Contraceptive Hypogonadotropic-Hyperprolactinemic Effect of Fenugreek Seeds, Animal Rat Model

Belqees A. Allaw[1*], Ahmed Kaid N. Allow[2], Bracamonte Maryam[3], Norbaiyah M Bakrim[4], Ayah Hellis[5], and Imad Al-Deen Mustafa[6].

[1]MSc Anatomy, preclinical department, Faculty of Medicine Widad University Colleague, Malaysia.
[2]PhD. MSc. Prof, Infertility consultant, Department of Physiology, Sana'a University; and Alaw Fertility Center Sana'a Yemen.
[3]MSc. Physiology, Physiology Dept, Faculty of Medicine and Health Sciences, Sana'a University, Yemen.
[4-5] MSc Physiology BMS. KOM, IIUM, Malaysia.
[6]PhD, Professor of Microbiology, BMS. KOM, IIUM, Malaysia.

***Corresponding Author*: belqees@widad.edu.my**

ABSTRACT

Seventy-two healthy mature Sprague Dawley female rats were used in this study to evaluate the effect of fenugreek aqueous extract (FSA) on the level of their reproductive hormones namely estrogen, progesterone, folliculo-stimulating, luteinizing and prolactin hormones. The effect of extract into the estrous cycle was evaluated. The rats were divided into 4 groups, 18 each. The first group was the control (CG), second was (SG1) given FSA 500 mg/kg/day and the third was (SG2) given FSA 750 mg/kg/day and the fourth was treated (SG3) with 1000 mg/kg/day) of FSA. The duration of this FSA treatment was 21 days. The results showed significant reduction in the estrogen, progesterone, LH and FSH and increment in the prolactin ($P<0.001$). In conclusion, treatment with FSA causes hyperprolactinemia and significant reduction in the estrogen, progesterone, FSH and LH levels in female rats.

Key words: Estrogen; Fenugreek Seed Extract; Hyperprolactinemia; Prolactin

CLINICAL IMPLICATIONS

We are investigating an alternative oral contraceptive plant product prepared from fenugreek seeds that might prove to be safer with fewer unwanted side effects than the hormonal-dependent oral contraceptive pills currently available in the pharmacies.

1. INTRODUCTION

Traditional medicine has evolved hand in hand with the development of human societies since ancient times. Plants and their products have been advantageously used as elements for traditional medicine. A plant with medical use is considered an effective constituents that is used for prevention or treatment of the body disorders. Nowadays the importance of phytochemicals which are mostly secondary plant metabolites being noticeable as scientists of different countries which are trying to recognize and study the properties of plants that can be enrolled in the medicine (Abdillahi HS, 2013). Fenugreek (*Trigonella foenum-graecum L.*) belongs to the *Leguminosae* family. It is an annual plant that is native to the Mediterranean region but currently has been widely cultivated in India, North Africa and Yemen. In Yemen-Rada'a-Furgan-Sabah fenugreek is known as Hulbah (Bukhari SB BM, 2008), while in Malay community it is called Halba. The seeds that mature in long pods, described as brown-greyish in colour with a sharp bitter taste, are the most common part of the plant used for various purposes (Allow A, 2016). Seeds of fenugreek have a lot of components which can be mentioned some of them oils, essence, alkaloids (Salah I Kheder, 2012), flavonoids, saponin, sapogenin (Allow A, 2016), mucilage, etc (Modaresi M, 2012).

Three putative bioactive compounds have been examined earlier; i.e., galactomannan, diosgenin (Abdillahi HS, 2013) (Allow A, 2016) sand 4-hydroxyisoleucine. These compounds are among the most frequently identified in the literature for their medicinal and health characteristics (Acharya S, 2006). Fenugreek has many pharmacological effects such as anti-diabetic and anti-hypercholesteremic properties. HDL showed no changes in its level while there significant reduction was observed in total cholesterol, LDL and triglyceride levels. Flavonoids of fenugreek seeds have known anti-oxidant activities (Bukhari SB BM, 2008). However, the physiological effects of different doses of fenugreek aqueous extract on the female reproductive hormones are not well established. Thus, the objective of the present study is to investigate the

effect of fenugreek seeds aqueous extract on the reproductive hormones and estrous cycle in the female rats.

2. MATERIALS AND METHODS

2.1 Animals

Seventy-two healthy mature Sprague Dawley female rats weighing 170-200 grams were involved in the present research work. The rats were kept under controlled environmental conditions such as a room temperature that was maintained at about 24^{o}C (±2) and exposed to 12 hour/day light program. The rats were housed in plastic cages “three rats per cage” and had free access to laboratory chow and water.

2.2 Experimental Design

The female rats were randomly divided into four groups of 18 rats/each. The first is control group (CG) was given normal diet plus plain water; the second group (first study group-SG1) was given 500 mg/kg FSA extract; the second studied group (SG2) was given 750 mg/kg FSA extract and fourth group (SG3) was given 1000 mg/kg FSA extract. The FSA extract was given daily for 21 days orally. Blood samples were collected from the animals by retro orbital puncture technique. Vaginal smear cytology was performed on each rat prior to blood collection to determine the phase of estrous cycle. The blood was collected only when the animals were in the pro-estrus phase. The rats were anesthetized with the inhalation anesthetic ethyl ether. As soon as the rat was removed from the inhalation chamber, unconscious animal was rested on a flat surface, and held gently but firmly by the nape of the neck. After about 3 ml of blood was collected, the nape of the neck was released slowly prior to withdrawal of the hematocrit tube to minimize hemorrhage from the puncture site. The animals were then placed into a separate recovery cage, observed for 20 minutes to ensure a safe recovery. Blood collected from each animal was divided into two tubes. One tube for assessment of FSH, LH, progesterone and estradiol and the second was for assessment of prolactin.

2.3 Hormonal Assays

The assays of reproductive hormones; FSH, LH and prolactin were performed by using immune radiometric (IRMA) assay kits (Beckman, Czech Republic). The assay procedure was done at the laboratory of Veterinary Faculty, University Putra Malaysia (UPM) in Selangor, Malaysia. The frozen serum was packed in an ice box for the three hours journey from Kuantan to UPM. The IRMA assay involved three main steps. Firstly, 100 uL of calibrator, control or test sample and 50 µL of tracer were added into the antibody coated tubes. Secondly, the mixtures were incubated for 90 minutes on a shaker incubator (Memmert, M65800, USA) at 200 rpm. The contents of the tubes were then aspirated carefully and the tubes washed twice with 2 ml of wash solution. Finally, the bound and total tracer radioactivity was counted for 1 minute using a five-detector gamma counter machine (Wallac Wizard, Model 1470, Finland). The hormonal assays of the three reproductive hormones: prolactin, estrogen and progesterone were performed using Enzyme Linked Immunosorbent Assay (ELISA) kit for rat hormones catalog number E-EL-R0391 from Elabscience,Mutiara Saintifik, Malaysia.

2.4 STATISTICAL ANALYSIS

The data from this study were analysed using Statistical Package for the Social Sciences IBM SPSS software, version 20.0. Statistically significant level was taken to be *p* value less than 0.05.

3 RESULTS

Following the administration of FSA extract the serum levels of estrogen and progesterone were significantly decreased in all studied groups compared to control group. The highest statistically significant ($p<0.03$) decrement of both hormones was obtained in both SG2 and SG3, which given 750 and 1000 mg/kg respectively, as compared to the control (Figure 1).

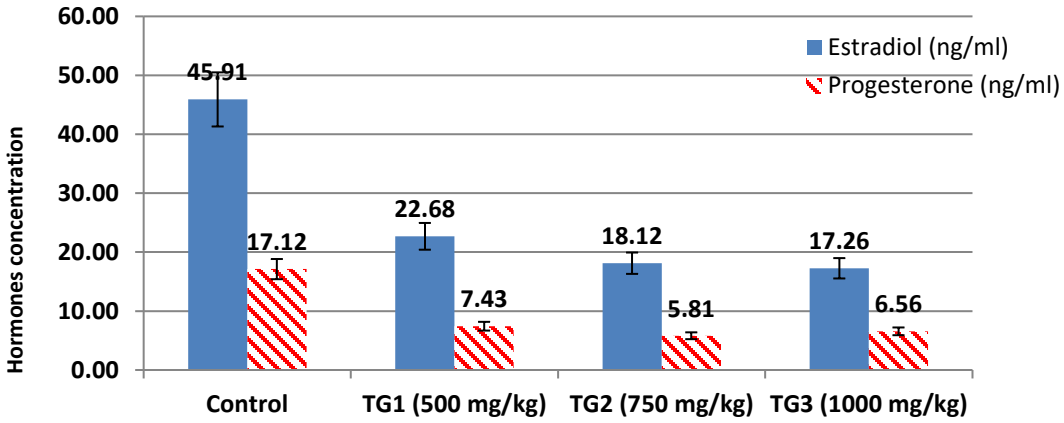

Figure 1: The effect of FSA extract on the concentration of estradiol and progesterone of female rats.

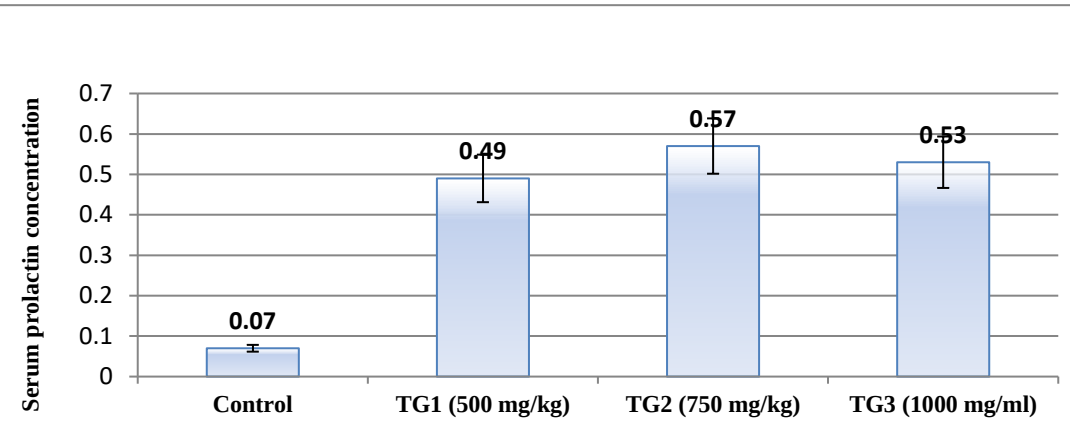

Figure 2: The effect of FSA extract on the serum prolactin level.

The results of the present study showed a statistically significant ($P<0.001$) increment in the serum prolactin level in all studded groups with FSA extract compared to control (Figure 2). No significant differences ($p=0.068$) were detected between the 500, 750 and 1000 mg/kg studied groups (Figure 2).

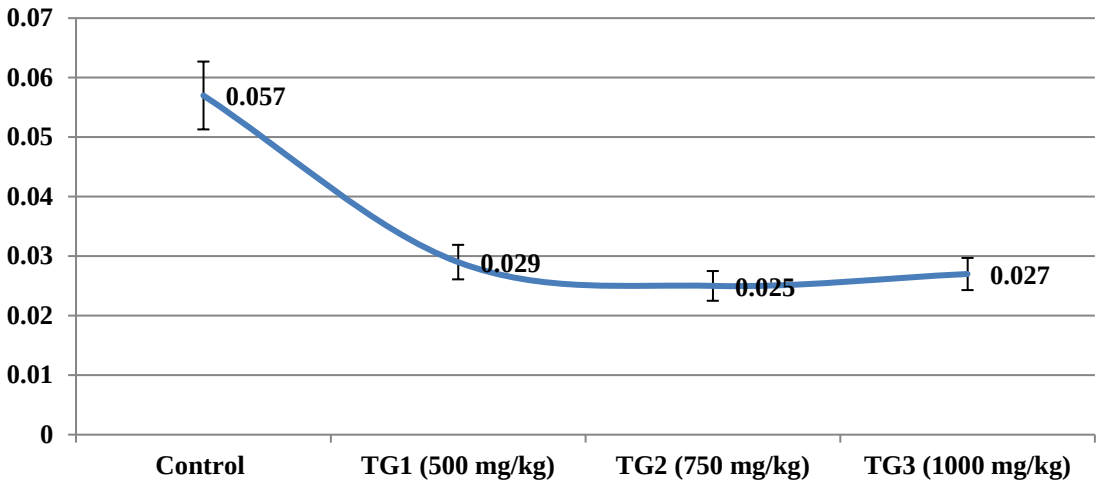

Figure 3: The effect of fenugreek seeds aqueous extract on the serum LH level.

Figures 3 and 4 show the effect of FSA extract on the serum levels of FSH and LH. Both hormones showed significant (P< 0.004) decrement in all studied groups with FSA extract as compared to the control (figure 3 and 4).

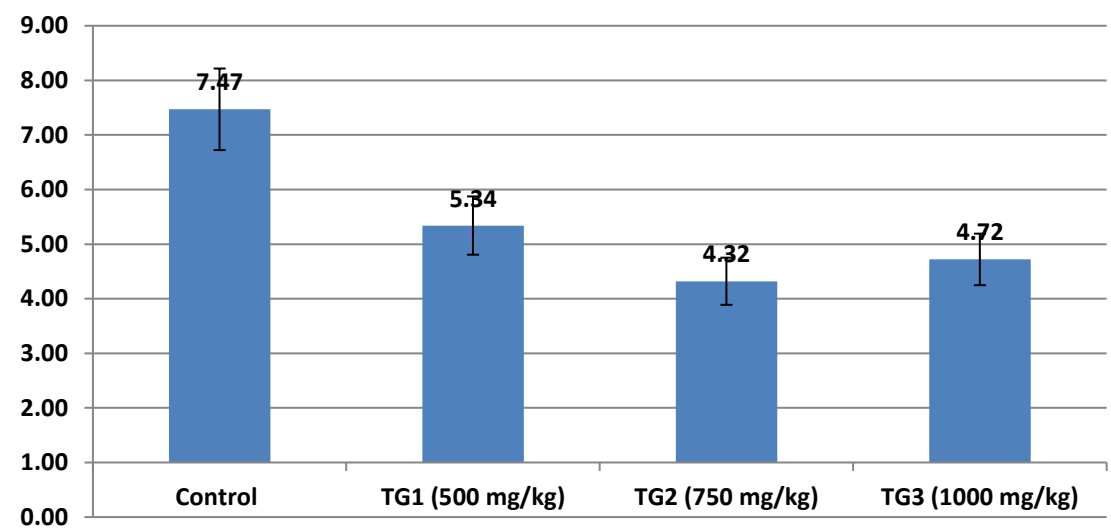

Figure 4: The effect of FSA extract on the serum FSH concentration.

4 DISCUSSION

The blood samples from all animals had been taken in the pro-estrus phase (Allow Ahmed Kaid, 2016). It is a short stage, normally occurring the day before estrus so it might be missed if smears are taken early in the morning. The decline in level of progesterone in this phase, which is released by the inhibition of LH secretion, allows pro-estrus phase to ensue. By increase in the size of ovarian follicle, the concentration of serum estradiol level will increase, too. This increment in the serum estradiol level will cause LH surge which will enhance the local ovarian enzymes to cause ovulation (Ouzir M, 2016). The high level of estrogen triggers LH surge that promotes ovulation (Bogle OA, 2012). Female rats are considered to be receptive for mating during pro-estrus as ovulation normally occurs at the end of pro-estrus (Cruz ME, 2015).

The effect of FSA-extract oral administration on the reproductive hormonal levels of experimental rats involved in the present study showed a significant reduction in the levels of estrogen and progesterone in all treated groups with FSA-extract (500, 750 and 1000 mg/kg/day FSA-extract). This reduction was more pronounced in the TG2 and TG3 (groups given 750 and 1000 mg/kg/day FSA-extract respectively). It was previously reported that a decrement in the estrogen level is associated with failure in the development of Graafian follicles (Kaya S, 2017). Medaresi and co-workers in 2012 also reported that a decrement in the number of Graafian follicles was associated with a decrement in the estrogen concentration in mice give fenugreek (Modaresi M, 2012).

Presence of diosgenin in the fenugreek seeds is thought to suppress the endogenous estrogen leading to decreased estrogen level in the serum of experimental animals (Hilles AR, 2016). Reduction in the serum concentration of estrogen and progesterone after 15 days of FSA extract treatment indicates disturbance in synthesis of these hormones (Allow A, 2016). The low concentrations of estrogen and progesterone in the treated animals as shown in the present study is consistent with the suggestion by Elseed et al., (2013) that saponin in the fenugreek is able to inhibit the expression of genes responsible for steroidogenesis (Elseed AF, 2013).

Fenugreek seeds aqueous extract was reported in the present study to cause an increment in the serum prolactin level (hyperprolactinemia) in all treated female rats (TG1, TG2 and TG3). This increment in the prolactin level is in good agreement with the data published by Modaresi et.al

2012, (Modaresi M, 2012). Increment in the prolactin level is probably via affecting gonadotrophin releasing hormone production and release causing reduction in luteinizing hormone production (Araujo-Lopes R, 2014).

Decrement in the level of serum FSH and LH in all treated groups of the present work is in a good agreement with reported results of Modaresi and his co-workers in 2012 (Modaresi M, 2012) (Allow A, 2016) (Salah I Kheder, 2012). LH stimulates theca cells in the ovarian follicle to convert cholesterol into androgen, while FSH stimulates granulose cells to convert androgen into estrogen (Dewailly D, 2016). LH also stimulates the synthesis of progesterone from cholesterol by both the large and small luteal cells in the corpus luteum. Therefore, the reduction in serum concentration of LH and FSH as demonstrated in the present study also contributes to the poor synthesis of estrogen and progesterone by ovarian tissues of treated animals. Even though there was only a minimal reduction of serum concentration of FSH and LH in the FSA treated animals and it was not significantly different from the control group, these minor changes were still sufficient to disrupt steroidogenesis in the target cells.

In conclusion, administration of FSA-extract to the experimental female rats caused significant reduction (contraceptive effect) in all circulating reproductive hormones including FSH, LH, estrogen and progesterone (hypogonadotropic effect) as well as causing hyperprolactinemia (hyperprolactinemic effect). This hyperprolactinemia probably has similar effects to the known physiological-lactation-hyperprolactinemia.

5 CONFLICT OF INTEREST

The authors have declared no conflict of interest.

6 ANIMAL ETHICAL APPROVAL

The proposal of the present work was taken from the animal ethical committee of the IIUM Kuantan campus.

7 ACKNOWLEDGMENT

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