ORIGINAL ARTICLE

Effect of trigonelline on fertility in female rats

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Antidiabetic activity of trigonelline and antifertility activity of fenugreek seeds have been reported. However, the effect of trigonelline on fertility is not reported. The aim of the study was to determine the estrogenic activity of trigonelline (75 mg/kg) as well as its effect on fertility in rats. The estrogenic activity was assessed by the vaginal cornification method. Trigonelline was administered on days 1–7 of pregnancy. The animals were anesthetized and laprotomy was carried out on day 10. The number of implantation sites was counted. The wound was sutured and animals were allowed to recover. The number of pups born after 21 days was counted. Trigonelline was safe up to dose of 5000 mg/kg orally. Trigonelline (75 mg/kg) did not alter the estrous cycle of rats. Nonsignificant difference in the number of implants and pups born was observed in the trigonelline group compared to the control group. The lack of abortifacient activity or teratogenicity confirms the safety of trigonelline during pregnancy in rats.

Key words: Trigonelline, implantation, antidiabetic

INTRODUCTION

Trigonella foenum graceum Linn., of family Leguminosae (TF) also known as Fenugreek, is an aromatic annual plant, 30–60 cm tall, found wild in Kashmir, Punjab, and the upper Gangetic plane and widely cultivated in many parts of India. Fenugreek has been used as cooking spice and flavoring agent for centuries.[1] It is used as an abortifacient,[2] antispasmodic, externally for abscesses, boils, galactagogue[3] appetite stimulant, blood cleansing, laxative, tonic[4]demulcent, emollient, expectorant, aphrodisiac[5]. The defatted seeds are rich source of steroids.[6] However, studies on fenugreek seeds[7] and its extract[8] have been reported to have antifertility activity in male and female rabbits.

Trigonelline is a major alkaloid present in fenugreek seeds and is reported to possess hypoglycemic effect.[9] Therefore, the seeds are widely recommended for type 2 diabetes patients. Fenugreek is thought to delay gastric emptying, slow carbohydrate absorption, and inhibits glucose transport. It has been shown to increase erythrocyte insulin receptors and improve peripheral glucose utilization, thus showing potential pancreatic as well as extra pancreatic effects. Some studies showed that trigonelline may exert hypoglycemic effect in healthy nondiabetic volunteers,[1] while others revealed no effect on fasting or postprandial blood glucose in nondiabetic patients.[10] The addition of trigonella seeds to the diet of diabetic patients was practiced by many Yemenites in Israel, who called it as "Chilbe".[9] Mishkinsky *et al.* (1967)[9] proved that trigonelline had some hypoglycemic effect on both alloxan-induced diabetic rats and diabetic patients. He also had stated that trigonelline did not inhibit the hyperglycemic effect of cortisone in rabbits.

As fenugreek seeds are contraindicated in pregnant female due to its abortifacient activity and trigonelline isolated from its seeds is used as an antidiabetic compound; our objective was to evaluate the effect of trigonelline (in a dose showing antidiabetic activity) isolated from fenugreek seeds on the estrous cycle in nonpregnant female rats and on pregnancy in pregnant female Wistar rats.

MATERIAL AND METHOD

Method of Extraction of Trigonelline

A 100 g of fenugreek seeds sourced from Indore, Madhya Pradesh, India, was flaked and defatted using 300 ml hexane followed by extraction carried out using aqueous alcohol (70:30, ethanol: water) 500 ml at room temperature for 8 hours of circulations. The extract was filtered and concentrated under vacuum to yield 9 g of a crude drug. The crude drug was dissolved in 500 ml deionized water and passed through strong acid cation exchange resin (100 ml) (T-42 M.P from Thermax, India). The resin bed was washed free of colors. The adsorbed compounds were eluted and showed the presence of amino acids major 4-hydroxy isoleucine and trigonelline (4HIT) with minor saponins. The solution was concentrated at 45°C in Buchii rota evaporator to give 900 mg of mixture. This mixture was dissolved in 50 ml of hot isopropyl alcohol followed by HCl

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gas saturation in the solution at 35°C. On cooling to –5°C, trigonelline hydrochloride was separated and filtered out (200 mg of trigonelline).

Chemicals

Anesthetic ether (TKM Pharma, Hyderabad, India) was purchased. Trigonelline was isolated and is patented by Indus Biotech, India.

Animals

Female Wistar rats of weight 150 ± 5 g and immature female Wistar rats weighing 55–60 g, were purchased from National Toxicology Centre, Pune, India, and used for the study. They were maintained at a temperature of 25 ± 1°C and relative humidity of 45–55% under 12:12-hour light–dark cycle. The animals had free access to food pellets (Chakan Oil Mills, Pune, India) and water was given ad libitum. The experimental protocol was approved by the Institutional Animal Ethics Committee (IAEC) of Poona College of Pharmacy, Pune, India, constituted under Committee for the Purpose of Control and Supervision of Experiment on Animals (CPCSEA).

Effect on Estrous Cycle-Estrogenic Activity

Effect of trigonelline (75 mg/kg p.o.) on vaginal cornification

The method described by the Vogel and Vogel, (2002)[11] was used. 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 three groups containing six animals per group. Group I, control group, was administered vehicle (distilled water), Group II was given trigonelline (75 mg/kg p.o.), and in Group III estradiol in olive oil was injected subcutaneously (0.5 μg/animal). Trigonelline was administered twice daily on two following days at 10:00 a.m. and 5:00 p.m. and once at 5:00 p.m. of the third day and at 10:00 a.m. on the fourth day. On fourth day, the smears were prepared on a glass slide and stained for 10 min with 5% aqueous methylene blue solution. The smears were examined microscopically and scored according to the following guidelines:

0: Metsestrous—the presence of mixture of leukocytes and epithelial cells.
1: Diestrus phase—mainly leukocytes with few epithelial cells.
2: Proestrus phase—the presence of nucleated or nucleated plus cornified cells.
3: Estrus phase—the presence of cornified cells only.

Only animals showing score 2 or 3 were considered to be positive for estrogenic activity.

Pregnancy Model: Effect of Trigonelline (75 mg/kg p.o.) on Pregnancy in Rats

Estrous cycle in female rats

Female Wistar rats, weighing 150–200 g were used. The animals were housed in group of four animals per cage. Every morning between 8:00 and 9:00 a.m., vaginal fluids were collected by inserting the tip of dropper filled with 1–2 ml of normal saline (NaCl 0.9%) into the rat vagina. A drop of vaginal fluid was smeared on the slide. An unstained vaginal smear was observed under light microscope, with 10x and 40x objective lenses. Three types of cells could be recognized: round and nucleated as epithelial cells; irregular ones without nucleus as cornified cells; and the little round as leukocytes. The proportion among them was used for the determination of the estrous cycle phases.[12]

Abortive activity of trigonelline (75 mg/kg p.o.)

Adult female rats of proven fertility weighing between 150 and 200 g were selected. Vaginal smear of female rats were examined daily. The rats in the proestrous phase of the estrous cycle were housed overnight for mating with adult male rats of known fertility. Vaginal smears of these female rats were examined the following morning for evidence of copulation; the presence of thick clump of spermatozoa in vaginal smear indicated pregnancy. The day on which spermatozoa were observed in the vaginal smear was designated as day 1 of pregnancy. The pregnant rats were separated out. The pregnant rats were randomly divided into four groups containing six animals per group. Trigonelline (75 mg/kg) was administered orally to the rats. The control group of animal received the vehicle (distilled water). The period of trigonelline/vehicle administration was from day 1 to 7 of pregnancy.

The animals were laparotomized under light ether anesthesia on day 10 of pregnancy. Implantation sites on both horns of uterus were recorded. The abdominal wounds were sutured layer by layer and animals were allowed to go term. After delivery, numbers of pups born were noted.[13-15]

Statistical analysis

Data for each of the parameter were analyzed by column statistics followed by paired *post hoc t*-test using Graph Pad, Prism software, version 4.03.

RESULTS

Acute Toxicity Studies

In acute oral toxicity studies, no mortality and changes in the behavior were observed in all treated and control groups of the mice up to the dose of 5000 mg/kg.

Effect of Trigonelline (75 mg/kg p.o.) on Vaginal Cornification

For the control group and estradiol (0.5 μg/animal)-treated rats showed estrous score 0 and 3, respectively. Oral

administration of trigonelline (75 mg/kg p.o.) showed estrous score 1. The score was given according to maximum numbers of animals in a given phase [Table 1].

Thus oral administration of trigonelline (75 mg/kg p.o.) to immature female rats did not show cornified and nucleated epithelial cells. Trigonelline does not exhibit estrogenic activity.

Effect of Trigonelline (75 mg/kg p.o.) on Pregnancy in Rats

The mean number of implants in trigonelline (75 mg/kg p.o.) treated rats was 9 ± 0.51. On the other hand, total number of implants was 8.83 ± 0.83 in the control group. The mean number of litters delivered in trigonelline group was 8.66 ± 0.80 while that in the control group was 8.33 ± 0.79. The average weight of pups in control and trigonelline (75 mg/kg p.o.) groups was 0.65 g and 0.66 g, respectively [Table 2].

DISCUSSION

Fenugreek is considered as a rich source of steroids. It is contraindicated in pregnancy due to its abortifacient activity. Chemical analysis of the seeds revealed the presence of anti-nutritional factors such as saponins[16] and alkaloids.[17] Feeding of fenugreek seeds to rats even upto 20% level has shown to have no toxic effects.[18]

Table 1: Effect on Estrous cycle

Group	Score
Control (n = 6)	0
Estradiol (0.5 μg/animal) (n = 6)	3
Trigonelline (75 mg/kgp.o) (n = 6)	1

Scores here are showing the estrous phase of group of animals. Score is given here according to maximum numbers of animals in a given phase at given time.

Table 2: Effect trigonelline (75 mg/kg p.o.) on no. of implants, no. of litters delivered, and wt. of litters born (g)

Animal no.	Treatment	No. of implants	No. of pups delivered	Wt. of litters (g)
1	Control	9	9	0.6
2		10	10	0.7
3		5	5	0.7
4		9	9	0.7
5		11	10	0.6
6		9	10	0.6
Mean ± SEM		8.83 ± 0.83	8.833 ± 0.79	0.66 ± 0.22
1	Trigonelline	10	10	0.6
2	(75 mg/kg	7	5	0.7
3	p.o)	8	8	0.6
4		9	9	0.7
5		10	10	0.7
6		10	10	0.7
Mean ± SEM		9.0 ± 0.51^{ns}	8.667 ± 0.80^{ns}	0.65 ± 0.22^{ns}

Data represented are expressed as mean ± SEM in female rats (n = 6) analyzed by column statistics followed by ***post hoc*** paired ***t***-test. ns, nonsignificant as compared to the control group.

In the present study, trigonelline (75 mg/kg p.o.) when fed to pregnant and nonpregnant female rats showed no deleterious effects in animals. The pregnant animals in the trigonelline (75 mg/kg p.o.) group showed nine implants and delivered mean 8.66 litters. There was no significant reduction in number of implants as well as numbers of litters as compared to that of control animals. The litters exhibited normal growth and survived. The dose of trigonelline employed in this study was 75 mg/kg p.o. which was equated to reported antidiabetic dose of trigonelline.[19]

Thus, it can be concluded that trigonelline (75 mg/kg p.o.) does not affect vaginal cornification and exhibit no abortifacient activity when given to pregnant female rats. Further studies are required to evaluate the effect in pregnant diabetic rats.

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