



GLOBAL JOURNAL OF ADVANCED RESEARCH
(Scholarly Peer Review Publishing System)

POSTCOITAL ANTIFERTILITY ACTIVITY OF STEROIDAL FRACTION OF FENUGREEK SEEDS IN RATS

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ABSTRACT

The seeds of fenugreek (*Trigonella foenum — graecum L*) are rich source of steroidal sapogenin. The steroidal fraction was extracted from fenugreek seeds by the modified method of Sarin et al. (1974) and was used for determination of its contraceptive efficacy in female rats. Normal cyclic female rats (*Rattus norvegicus*) were kept for mating with proven fertile male in the ratio of 2:1, next day in the morning the vaginal smear was checked for the presence of spermatozoa. The female rats with positive mating were divided in to four groups. Group I-control received only vehicle and Group II and III treated with plant extract (50mg and 100mg) for 12 days respectively and Group IV, treated with phytodrug (100mg) and ascorbic acid (100 mg) for 12 days. The data revealed that lower dose (50 mg) of phytodrug resulted in reduction of total embryo implantation and increased in number of reabsorbed embryo as compared to control; thereby inhibit fertility upto 50%. However, higher dose (100 mg) of seed extract showed no implantation site and reabsorbed embryo in all the rats studied, revealing 100% negative fertility in female rats studied.

Ascorbic acid is known for its antioxidant properties and it was given orally to female rats along with phytodrug. The observations revealed a negative impact of it i.e. the fertility rate remained 100% negative. The data suggests that steroidal fraction of fenugreek exhibited remarkable anti-implantation and early abortifacient activities in female rats leading to negative fertility. So the phytodrug can act as a potent post-coital emergency contraceptive agent in females.

General Terms

Fenugreek, Phytodrug, Rat, Fertility, Antioxidants

Keywords

Trigonella foenum-graecum, steroidal fraction, antifertility, anti-implantation, ascorbic acid.

1. INTRODUCTION

Fertility regulation is certainly an essential pathway but not sufficient in itself to the optimization of population rise. A large variety of substances both natural and synthetic have been shown to intercept pregnancy either during the pre-implantation or post-implantation stages of pregnancy. Postcoital contraceptive efficacy of certain compounds has also been related to a direct action on the blastocyst or to their indirect action on the pituitary gland (Pincus, 1965).

Various phytosteroids affect gonadotrophins of the sex organs, thus serving as precursor of sex hormones (Chopra and Handa, 1963). *Trigonella foenum graecum* (fenugreek) is considered to be a rich source of steroidal sapogenins (Hardnan, 1969). It is also considered to be hypoglycaemic (Jain et al., 1987) and antifertility agent (Setty et al. 1977; Kamal et al. 1993; Sharma et al., 1994; Bhinda 2005; Kassem et al; 2006).

Vitamin C acts as an antistressor and detoxicant to maintain biochemical homeostasis within the body. The present work has been undertaken to highlight the postcoital antifertility effect of fenugreek steroidal seed extract and ascorbic acid in female albino rats.

2. MATERIALS AND METHODS

Healthy adult female albino rats (*Rattus norvegicus*), each weighing between 150-180 gm were used for experiments. The animals were maintained under standard husbandry condition on a standard diet (Ashirwad Limited, Chandigarh) and water *ad libitum*. The animals were exposed to light 14h a day. The dried seeds of fenugreek were procured from market, powdered, weighed and used for extraction of phytodrug. The known amount of *Trigonella foenum graecum* (TF) seeds powder was hydrolyzed with 2N HCl (v/v) for 4h on water bath at low temperature (50° — 60°C). The mixture was neutralized with sodium bicarbonate (NaHCO₃) till neutrality (pH-7), washed with double distilled water and filtered. The residue was dried and soxhlet extracted with chloroform for 16h and dried in vacuum. The steroidal seed extract of seeds was fed orally to female rats that have mated. The experimental animals were divided into four groups. The group I - control received only vehicle, whereas II and III groups of rats received dose of steroidal extract of concentration 50mg and 100mg per day per rat respectively for successive 12 days. In group IV TF treated female rats were fed with ascorbic acid (100mg) for 12 days. On day 14th, animals were laprotomized under light by ether anesthesia and foetal implants in each horn of uterus of each animal were recorded. The incidence of absence of implantation was taken as antifertility index. Average of such implants in each group was also taken in to consideration. The results were analyzed statistically using student's 't' test.

3. RESULTS AND DISCUSSION

Post-coital (PC) contraceptive efficacy of *T. foenum-graecum* was carried out in female albino rats. The data revealed that the control uterine horns showed normal implantation and no sign of reabsorption of embryo. The average no. of implantation for rat was 9.66 ± 1.23 . The data indicate that 100% positive fertility rate was noted in control rats (Table 1, Plate 1, and Fig.1). The lower dose (50 mg) of *T. foenum-graecum* resulted in reduced implantation sites and reabsorption of embryos. The average number of implantation was found to be 5.66 ± 0.36 (Table 1, Plate 1 and Figs. 2-3) with reabsorbed embryos, leading to negative fertility. This may attribute to change in the internal environment of the uterus. The phytosteroid caused adverse effect on physiology of female reproduction making uterus hostile for development of embryo, inhibited fertility upto 50%. The number of litters was reduced as compared to control rats. This indicates that the phytodrug brought about antiimplantation and pregnancy interceptory effects in female rats. Badami et al. (2003) also reported antifertility activity of mixture of powdered roots of *Cassia occidentalis*, *Derris brevipes* variety *Coriacea* and *Justicia* simply in fertile female rats. The female rats treated with high dose of TF (100mg) and TF + ascorbic acid (100 mg) for 12 days PC, did not show any sign of resorption of embryos or implantation sites in any of the uterine horn, elucidate antiimplantation action of phytodrug revealing 100% negative fertility following fenugreek (100 mg) treatment (Table 1, Plate 1 and Fig. 4). This indicates that higher dose of plant extract was more effective in changing the internal milieu of the uterus, hence, no implantation sites was observed, therefore no development of embryo. The uterus was not conducive enough for the reception of fertilized ova. Therefore, phytosteroids brought about abortifacient effect in female albino rats. Similar postcoital antifertility activity of *Acalypha indica* L. was investigated by Hiremath et al. (1999) in female albino rats. Similar studies have been reported by Hiremath in 1995 of the plant *Striga desiflora* on female albino rats.

Antifertility effects of *Achyranthus aspera* on rats have been reported earlier in similar studies (Vasudeva et al., 2006). Dhanasekaran et al. (1993) also reported abortifacient activity of *Ailanthus excelsa* in rats. Carcia (papaya) seed extract produced antiimplantation activity in rats (Jacob et al. 1986, Singh and Singh (1992), Kesary et al. (1993). K. D. Rajan and colleagues (2014) have also reported antifertility effects of mangrove plant (*Avicennia alba*). Antiestrogens with weak estrogenic activity administered early in pregnancy may also interfere with implantation by altering the normal pattern of hormonal conditioning required for conception (Psychogos and Prepas, 1987). Antifertility effects of *Ficus religiosa* on goat uterus have been reported by A.K. Goyal (2014). Pattanayak and Majumdar (2006) studied antifertility effect of *Dendrophthoe falcata* extracts on female reproductive system in Wistar rats. However, ascorbic acid treatment along with Phytodrug, did not improve the fertility, but remained declined (Table 1, Plate 1 and Fig. 5). The inhibition of fertility cent percent indicates that ascorbic acid did not interfere with contraceptive efficacy of phytodrug. Ascorbic acid has been proved to be involved in maintaining normal physiology of rats without interfering with contraceptive efficacy of phytodrug as reported earlier (Sharma 1981, Bhinda and Sharma 2004, Jain 2005, Bhinda et al. 2005). The data suggests that higher dose (100 mg) of fenugreek is more effective in reducing fertility and can be used as emergency contraception in female rats.

Table 1: Post-coital contraceptive efficacy of *T. foenum-graecum* (TF, 50 and 100 mg) and TF + Ascorbic acid in female albino rats.

Group	Treatment	Mode of Administration	No. of rats used (6)	No. of implantation	Total No. of implantation	No. of reabsorbed embryo	Total number of reabsorbed embryo	Implantation per rat	Fertility (%)
I	Control (received vehicle for 12 days) PC	Oral	1	12	58	NIL	NIL	9.66 ± 1.23	100% +ve
			2	9		NIL			
			3	8		NIL			
			4	8		NIL			
			5	11		NIL			
			6	10		NIL			
II	<i>T. foenum-graecum</i> (50 mg/day/rat for 12 days) PC	Oral	1	5	17	2	50	5.66 ± 0.36	50% +ve
			2	NIL		16			
			3	NIL		15			
			4	8		3			
			5	4		6			
			6	NIL		8			
III	<i>T. foenum-graecum</i> (TF) (100 mg/day/rat for 12 days) PC	Oral	1	NIL	NIL	NIL	NIL	NIL	100% -ve
			2	NIL		NIL			
			3	NIL		NIL			
			4	NIL		NIL			
			5	NIL		NIL			
			6	NIL		NIL			
IV	TF + Ascorbic acid (100 mg/day/rat 12 days) PC	Oral	1	NIL	NIL	NIL	NIL	NIL	100% -ve
			2	NIL		NIL			
			3	NIL		NIL			
			4	NIL		NIL			
			5	NIL		NIL			
			6	NIL		NIL			

Values are mean ± S.E.; PC – Post-coital

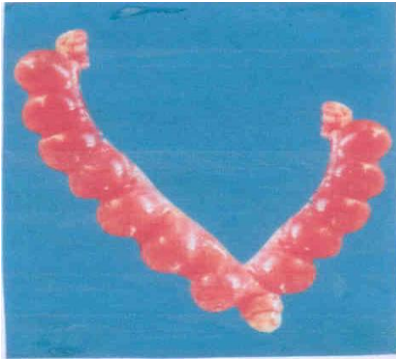


Figure 1: Photograph showing implantation sites of control rat. Note that both uterine horns contain healthy developing embryos.

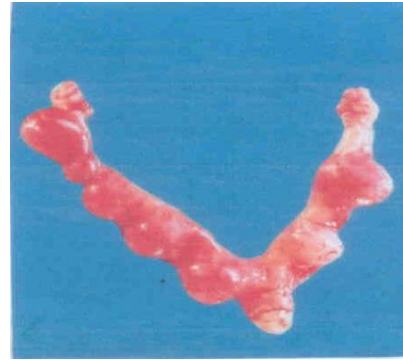


Figure 2: Photograph of *T. foenum-graecum* (50 mg/day for 12 days *pc*) treated rat uterine horns. Note resorption embryo and reduced number of implantation compared to control rat.

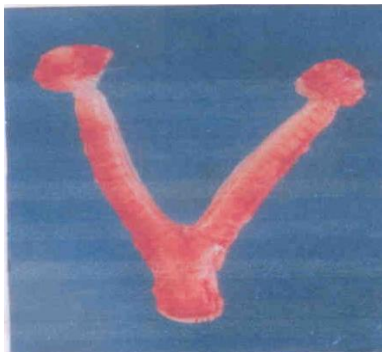


Figure 3: Photograph showing implantations in both uterine horns. Note abortifacient activity of drug.



Figure 5: Reproductive tract of female rats after *T. foenum-graecum* + Ascorbic Acid (100 mg/day/rat for 12 days *pc*) treatment

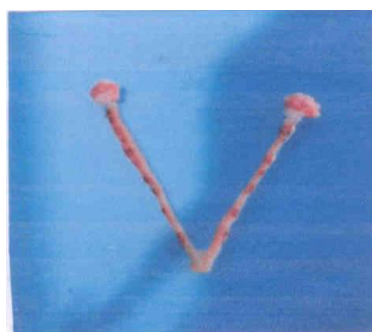


Figure 4: Photographs of female reproductive tract of rat uterus showing vascularization in uterine horns. Note absence of implantation and embryo.

4. CONCLUSION

It can be concluded from the above study that extracts of fenugreek seeds can be used as potential drug for abortifacient activity as postcoital contraceptive. Though more research is required on dosage optimization and their effects on human beings, it has been

proved in the study that fenugreek contains metabolites which serves as anti-implantation chemical which is plant based and some-day can replace use of regular chemical contraceptives in market.

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Fig.1

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