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Original Research Article

EFFECT OF FENUGREEK SEEDS ON RAT'S OVARY: HISTOLOGICAL STUDY

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ABSTRACT

Background and objective: The Fenugreek seeds effect on vagina rat's estrous andits galactogogualactivity were suggested to their estrogen and/or progesterone like action. Accordingly, this study is concerning with the role of different doses of Fenugreek seeds, that may play, on mature,perimature immature rat's ovary.

Materials and Methords: A total of 135, healthy virgin female Norway albino rats were used for the study. Fenugreek seeds were administrated by oro-gastric tube to the experimental rats. The rats were divided according to their age into three groups, namely, mature,perimature and immature. Each group was subdivided into subgroups according to the dose of Fenugreek seeds (0.8, 1.6 and 3.2 mg/g bod weight) used. Ovaries of these rats were processed for histological study.

Results: This study showed an increase on ovarian size of all experimental subgroups, compared with control groups. Histological study of the ovaries revealed marked increase in the mean number of all types of growing follicles (folliculogenisis) and the total structures in the ovaries. The increase in the thickness of theca interna was prominentin all experimental subgroups.

Conclusion: The data obtained from this study elicit that crude Fenugreek seeds possess estrogen like action which can be performed by their direct action or through diosgenin biotransformation to estrogen inside the ovary. This estrogen enhance folliculogenisis indirectly. The histological findings of mature, Perimature and immature rats treated with Fenugreek seeds was nearly identical, irrespective of the doses used in this study.

KEY WORDS: Fenugreek Seeds, Ovary, Histology, Estrogen And Progesterone Hormones.

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INTRODUCTION

Fenugreek (Trigonell a Foenum-graecum L) is an annual herb of Leguminosae family. The effective medicinal part of the plant is their dried ripe seeds which possess estrogen and, or progesterone like effect [1]. Fenugreek seeds contain several possible active chemical constituents such as steroid saponins (C_{27}-steroidal sapogenin-peptide ester), on acid hydrolysis; it contains diosgenin, alkaloids, flavonoids, tannins, amino acids and trigonilline [2,3]. Literature review for the effect of Fenugreek seeds reveled that under various experimental circumstances, it possessgalacto-gogual activity [4], hypolipidemia, hypoglyce-mic[5,6,7]anti-inflammatory [8] and an antifer-tility agent [9]. It is also used for treatment of wounds, gastrointestinal ailments [8] and can reduce the severity of dysmenorrhea [10].

In rats, the ovaries are lentiform, lie on each side of bicornuate uterus in relation to the lateral abdominal wall. Ovaries mainly produce haploid female gametes (oogenesis) and steroid hormones [11]. The ovary has peripheral greater cortex and narrow central medulla. The ovarian surface is covered by germinal epithelium under which there is tunica albuginea. The cortex is formed by numerous follicles ofdifferent stage embedded instroma of connective tissue contains stromal cells, collagenous and reticular fibers (primary interstitial tissue).The medulla consists of typical dense,irregular connective tissue in which are present rich vascular bed [12]. Ovarian follicles are:-Primordial follicle (primary oocyte surrounded by a single layer of flattened follicular cells); Primary follicle (primary oocyte surrounded by a single layer of cuboidal cells); Secondary follicle (primary oocyte surrounded by more than one layer of granulosa cells); Tertiary follicle known as antral follicle (primary oocyte surrounded by more than one layer of granulosa cells and having antral formation - fluid filled space) and Graafian follicle known as ovulatory follicle (large antral follicle approximately 0.9–1.0 mm in diameter, where the primary oocyte is surrounded by a cumulus oophorus). While these modifications are taking place, the stroma immediately around the follicles differentiate to form the theca follicul (theca interna and theca externa [12]. The laboratory rat is sexually mature at 6 -7 weeks, when the first estrous cycle appear. In mature rats, the estrous cycle recurs every four or five days. It has no breeding season. Multiovulation is the rule in the female rat [13]. After ovulation, the granulosa cells and cells of theca interna, that remain in the ovary form a temporary endocrine gland called the corpus luteum (yellow body). Most ovarian follicles undergo atresia from the time of birth [12].

Gonadotropin-releasing hormones (GnRH) is secreted by the Hypothalamus. It stimulates the secretion of pituitary gonadotropins [Follicle-Stimulating hormone (FSH) and Luteinizing hormone (LH)]. FSH is responsible for early maturation of ovarian follicles.As the follicle grows in size, estrogen is secreted. Estrogen inhibits further production of FSH andStimulates the pituitary gland to secrete luteinizing hormone (LH). LH influence follicular stage progresses, the developing follicle increases in size and becomes a mature follicle, lead to high level of estrogen and further release of LH (high concentration of LH in the blood), then ovulation occurs.The high concentrations of LH that brings about ovulation has an effect on the follicle cells that remain in the ovary and its transformation into corpus luteum. Corpus luteum secretes some estrogen and a large amount of progesterone. High concentrations of estrogen and progester-one inhibit production of FSH and LH. Without FSH and LH the cells of the corpus luteumde generates and less progesterone and estrogen is secreted. With less estrogen and progester-one, the FSH is no longer inhibited, and the cycle can start again [14].

In female, estrogen secretion comes from the ovaries (Theca intern, granulosa cells of the ovarian follicles and corpus lutein), beside significant amount from adrenal gland, adipose tissue, liver,skin and other tissues [15]. Progesterone is secreted by the corpus lutein cells, provide preprogesterone to the granulosa lutein cells, which convert it to progesterone), the placenta (syncytiotrophoplast) and in small amount by the ovarian follicles (granulosa cells) [16]. Since literature review shows that Fenugreek seeds contain rich amount of steroids

and precursor of steroid hormones, the purpose of this study was to evaluate the effect of different doses of Fenugreek seeds on mature, perimature and immature rat's ovary.

MATERIALS AND METHODS

This study is an experimental, prospective, observational work. A total of 135, healthy virgin Norway albino rats were used. They were grouped according to their age into three groups (Mature,Perimature and immature). Each group was subdivided into subgroups according to the dose of Fenugreek seeds (Table 1). All animals were kept in Animal House , College of Medicine, University of Baghdad, under identical conditions.

Fenugreek seeds were cleaned, standardized in the " Iraqi National Herbarium" and were ground in a coffee grinder. The seeds powder (dose) (Table 1) was mixed with four milliliter distilled water by glass rod and given through an oro-gastric tube (5.5 cm length, 1.3mm diameter). All subgroups were administered dose for continuous 14 days [17]. The animals were sacrificed at the end of this period.

Fifteen rats for each experimental subgroup of mature, perimature and immature groups, were used for histological study. During the study period, general behavior of each rat was closely observed, and approximately the amount of diet consumed daily by each was recorded. Also the body weight for each rat was measured at the beginning and end of the experimental work, using electronic balance (Sartorius type, 1265 MP).

For histological study, all rats were anaesthetized using open ether [diethyl ether, $(C^2H^5)_2O$, General Purpose Reagent, BDH chemicals Ltd.]. From each anaesthetized rat, both ovaries and a piece of liver were removed. During dissection of the tissue blocks, they were placed in cold normal saline. A very thin slice from the surface of each ovary was removed by a sharp blade to ensure perfect fixation. Liver was sliced into small pieces that have at least 1-3 mm thickness in one of their dimensions. For immature rats, the ovaries were placed in an already made hole in liver tissue to ease orientation and sectioning. All tissues were fixed for one hour in 2% paraformaldehyde $[(H.CHO)_n$, Laboratory Reagent, BDH chemicals Ltd.] in 0.067M phosphate buffer, pH 7.3, containing 7.5% sucrose [18].The fixed tissue blocks were rinsed for one hour in 0.067M phosphate buffer, pH 7.3, containing 7.5 sucrose at 4^0 c [18]. Then they were arranged on corks (22 mm in diameter, and three mm in thickness) and few drops of 10% gum acacia (BDH chemicals Ltd) were dropped over them. Each cork with tissue block on top, was quenched in liquid nitrogen, mounted on cryostat metal chuck with a drop of distilled water and left in the sealed cryostat cabinet whose temperature was pre-adjusted to -20^0c and left there for 15 minutes before sectioning. Such tissue is prepared for histological and histochemical studies. Serial sections were cut at 10-12µ, mounted on clean coverslips without adhesive and left for 30 minutes to dry at room temperature.

Tissue sections were stained by rapid Haematoxylin eosin stain[19]. sections were rinsed in tap water, stained by Haematoxylin $[(C_{16}H_{14}O_6.3H_2O)$, for Microscopy Fluka AG, Buchs AG] for two minutes, rinsed in saturated lithium carbonate $[(Li_2CO_3)$, Laboratory Reagents, BDH chemicals Ltd], then in tap water, and stained for ten seconds in eosin $[(C_{20}H_6Br_4Na_2O_5)$ for Microscopy, Fluka AG, Buchs SG]. After thatsections rinsed in tap water, dehydrated in graded ethyl alcohol, cleaned in 50%/50% alcohol/xylene and 100% xylene (Fluka AG, Buchs SG) and lastly mounted in DPX (Biological Reagent, Hopkin and Williams, Searle Co.). Six sections from different parts of each ovary were stained.

Following examination and assessment, ovary sections were photographed using Olympus microscope SC 35 camera.

The significance of difference between two groups mean , with continuous variables, was calculated by student t-Test, and among three groups or more by F-Test, using the least significant difference (LSD) if it was significant [20]

RESULTS

Rats were generally resisting the oro- gastric intubation, particularly in the first few trials. Yet, the selection of the appropriate size and length of the oro- gastric tube, with good animal

handling, made the procedure of the intubation relatively easier. Out of 135 intubated rats, three died probably from suffocation. The rest maintained good health as assessed by normal activity and food intake. There was some increase in food intake in experimental groups, which turned to be more active than the controls.

Data on body weight of normal mature, perimature and immature groups. Whether taking fenugreek seeds or not are summarized in table (2). Progressive increase in body weight was observed in all rats. There was no statistical significant difference in the initial body weight of immature, perimature and mature groups. Also there was no statistical significant difference in body weight in immature, perimature and mature subgroups compared with its controls. In mature group there was no statistical significant difference in body weight of subgroups I.C, I.E.A and I.E.B, but there was significant difference in body weight between these three mature subgroups in weight of rats which received a dose of 3.2/ g.b.w. (I.E.D) ($P < 0.05$) (Figure 1)., there is significant decrease in weight gain, in all others (immature, perimature and mature) subgroups the weight gain is approximately the same.

Regarding general morphology of studied ovaries, by low objective (×4), rats ovarylooks as an oval or elliptical organ containing follicles of all types [primordial, primary, secondary, mature (graafian) and atretic] scattered through its stroma [figures 3 -A, B and C]. By higher power objectives, the ovary seems to contain large number of cells of different shape, size and nuclei characters. Some of these cells form follicles [Primordial, Primary unilaminar, Primary multilaminar, Secondary (Antral) (figures 5- A,B and C) and Mature (Graafian], corpus luteum and corpus albicans. Primordial and primary unilaminar follicles appeared at the peripheral of the ovary (figure 5- A). A few layers of spindle-shaped or elongated cells surrounded the granulosa cells layers of the primary multilaminar, antral follicles (figures 5- B and C) and mature follicles, these are the theca interna cells (figures 5- B and C). Theca externa was not clear and it could not be differentiated from the surrounding interstitial tissue. Corpus luteum appeared at the peripheral of the ovary , as a solid, relatively large and more or less rounded. More than one corpus luteum (up to 14) was seen in the mature ovary. Luteal cells were larger than granulosa cells (figure 6-A). Few corpora albicans were seen in some sections of mature ovaries, as small and usually center structures (figure 6-B). Different stages of follicular atresia were seen in each ovarian section. Interstitial cells were found to be of two types, the primary and secondary interstitial tissue cells (figure 6-C).

Morphological appearance in the ovaries of control subgroups revealed that, although the ovaries of the three control subgroups were similar in their general structure, yet they were different in size, shape (figures 3 –A, B and C) and numbers of their inside structures and cells (Table 3; figure 2). The immature ovaries showed: very small size (c. 1×0.7×0.5 mm), scantly fatty tissue around them, smooth surface and are friable on touch. Microscopically, different types of follicles exist (folliculogenesis), however they are lacking Graafian follicles, corpus luteum and corpus albicans (Table 3; figures 2 and 3-A). The perimature ovaries revealed similar morphological features of the immature, but with slight decrease in the number of growing and atretic follicles. In addition, this subgroup manifested the appearance of small number of Graafian follicles and corpora lutea (table 3; figures 2 and 3-B). Lastly, mature control subgroup elicited, in general, same picture as immature and perimature control subgroups, but there is increase in the number of growing follicles and corpora lutea (Table 3; figures 2 and 3-C).

In comparison to the control subgroup of the same group, changes in the ovaries of experimental subgroups are the follows: Experimental immature subgroup showed, marked increase in the size of the ovaries (c. 2.5×), with more fatty tissue around them and they become firm to touch. Microscopically, there is increase in the number of growing follicles, atretic follicles and the total structures in the ovaries (table 3; figure 2), and it is clear that there is increase in the thickness of theca interna. Experimental perimature subgroup revealed, increase in the number of growing,

atretic follicles, corpora lutea and the total structure in the ovary, beside increase in the size of the ovary (c. 1.5×) (table3; figure 2), and increase in the thickness of the theca interna. This picture is approximately the same, irrespective to the dose of crude fenugreek seeds. Experimental mature subgroup elicited an increase in the number of growing follicles, corpora lutea and the total structures in the ovary, but the number of atretic follicle is approximately the same. Also there is increase in the thickness of the theca interna and the size of the ovaries (c.1.3×). This picture is approximately the same indifferent to the dose of crude of fenugreek seeds (Table 3, figure 2).

In general, statistical analysis shows; the mean number of growing follicles and corpora lutea per ovary were different in all groups according to the age of the rat. In immature group, there is statistical significant increase in the mean of atretic follicles and total structures of experimental rats in comparison to control (P<0.05), while the increase in mean of total follicles is statistically significant only at (P<0.01). In perimature group, there is no statistical significant difference between the mean of growing follicles, atretic follicles and corpora lutea in all subgroups, even at (P<0.01). Also there is no statistical significant difference between the mean of total structures in the ovary of experimental subgroups, but there is a statistical significant increase in the mean of total structures in experimental subgroups in comparison to control (P<0.01). In mature group, there is no statistical significant difference between the mean of atretic follicles and corpora lutea in the ovary of all subgroups, even at (P<0.01). Also there is no statistical significant difference between the mean of total follicles in the ovary of experimental subgroups, but there is a statistical significant increase in the mean of these follicles in experimental subgroups in comparison to their control (P<0.05). The difference in mean of total structures in experimental subgroups was not statistically significant even at (P<0.1), but there is a statistical significant increase in the mean of these structures in experimental subgroups in comparison to their control at (P<0.1) and not at (P<0.05).

Table 1: Showing the animal groups and subgroups used with the dose given for each, in this study.

Group	Age (weeks)	Subgroups	Dose: Fenugreek seeds mg/g body weight	No. of Rats
I (Mature)	10-12	Control (I.C)	4 ml distill water only	10
		Experimental (I.E.A)	1.6	15
		Experimental (I.E.B)	0.8	15
		Experimental (I.E.D)	3.2	15
II (Perimature)	4	Control (II.C)	4 ml distill water only	10
		Experimental (II.E.A)	1.6	15
		Experimental (II.E.B)	0.8	15
		Experimental (II.E.D)	3.2	15
III (Immature)	3	Control (III.C)	4 ml distill water only	10
		Experimental (III.E.A)	1.6	15

Table 2: Showing the means of initial body weight and changes produced at the end of experimental period, in mature, perimature and immature groups.

Group	Subgroups (for each, ten rats)	Initial body weight(gm) ± SD	Final body weight (gm) ± SD	The difference in weight (gm) ± SD
I (Mature)	Control (I.C)	103.4± 22.7	127.7 ± 18.5	24.3 ± 1.16
	Experimental (I.E.A)	105.5 ± 19.1	134 ± 20.5	28.5 ± 7.14
	Experimental (I.E.B)	98 ± 20.4	118.5 ± 19.2	20.5 ± 12.15
	Experimental (I.E.D)	115.5 ± 11.3	119 ± 21.5	3.5 ± 9.95
II (Perimature)	Control (II.C)	52 ± 7.8	119 ± 6.6	67 ± 9.17
	Experimental (II.E.A)	55 ± 6.2	134.2 ± 15.9	79.2 ± 12.15
	Experimental (II.E.B)	62 ± 4.4	129 ± 9.8	67 ± 9.42
	Experimental (II.E.D)	45 ± 13.9	110 ± 23.6	65 ± 10.31
III (Immature)	Control (III.C)	26 ± 2.8	61.5 ± 2	35.5 ± 0.71
	Experimental (III.E.A)	23.7 ± 2.2	60.7 ± 3.6	37 ± 1

Fig. 1: Histogram showing the means of weight gain in mature, perimature and immature groups in relation to the dose of fenugreek seeds.

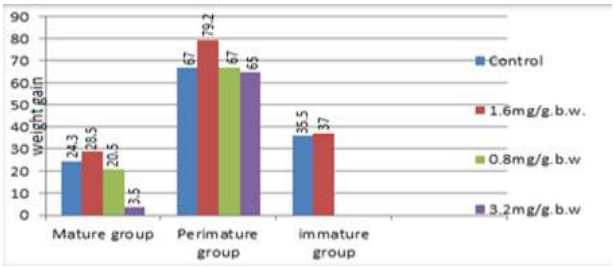


Table 3: Showing the mean number of various ovarian components for control and experimental subgroups in immature, perimature and mature groups.

Various ovarian components	Mature group				Perimature group				Immature group	
	Experimental (I.E.D)	Experimental (I.E.B)	Experimental (I.E.A)	Control (I.C)	Experimental (II.E.D)	Experimental (II.E.B)	Experimental (II.E.A)	Control (II.C)	Experimental (III.E.A)	Control (III.C)
Primary unilaminar follicles	5.0 ± 2.5	6.3 ± 3.2	4.7 ± 0.8	5.6 ± 2.2	5.7 ± 1.4	5.5 ± 0.8	10.0 ± 1.4	11.5 ± 4.1	11.0 ± 1.4	11.1 ± 1.96
Primary multilaminar follicles	6.0 ± 2.9	7.5 ± 1.96	4.4 ± 1.9	4.9 ± 1.9	4.7 ± 1.6	5.5 ± 2.4	11.2 ± 2.9	13.5 ± 2.5	13.4 ± 1.1	13.4 ± 4.2
Secondary (Antral) follicles	4.0 ± 1.7	5.4 ± 1.5	4.4 ± 1.8	6.4 ± 1.7	6.1 ± 1.7	6.4 ± 2.4	7.2 ± 1.1	9.5 ± 1.9	9.4 ± 0.9	9.5 ± 1.6
Graafian (mature) follicles	0.0 ± 0.0	0.6 ± 0.8	0.6 ± 0.5	0.5 ± 0.7	0.5 ± 0.5	0.3 ± 0.5	0.7 ± 1.1	0.8 ± 0.9	1.2 ± 0.8	1.1 ± 0.6
Total follicles (growing follicles)	15 ± 5.24	19.8 ± 2.5 x	14.1 ± 3.7	17.4 ± 3.0 =	17 ± 2.5 =	17.7 ± 4.9 =	29.1 ± 3.6	35.3 ± 7.6 = √	35.0 ± 1.4 = √	35.1 ± 3.4 = √
Corpora lutea	0.0 ± 0.0	0.0 ± 0.0	5.9 ± 1.2	6.8 ± 1.5 =	6.0 ± 1.8 =	7.0 ± 2.7 =	10.8 ± 1.7	12.0 ± 1.2 =	11.0 ± 0.0 =	11.6 ± 2.1 =
Atretic follicles	8.0 ± 2.8	12.8 ± 4.6 √	6.8 ± 2.2	7.9 ± 3.5 =	8.1 ± 1.7 =	7.5 ± 2.39 =	8.0 ± 1.3	8.0 ± 1.6 =	7.8 ± 1.1 =	7.8 ± 1.7 =
Total	23.0 ± 6.5	32.6 ± 9.5 √	26.8 ± 3.8	32.1 ± 5.1 = x	31.1 ± 3.8 = x	32.2 ± 4.9 = x	47.9 ± 5.4	55.3 ± 8.4 =	53.8 ± 1.9 = x	54.5 ± 4.0 = x
Ratio secondary/primary interstitial tissues	4/3	3/1	3/2	3/1	3/1	3/1	4/1	5/1	5/1	5/1

Total = Total follicles + Corpora lutea + Atretic follicles.*
Data expressed as mean of at least forty sections (ten rats) + Standard deviation.*
These results were obtained from serial sections.*
Approximately same.=
" Statistically significant at P < 0.05.
X Statistically significant at P < 0.1.

Fig. 2: Histogram showing the mean of different structures in the ovary of immature, perimature and mature groups in relation to the dose of fenugreek seeds. Mean obtained from forty sections of ten rats.

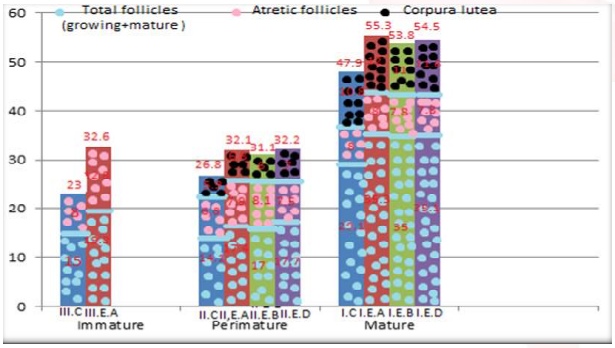


Fig. 3: Showing ovaries sections (six micron thickness), stained by Haematoxylin and eosin. A: Normal immature (five weeks old) rat's ovary with follicles in various stages of differentiation, but no corpus luteun. B: Normal perimmature (six weeks old) rat's ovary with follicles in various stages of differentiation, with corpus lutea in the cortex. C: Normal mature rat's ovary with follicles in various stages of differentiation, with many corpus lutea. (×22).

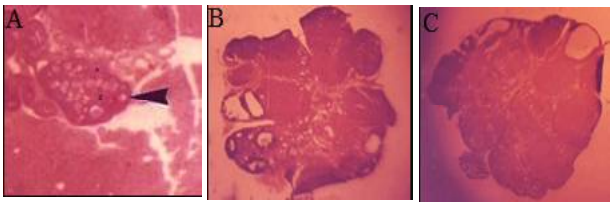


Fig. 4: Showing ovaries sections (six micron thickness), stained by Haematoxylin and eosin, after two weeks treatment with fenugreek seeds. A: Immature (five weeks old) rat's ovary with bigger ovarian size and marked increase in the number of growing follicles, but no corpus luteun, cf. figure 3-A. B: Perimmature (six weeks old) rat's ovary with bigger ovarian size and increase in the number of growingfollicles and corpus lutea in the cortex. Cf. figure 3- B. C:Mature rat's ovary with bigger ovarian size and marked increase in the number of growingfollicles, cf. figure 3- C. (×22).

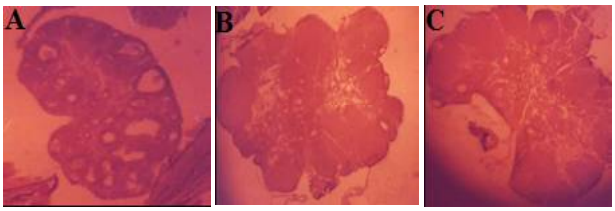


Fig. 5: Showing; A: Primordial follicle (red arrow), a primaryunilaminar follicle (white arrow). Note their peripheral position in the ovary and the oocyte is surrounded by single layer of granulosa cells. B: Primary mutilaminar follicle (arrow). Note the oocyte is surrounded by multilayers of granulosa cells and thetheca folliculi are clearly demonstrated. C: Secondary (antral) follicle, showing the oocyte, granulosa cells, theca interna and big antrun. (×1320).

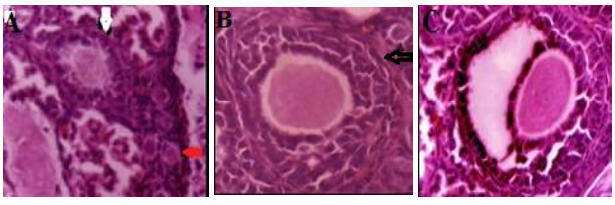
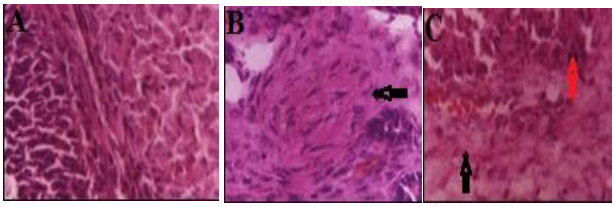


Fig. 6: Showing in mature rat's ovary; A: Part of corpus luteum (right) and part of secondary (antral) follicle (left). Observe the relatively large luteal cells in comparison to granulosa cells. B: corpus albicans (arrow). C: Primary (black arrow) and secondary (red arrow) interstitial tissue. (×1320).



DISCUSSION

The Fenugreek seeds effect on vagina rat's estrous and its galactogogual activity were suggested to their estrogen and/or progesterone like action [1,17]. Accordingly to this suggestion, and the fact that the ovary is the main organ which secretes estrogen and progesterone [15,16] , this study is concerning with detection of effect of different doses of fenugreek seeds on mature, premature and immature rat's ovary, using histological method.

Historically, fenugreek seeds have been administered oraly (mixed with diet, or less commonly infused with drinking- water) almost routinely, to coincide with the traditional method of preparation [21,22]. In 1996, Al-Khateeb [17] used oro- gastric tube intubation to administer fenugreek seeds. In this work, oro- gastric intubation was used for administration of fenugreek seeds to ensure a precise daily dose, and to a less extend, to avoid rat's annoyance and rejection of the bitter taste of the fenugreek seeds [17]. In the present study, the three rats were deaths, which account for 2.2% of total intubated rats, were most probably caused by a faulty oro- gastric intubation and hence suffocation. However, the low death percentage is negligible weighed against the dose accuracy gained by using this route of administration.

In this work, all fenugreek- treated rats revealed good general health, apparently hyperactive and an increase in food intake. However, no significant changes in weight gain were recognized, except in mature rats which received 3.2mg fenugreek seeds/ gm b. w, where there was significant decrease in gain of body weight. It had been reported that fenugreek seeds are nutritive and have an appetizer properties[17], Furthermore, they were found to enhance motivation to eat and food consumption[22], however no significant gain in body weight was observed[17]. Fortunately, no side- effect were reported for fenugreek seeds [10,23] in doses used. However Nakhla et. al. [24] earlier in 1991 reported that; crude fenugreek seeds, when administrated to Hisex- type chicken, produce body weight depression and pathological changes in liver and kidney. Significant decrease in body weight, reported in this work and by Nakhla et. al. [24], suggested that, high doses of fenugreek seeds may interfere with absorption and/ or metabolism of food, which needs more investigations.

Histological findingshaematoxyline and eosin stained sections of rats ovary of control subgroups, are similar to the classical picture of this organ described elsewhere [25]. Table (3) was done to present another view of the normal rats ovary picture in immature, perimature and mature groups and to compare their results, with those obtained from treated rats, according to the dose of the fenugreek seeds. Results obtained from control subgroups elicited precocious maturation (six weeks), in female albino, Norway rats, which were breeding in Iraq [26]. This may be due to maturation of the neural pathways involved in photoperiodic control of LH and prolactin secretion [27], or it might be due to the effect off ood intake [28], or combination of both factors.

Comparing the ovarian morphology of experimental immature rats (figure 4-A) with their controls (figure 3-A) and from examination of table (3), it is obvious that there is increase in the mean number of growing, atretic follicles and the total structures in the ovaries of the fenugreek- treated immature rats. Increase in atretic follicles and total structures were statistically significant ($P<0.05$). Also there was marked increase in the size of the ovaries for the fenugreek- treated rats of the same group. Picut, Dixon et.al. in 2014 [25] describe the histological characters of the immature rat's ovary, through the developmental periods,as follows: During the neonatal stage (postnatal day [PND] 0–7), ovarian follicle development is independent of pituitary gonadotropins (luteinizing hormone [LH] or follicle-stimulating

hormone [FSH]), and follicles remain preantral. Antral development of ''atypical'' follicles occurs in the early infantile period (PND 8–14) when the ovary becomes responsive to pituitary gonadotropins. In the late infantile period (PND 15–20), the zonapellucida appears, the hilus forms, and antral follicles mature by losing their ''atypical'' appearance. The juvenile stage (PND 21–32) is the stage when midsized antral follicles are a prominent feature, Interstitial glands apparent and atresia of medullary follicles occurs. These corresponding to a nadir in FSH levels. In the peripubertal period (PND 33–37),Graafian follicles develop (0.9–1 mm diameter), no corpora lutea,reduced prominence of midsized antral follicles,reduced number of atretic follicles andhilus well developed,as FSH levels rebound, and LH begins its bimodal surge pattern leading to ovulation. From this review, there was increase in the number of growing follicles, but there was no corpora lutea. These results suggest that fenugreek seeds are responsible for folliculogenisis and not for maturation. These lead to increase in the number of atretic follicles and the total number of structures in the ovary, which is reflected on its size.

Comparing the morphological feature of the experimental perimature subgroups (figure 4-B) with their control (figure 3-B) and from examination of table (3), it is clear that there is increase in the mean number of growing, atretic follicles and corpora lutea, but it is only significant in total structures ($P<0.1$), beside these there was increase in the size of the ovaries in fenugreek- treated perimature subgroups. This picture is approximately the same irrespective to the dose of crude fenugreek seeds. These results suggested that fenugreek seeds enhance the normal process of folliculogenisis with apparently normal follicular maturation. This process lead to increase in the number of all ovarian structures and the size of the ovary.

Comparing the ovaries of control mature subgroups (figure 3-C) with that of fenugreek-treated mature subgroups(figure 4-C) and from examination of table (3), revealed, no changes in the number of atretic follicles, with slight increase in corpora lutea, but there is significant different in the number of growing follicles ($P<0.05$) and the increase in total structures in the ovaries is significant only at ($P<0.1$). These findings are more or less the same irrespective to the dose of crude fenugreek seeds used in this study. These results also demonstrate that fenugreek seeds increase the normal process of folliculogenisis with apparently normal follicular maturation. Mature ovaries secrete adequate amount of estrogen which is enhanced by crude fenugreek seeds and this lead to significant increase in growing follicles, with increase in the total ovarian structures and ovarian size.

The increase in the thickness of theca interna, which was demonstrated in all experimental subgroups of immature, perimature and mature groups, suggest that crude fenugreek seeds may play role in steroidogenisis. This is due to the fact that estrogen in ovary, is secreted by theca interna, granulosa cells of ovarian follicles and corpus luteum [15].

CONCLUSION

Morphologically, normal rat's ovary showed gradual increase in ize, in relation to age. Immature rats ovaries elicit folliculogenisis process, but no mature follicles or corpora lutea. Folliculogenisis decreases slightly and there were corpora lutea at six weeks old rat's. Then folliculogenisis increase again and the number of corpora lutea in relation to age.

Fenugreek seeds maintained good general health, apparently hyperactivity and increased food intake, but no significant changes in body weight gain. However high doses reduce the mature rat body weight gain. Fenugreek seeds enhance folliculogenisis in mature, perimature and mature groups, but they do not affect follicular maturation. Fenugreek seeds have estrogen like action. Fenugreek seeds, indifferent to the dose used, have the same effect on the ovary.

Conflicts of Interests: **None**

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