycemic [10], antibacterial [11], wound

healing [12], and estrogenic [13], activities. In the present study, we report the antiimplantation and estrogenic activities of *Trigonella foenum graecum* seeds.

MATERIALS AND METHODS

Trigonella foenum graecum seeds were procured from local market (Sagar, MP), India, in the month of December. Procured seeds were identified and authenticated by Department of Botany, Dr. H. S. Gour University Sagar India. Voucher specimen (TRS/101) was deposited in the laboratory of Department of Pharmaceutical Sciences for further reference. Seeds were dried, moderately powdered and stored. One kg of Seed powder was successively extracted with petroleum ether (60-80°), ethanol (90%) and water. The extracts were concentrated into viscous liquid under vacuum. The yields were found to be 2.38%, 10.24% and 2.48% respectively.

Phytochemical examination

Phytochemical tests of various extracts of TFG were carried out as described by Harbone [14]. Drangendroffs, Mayer, Wagner and Hager reagents were used for alkaloids, Libermann-Buchard reagent for steroids, shinoda test for flavonoids, Molish test for carbohydrate and ferric chloride reagent for phenolics and foam test for saponins.

Animals

Adult healthy female albino rats of Wistar strain weighing 100 ± 10 g were used for antiimplantation activity. Twenty one day old Swiss mice (20 ± 5 g) of either sex were used for dose fixation and estrogenic or antiestrogenic activity. All the animals were maintained under controlled standard animal house conditions with access to food and water *ad libitum*. The Institutional Ethical Committee for Animal Care and Uses approved all experimental procedure (Reg No. 397/01/ab/CPCSEA).

Dose fixation

Dose fixation was carried out by staircase method [15] on Swiss mice weighing 20 ± 5 g. All the extracts were homogenized in 1% carboxy methyl cellulose (CMC) in distilled water, and they were administered to mice orally by means of intragastric catherter. It was observed that all the extracts were found non toxic up to 5000 mg/kg body weight. Hence one-tenth of 5000 mg/kg (500 mg/kg) body weight dose was fixed as dosage for all the extracts.

Antiimplantation activity

Healthy adult Wister strain female albino rats (100 ± 10 g) of proven fertility and regular estrus cycles were selected and caged with male of proven fertility in the ratio of 3:1 in the evening of estrus phase and examined the following day for the evidence of copulation. Those rats showing thick clumps of spermatozoa in their vaginal smears were separated and that day was designed as d 1 of pregnancy. Such rats were divided into four groups containing six rats in each. Group I received vehicle only (1% CMC suspension 0.2ml) and served as control. Group II, III and IV received pet. ether, ethanol and water extract at 500 mg/kg body weight dose respectively. Doses were given from d 1 to 10 of pregnancy and after 24 h the last dose laprotomy was performed under light ether anesthesia using sterile conditions. The uteri were examined to number of implantation sites. The abdominal wound was sutured and the animals were allowed to recover and deliver after full term [16].

Estrogenic / Antiestrogenic activity

Ethanolic extract at 500 mg/kg dose was found to be the most effective among all the extracts which were used for antiimplantation activity testing. Therefore it was subjected to detailed investigation for potential estrogenic or antiestrogenic activity [17]. Colony bred 21 d old mice (20 ± 5 g) bilaterally ovariectomized was divided into four groups consisting six in each. Group I received vehicle only (1% CMC suspension 0.2 ml) and served as control. Second group received ethinyl estradiol in olive oil, 1µg/mice/d, subcutaneously. The third group received ethanol extract 500 mg/kg orally with the help of intragastric catheter and group IV received ethanol extract and ethynilestradiol at the above mentioned dose. Doses were given for 7 d, on the 8th d of the experiment, all the animals were sacrificed under light ether anesthesia and uteri were dissected out, surrounding tissues removed, blotted on filter paper and weighed quickly on a sensitive balance. A portion of uterine tissues from control and treated animals were fixed in Bouin's fluid for 24 h, dehydrated in alcohol and then embedded in paraffin wax. The paraffin sections were cut at 6 micron and stained with haematoxylin-eosine for histological examinations. Another part of uteri was processed for glycogen [18] and protein [19] estimation.

Statistical Method

Statistical analysis was carried out using Student's't' test. The results were judged significant if $P<0.05$.

RESULTS AND DISCUSSION

Results are tabulated in Table 1 to Table 3 and in photomicrograph 1 to 4.

Table 1 Phyto-chemical screening of crude extracts of *Trigonella foenum graecum* seeds

Class of compound	PE	EE	WE
Alkaloid	- ve	+ ve	+ ve
Steroid	+ ve	- ve	- ve
Flavonoid	- ve	+ ve	+ ve
Tannins and Phenolic	- ve	+ ve	+ ve
Carbohydrate	- ve	+ ve	+ ve
Saponin	- ve	+ ve	+ ve

PE = Petroleum ether, EE = Ethanol extract, WE = Water extract, - ve = Negative, + ve = Positive

Table 2 Antiimplantation activity of *Trigonella foenum graecum* seeds

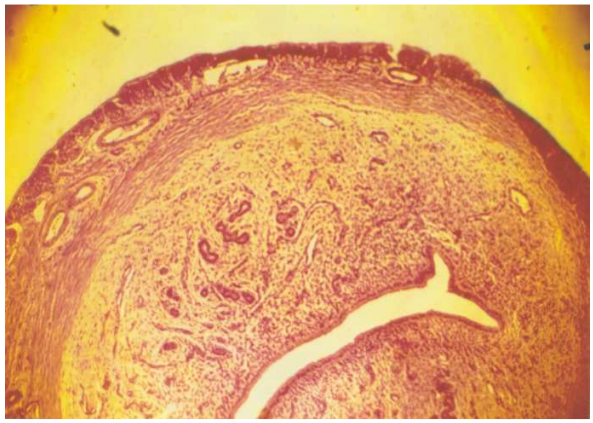
S. No.	Dose mg/kg	Treatment (in days)	No. of rats without implants on day 11/ No. of rats used	No. of implants on day 11	% Antifertility activity
1	Control CMC 0.2ml	10	0/6	6.50±0.42	-
2	PET 500	10	2/6	4.50±0.25	33.33
3	EE 500	10	4/6	3.16±0.48*	66.66
4	WE 500	10	1/6	5.26±0.71	16.66

Values are Mean ± S.E., = $p<0.05$ when compared to control, PET = Petroleum ether extract, EE = Ethanol extract, WE = Water extract, CMC = Carboxy methyl cellulose*

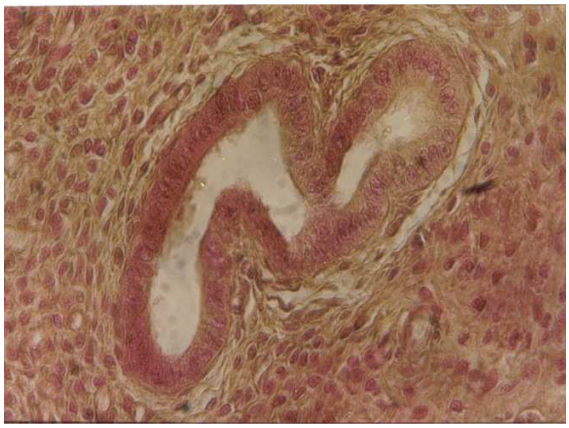
Table 3 Estrogenic activity of ethanol extract of *Trigonella foenum graecum* on immature ovariectomized mice

Treatment	Uterine wt. (mg/100 g)	Vaginal status	Vaginal cornification	Glycogen units/mg tissue wt.	Protein units/mg tissue wt.
Control CMC-0.02ml	16.21±0.11	Not opened	Nil	73.23±7.11	65.11±4.03
EED 1µg/rat/day	38.08±2.40*	Opened	+++	113.01±7.56*	98.21±3.21*
EE 500mg/kg b.w.	27.31±1.20*	Opened	++	91.22±5.41*	79.11±4.32*
EED + EE	49.11± 2.16*	Opened	++++	134.16±7.53*	126.36±3.76*

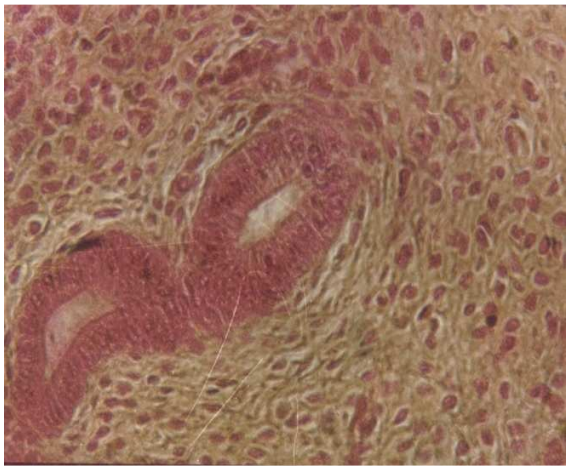
*Values are Mean ±S.E, * = p<0.05 when compared to control, CMC = Carboxy methyl cellulose, EED = Ethinyl estrdiol, EE = Ethanol extract, b.w. = Body Weight*



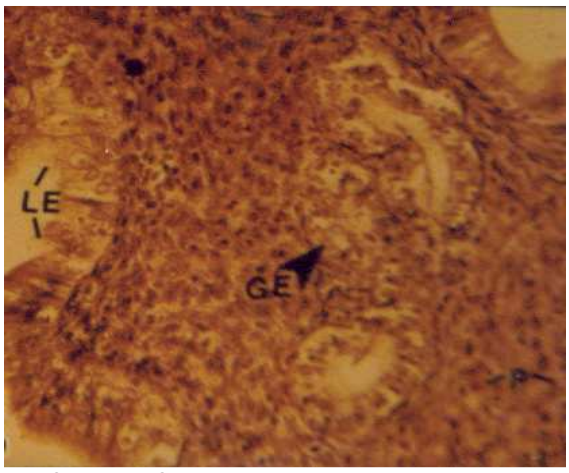
Photomicrograph 1 Section of Control uterus of group I (x100) showing all the normal conditions of uterus



Photomicrograph 2 Section of uterus of group II (x100) shows stimulatory effect on uterus after 7 d treatment with ethinyl estradiol at the dose of 1µg/mice/d



Photomicrograph 3 Section of uterus of group III (x100) showing stimulatory effect on endometrium and luminal epithelium after 7 d treatment with ethanol extract of *Trigonella foenum graecum*



Photomicrograph 4 Section of uterus of group IV (x100) showing over all stimulatory effect after 7 d treatment with ethinyl estradiol and ethanol extract of *Trigonella foenum graecum*

The process of implantation of an egg to the uterine wall depends upon the hormonal milieu of the uterus [20]. Antiimplantation agents are effective by virtue of their hormonal attributes, namely estrogenic or progestational properties or by antagonizing the effects of female sex (estrogen and progesterone) hormones [21]. A number of plants have been reported to inhibit implantation by their estrogenic mode of action [22]. It has been well established that some conventional properties of a typical estrogen such as increase in uterine weight and vaginal cornification are used as tools for detection and confirmation of the hormonal nature of an antifertility agent [23]. Besides this, some uterine biochemical parameters and induction of implantation after administration of estrogenic substances in experimental animals are also used as tools for detection and confirmation of the hormonal nature of an antifertility agent [24]. *Embelia ribes* fruits were reported to alter the level of estrogen and progesterone leading to improper implantation [25]. Therefore in any of the conditions, the secretion of estrogen and/or progesterone governs all the preparatory changes in the uterus for implantation. In the present investigation pet ether and ethanol extracts of TFG exhibited 33.33% and 66.6% antiimplantation activity respectively. In immature female mice ethanol extract exhibited definite estrogenic

activity at 500 mg/kg dose. Hence, the antiimplantation activity of ethanol extract may be due to an imbalance of endogenous estrogen and progesterone levels as evidenced histologically by unfavourable uterine milieu viz significant increase in uterine weight and stimulatory effect on endometrium and epithelial cells (photomicrograph 3 and 4). From the observation of reduced implantation sites and increased uterine weight confirms antifertility activity of ethanol extract of *Trigonella foenum graecum* seeds.

Glycogen is mainly located in the circular muscles in the rats and its physiological function may be involved in the rhythmic uterine contractions. This is further supported by the fact that many of the potent estrogenic compounds increased the uterine contractibility and propels the blastocysts from the pregnant uterus and thus provokes their antiimplantation activity. Therefore, the increased uterine glycogen level as observed in the present study may be involved in providing the readymade energy for uterine contractions and thus help in the expulsion of fertilized eggs from the uterus. This explanation is further strengthened by the fact that the administration of exogenous glycogen in the uterus of pregnant rats terminates the pregnancy [13].
Protein contents in the female reproductive organs are also regulated by the ovarian hormones. Estrogen and progesterone separately or in combined form significantly increased the protein contents of reproductive organs [26]. Our present findings also support the aforementioned information that alcoholic extract behaved in estrogenic way as it increased uterine protein contents. Increase in protein content of reproductive organs generally led to increased uterine weight [27] which produces unfavorable conditions for implantation as indicated in histological features.

Phytochemical examinations revealed the presence of steroid, flavonoids and saponins. Flavonoids [28] have been reported to possess the antifertility activity. Therefore the antifertility activity of TFG may be due to flavonoids. On the basis of these observations it may be concluded that ethanolic extract of TFG owing to its estrogenic nature alters the biochemical milieu of the uterus which lead to a change in the normal status of reproduction in female reproductive tract of rats and thus produce significant antifertility activity.

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