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Antifertility Activity of Steroidal Extract of *Trigonella foenum-graecum* (seeds) in Female Rats

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Abstract : Healthy adult female albino rats (*Rattus norvegicus*) were fed orally with steroidal extract of *Trigonella foenum-graecum* (100 mg/day/rat for 15 days). The data revealed that the body weights were not affected but the weights of ovary and uterus declined. The biochemical parameters *viz.*, protein, sialic acid, glycogen and ascorbic acid were reduced in ovary and uterus, however the concentration of cholesterol was increased in ovary and uterus after fenugreek treatment. The enzyme activity of acid and alkaline phosphatase of ovary and uterus got reduced. The vaginal smears examined daily during the treatment should that female rats were mostly either in metoestrus or diestrus stage. The fertility test was 100 % negative following fenugeek treatment. The data suggests that *T. foenum-graecum* seeds extract exerts antiestrogenic and antifertility activity in female rats.

Introduction :

Fenugreek (*Trigonella foenum-graecum*) is considered to be a rich source of steroidal sapogenins (Hardman, 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.*,1994a, 1994b). Dhawan *et al.* (1977) reported spermicidal activity of fenugreek in albino rats. Preliminary studies carried out from this laboratory on albino rats revealed that steroidal seed extract of fenugreek brought about anti-implantation effects in rats (Sharma and Kamal, 1992). Information on antifertility aspect of fenugreek in female rats is scanty. Therefore, the present work the effects of fenugreek steroidal seeds extract on fertility is an attempt to in vestigate on of female albino rats.

Materials and methods

Healthy, adult female albino rats (*Rattus norvegicus*), each weighing between 150-180 gm were used for the experiments. The animals were maintained under standard husbandary conditions on a standard diet

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(Hindustan Lever Ltd., Bombay) and water *ad libitum*. The animals were exposed to 14 day light hours. The dried seeds of fenugreek were procured from market, powdered weighed and used for extraction. The powder of fenugreek seeds were hydrolysed with 2N HCl for 4h on water bath. The residue was dried and soxhlet, extracted with chloroform with 16h. The animals were divided into two groups control and treated, containing ten animals in each group. The control animals received only vehicle, whereas the treated group was fed orally with steroidal seed extract of fenugreek (100 mg/day/rat for 15 days). The vaginal smears were checked daily during the experimentation. After completion of the treatment half of the animals were kept for fertility test. Treated female rats were caged with one normal male of proven fertility. Mating was confirmed next day either by observing vaginal plug or by checking the vaginal smear for the presence of spermatazoa. Female rats showing positive mating test were kept separatly for 22 days at laboratory conditions (The number of females delivered was recorded. Fertility test was taken as positive if the females delivered else it was taken as negative). The remaining half of the animals were autosied on day 16th and reproductive tissues (ovary, uterus) excised, blotted free of blood, weighed and used for tissue biochemistry. The following parameters were studied: Protein (Lowery, *et al*.,1951), glycogen (Montogomery, 1957), sialic acid (Warran, 1959), cholesterol (Zlatkis, *et al;* 1953), ascorbic acid (Roe and Kuether,1943), acid phosphatase and alkaline phosphatase (Oser, 1979). Minimum of six replicates were analysed for each tissue and parameter. The results were anlyzed statistically using Student's **'t'** test.

Result :

The data revealed that the body weights of rats were not found altered but the weights of reproductive organs declined following treatment as compared to control rats. A significant reduction was observed in weights of ovary ($P<0.001$) and uterus ($P<0.001$) (Table 1).

The oestrus cycle of control rats showed normal oestrus cycle whereas the treated rats remained either in metoestrus or diestrus stage

during the experimentation. Fenugreek treatment for 15 days resulted in 100 % negarive fertility rate (Table 1).

Table 1 : Body and organ weights, oesterus cycle and fertility rate of control and *T. foenum-graecum* (100 mg/day/rat for 15 days) treated rats.

Parameters		Control	*T. foenum-gracecum*
Body weights (gm)	**Initial**	163.05 ± 2.68	152.88 ± 2.75
	Final	172.55 ± 2.14	169.66 ± 2.46
Organ weight (mg/gm b wt)	**Ovary**	23.44 ± 0.21	13.66[a] ± 0.64
	Uterus	93.88 ± 1.63	75.08[a] ± 3.38
Oestrus cycle		Regular oestrus cycle	Meta-oestres or diestrus
Fertility rate %		90-100% +ve	100% -ve

Values are Mean ± S.E.
a = P < 0.001

The concentration of protein, glycogen and siaclic acid also declined significantly following fenugreek treatment for 15 days in ovary (P<0.001) and uterus (P<0.001). However, the cholesterol content of ovary and uterus increased after *T. foenum-graecum* treatment. The significant increase was observed in ovary (P<0.01) and uterus (P<0.001) (Table 2).

The ascorbic acid concentration also declined significantly in ovary (P<0.001) and uterus (P<0.001) after fenugreek treatment. The enzyme activity of acid and alkaline phosphatase got reduced following fenugreek treatment. However, the reduction was significant in ovary (P<0.001) in both the enzymes studied, whereas in uterus the acid phosphatase declined significantaly (P<0.001) and alkaline phosphatase enzyme activity reduced at P<0.05 level as compared to those of control rats (Table 2).

Table-2 : Protein, glycogen, sialic acid, cholesterol, ascorbic acid concentration and enzyme activty of acid and alkaline phosphatase of control and *T. foenum-graecum* (100 mg/day/rat for 15 days) treated rats.

Parameters	Tissue	Control	*T. foenum-graecum*
Protein (mg/g)	**Ovary**	219.48 ± 2.10	199.02^{a} ± 2.54
	Uterus	207.24 ± 2.10	182.50^{a} ± 1.28
Glycogen (mg/g)	**Ovary**	5.59 ± 0.21	3.76^{a} ± 0.23
	Uterus	7.07 ± 0.23	3.54^{a} ± 0.21
Sialic acid (mg/g)	**Ovary**	0.964 ± 0.01	0.744^{a} ± 0.01
	Uterus	0.963 ± 0.01	0.770^{a} ± 0.01
Cholesterol (mg/g)	**Ovary**	8.84 ± 0.55	10.91^{b} ± 0.33
	Uterus	4.15 ± 0.18	7.07^{a} ± 0.19
Ascorbic acid (mg/g)	**Ovary**	12.45 ± 0.64	8.04^{a} ± 0.68
	Uterus	17.22 ± 1.22	6.26^{a} ± 0.31
Acid Phosphatase (mg^{pi}/g/h)	**Ovary**	4.87 ± 0.62	2.14^{a} ± 0.24
	Uterus	3.98 ± 0.32	2.13^{a} ± 0.28
Alkaline phosphatase (mg^{pi}/g/h)	**Ovary**	4.21 ± 0.23	2.52^{a} ± 0.19
	Uterus	7.01 ± 0.42	5.82^{c} ± 0.39

Values are Mean ± S.E. a = $P < 0.001$, b = $P < 0.01$, c = $P < 0.05$

Discussion :

The data revealed that oral administration of steroidal fraction of fenugreek seed extract to female rats for fifteen days brought about a decrease in the weights of reproductive organs, indicating that the level of estrogen was not enough to maintain the weights of reproductive organs.

The structural and functional integrity of reproductive tissues depend on the circulating level of estrogen and therefore any small change in estrogen level may result in reduction in the weights of the reproductive organs. 100% negative fertility rate could achieved which may be attributed to anoestrus condition of female rats. Anoestrus vaginal smear appear to be due to the absence or decrease of circulating gonadotrophins (Behrman *and* Armstrong,1969).

The decrease in most of the estrogen dependent parameters of female reproductive organs revealed that the internal phsiology of female reproductive organ is disturbed because of the insufficient level of circulating estrogen which is essential for maintainence of their physiology integrity (Chatterjee, 1995; Rang *et al.*, 1999; Tamooki and Pincus, 1961; Guillemin and Sakiz, 1961).

Cholesterol is the precursor of sex hormones and is utilised during steroidogenesis. During the investigation the cholesterol concentration of ovary and uterus increased after fenee greek treatment, indicating non-utilization of cholesterol by the system. Hence reduced level of circulating estrogen contributes to altered physiology of female reproductive system. Thus, the present investigation suggests that steroidal fraction of fenugreek seeds extract exerts antifertility and antiestrogenic activity in female rats.

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