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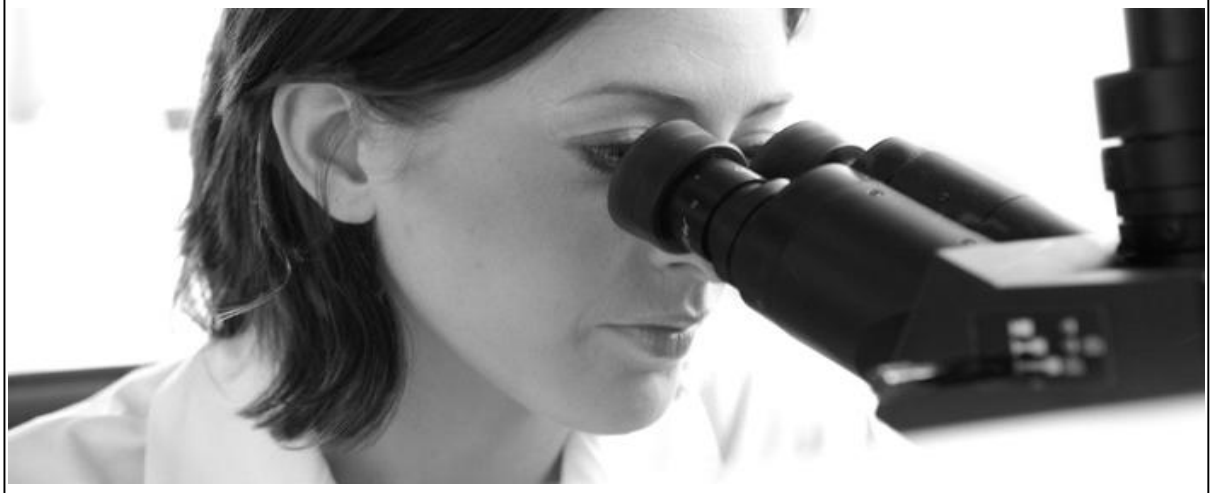

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RESEARCH ARTICLE

EFFECT OF FENUGREEK SEEDS ON RAT'SVAGINA; CYTOLOGICAL STUDY

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ABSTRACT

Background and objective: It has been shown in literature that Fenugreek seeds contain rich amount of steroids and precursor of steroid hormones. The objective of this study was to evaluate the effect of different doses of Fenugreek seeds on mature and perimature rat's vagina. **Methodology:** A total of 80, healthy virgin Norway albino rats were used. They were divided according to their age into two groups, namely, mature and perimature. Each group was subdivided into subgroups according to the dose of Fenugreek seeds (0.8, 1.6 and 3.2 mg/g bod weight).
Results: It has been observed that in experimental perimature subgroups, there was precocious vaginal opening. The appearance of the first estrous phase occurs earlier than the control subgroup, with normal first vaginal cycle and prolong of the second estrous phase. Comparing the cytological results obtained from experimental mature subgroups with their controls, it was obvious that there was ashortening of the vaginal cycle, prolongation in estrous and proestrous phases. In addition, a decrease in the amount of mucus and leukocytes with an increase in the number of cornified and nucleated epithelial cells were identified.
Conclusion: The data obtained from this study revealed that crude Fenugreek seeds possessoestrogen and, or progesterone like effect. The vaginal cytology of both mature and Perimature rats treated with Fenugreek seeds was nearly identical, irrespective of the doses used in this study.

INTRODUCTION

Fenugreek (English name), Helba (Arabic name) or Methi (Indian name) is part from Leguminosae family. It's scientific name is TrigonellaFoenum-graecum L. Their plant is an aromatic annual herb which produce small flowers, white or yellowish-white, contain 10-20 seeds [1, 2, 3]. The effective medicinal part of the plant is their dried ripe seeds which emitting a characteristic odor, with an oily bitter, farinaceous taste[1,2]. Fenugreek is cultivated in Mediterranean countries, Asia, Southern Europe, Northern Africa and USA (California)[3]. The analysis of a sample of Fenugreek seeds from Saudi Arabia market gave the following value: moisture 7,71%, crude protein 12.91%,crude fat 4.51%, crude fiber 13.14%, carbohydrates 57.5% and ash 4.23%. It also contains: potassium 603 mg/100g, calcium 75 mg/100g, Magnesium 42 mg/100g and iron 25.8 mg/100g. However, zinc, manganese and copper were found 2.4, 0.9 and 0.9 mg/100g respectively. The major fatty acids in Fenugreek seeds was the lenoleic acid, oils being 34.85%. Total unsaturated fatty acids were 92.99% [4].Fenugreek seeds contain several possible active chemical constituents such as saponins, alkaloids, steroids, flavonoids, tannins, amino acids and trigonilline [5]. Fenugreek seeds has been scientifically used for increasing milk supply [6], wounds, inflammation, gastrointestinal ailments, cholesterol decrease agent [7], diabetes [8] bronchitis, inflammation, chronic cough, liver disorders [9] and as an antifertility agent [10].

Grossly, rat's vaginal orifice lies just ventral to the anus. It open's to outside separately from the urethra. In young female rat the vagina is sealed caudally by transvers epithelium septum which started to degenerate at 20-35 days of age and progressive disintegration of the septum lead to formation of a continuous lumen at 40- 80 days [11]. The estrous cyclic changes in the vagina is under the action of oestrogen and progesterone [12] and it is reflected by theratio of nucleated epithelial cells, cornified squamous epithelial cells, and leukocytes observed in smears. In Pro-estrous, cells are almost exclusively clusters of round, well-formed nucleated epithelial cells. During estrus, cells are predominantly cornified squamous epithelial cells. During metestrus, small darkly stained leukocytes predominate. Cornified squamous epithelial cells may be observed, often in fragments. During diestrus, rare cornified squamous epithelial cells may still be present, however leukocytes still predominate. Metestrus can be distinguished from diestrus by the appearance of nucleated epithelial cells in diestrus [13, 14].

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Table 1 Showing types of cells found in vaginal smear during estrous cycle [13].

Phase	Time	Histological features
Proestrous	12 hours	Nucleated and relative small epithelial cells, mucous cells and degenerating leukocytes.
Estrous	12 hours	Moderate number of large cornified squamous cells, non-nucleated, with total absent of leukocytes and clear background.
Metestrous	21 hours	Cornified cells and numerous polymorphonuclear leukocytes.
Diestrous	57 hours	Small epithelial cells, some mucous cells and high concentration of leukocytes.

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 and perimature rat's vagina.

MATERIAL AND METHODS

This study is a prospective, observational and experimental work. A total of 80, healthy virgin Norway albino rats were used. They were grouped according to their age into two groups (Mature and Perimature). Each group was subdivided into subgroups according to the dose of Fenugreek seeds (Table 2). 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 2) 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 [15].

Ten rats for each subgroup of mature and perimature groups, were used for vaginal cytology. Vaginal smear from each rat was examined daily during the 14 days of experimental period. For the perimature group, the vaginal septum was perforated at four weeks of age, by blunt needle for only five rats in each subgroup.

The material for vaginal cytology was obtained by good gently scrap of the vagina using cotton swab pre-moistened in normal saline, then smeared on clean glass slide, and fixed immediately in 95% ethyl alcohol for 24 hours. After fixation the vaginal smear was stained by Papanicolaoustaining method [16].

Photography was taken using Olympus microscope with Olympus SC 35 camera.

Ethical consent: The study was approved by the biomedical ethical committee, faculty of Medicine, University of Baghdad, Iraq.

RESULTS

Vaginal smear study of mature control subgroup shows, the four stages of the estrous cycle with 4-5 days duration. The characteristic smear findings are:

1. Estrous: Flat, rather large, anucleatedcornified cells, with clear cytoplasm and isolated in a clear background (figure 6).
2. Metesrous: Numerous leukocytes, few nucleated cells, with some cornified cells (figure 7).
3. Diestrous: High concentration of leukocytes with mucus and remains of large cornified cells (figure 8).
4. Proestrous: Nucleated epithelial cells mucus and few leukocytes (figure 9).

Vaginal smear of fenugreek seeds-treated rats shows, shortening of vaginal cycle with prolongation in estrous and proestrous phases (figure 2). In general there is decrease in the amount of mucus and leukocytes with an increase in the number of cornified cells and nucleated epithelial cells. The vaginal cytology of fenugreek seeds- treated mature rats, was approximately identical irrespective to fenugreek seeds doses used in this study.

In perimature control rats, daily vaginal smear showed, in the first day of the experimental work (figure 4), high concentration of leukocytes with thick mucus, numerous superficial cells, intermediate cells and some parabasal cells. In the following days, the amount of the superficial cells, leukocytes and mucus decreases gradually, while that of intermediate and parabasal cells increase (figure 5). The first estrous phase appeared at about 38-40 days of age, with scantly superficial cells and clear background, but till the end of the experiment there is no clear estrous cycle (figure 1). On the other hand, fenugreek seeds- treated perimature rats, showed the same picture as the control subgroup. However, the first estrous phase appeared at 33-35days of age and was of one day duration. The second estrous phase started at 37-39 days of age and was of two day duration. Between the two estrous phases there was normal estrous cycle (figure 1).

Table 2 Showing the animal groups and subgroups used with the dose given for each, in this study:

Group	Age (weeks)	Subgroup	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	10
		Experimental (I.E.B)	0.8	10
		Experimental (I.E.D)	3.2	10
II (Perimature)	4	Control (II.C)	4 ml distill water only	10
		Experimental (II.E.A)	1.6	10
		Experimental (II.E.B)	0.8	10
		Experimental (II.E.D)	3.2	10

-------All animals were sacrificed after two weeks.

This appearance was approximately similar in all rats which received fenugreek irrespective to the dose used in this work. The mean age of complete vaginal septum degeneration, with opening of the vagina is showed in table (3).

Table 3 Showing age for complete vaginal septum degeneration in control and experimentals of perimature group

Subgroups	Age of complete vaginal septum degeneration (days)
Control (II.C)	40+
Experimental of 1.6 mg/g body weight (II.E.A)	36.5
Experimental of 0.8 mg/g body weight (II.E.B)	36
Experimental of 3.2 mg/g body weight (II.E.D)	37

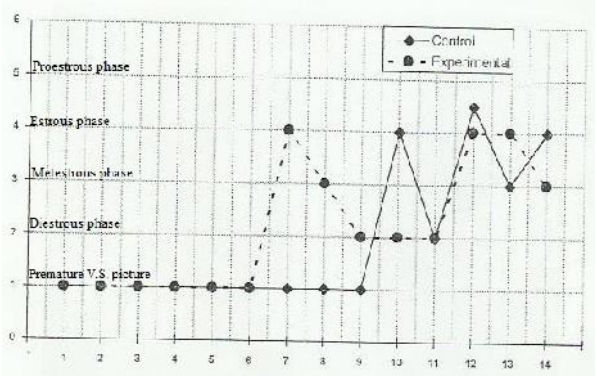

Fig. 1 A graphic study of daily vaginal cyclic changes in control and experimentals perimature rats.

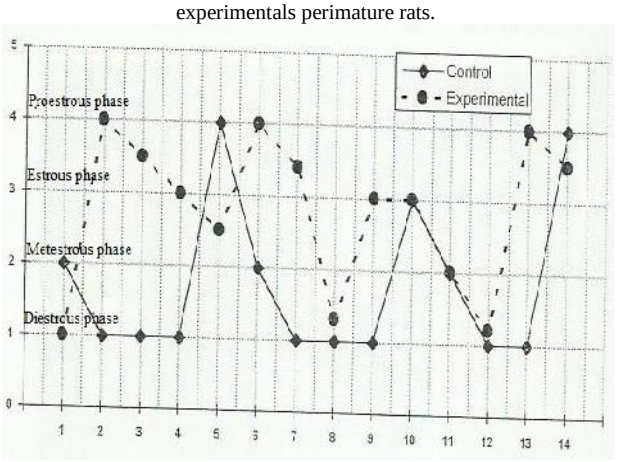

Fig. 2 A graphic study of daily vaginal cyclic changes in control and experimentals mature rats.

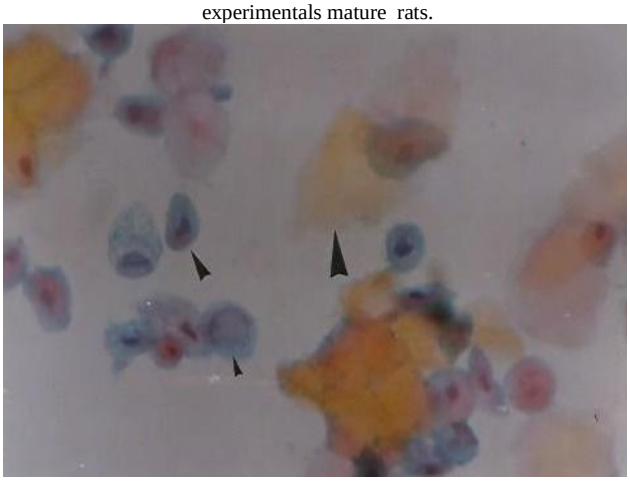

Fig. 3 Vaginal smear, showing only the three types of epithelial cells, which are :superficial or cornified (big arrow), intermediate (medium arrow) and parabasal(small arrow).(x1320).

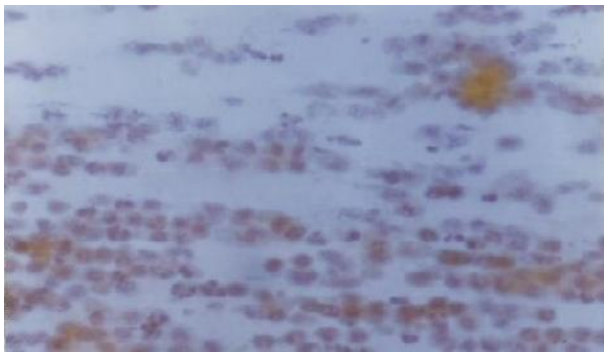

Fig. 4 Vaginal smear of perimature rats in the first day of experimental work. Note high concentration of leucocytes with thick mucus, numerous superficial cells and some intermediate and parabasal cells. (x 330).

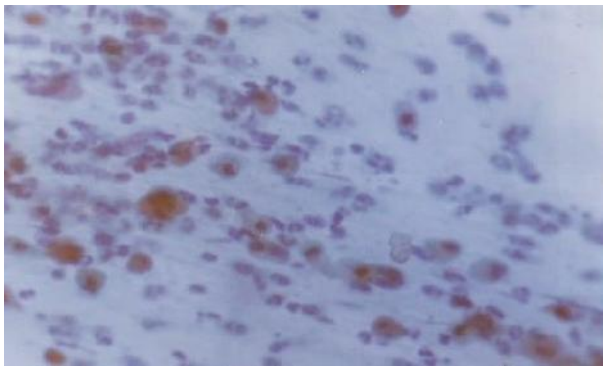

Fig. 5 Vaginal smear of perimature rats in the fifth day of experimental work. Note the decrease in the number of leucocytes, superficial cells and mucus with higher concentration of intermediate and parabasal cells, cf. fig. (4). (x 330).

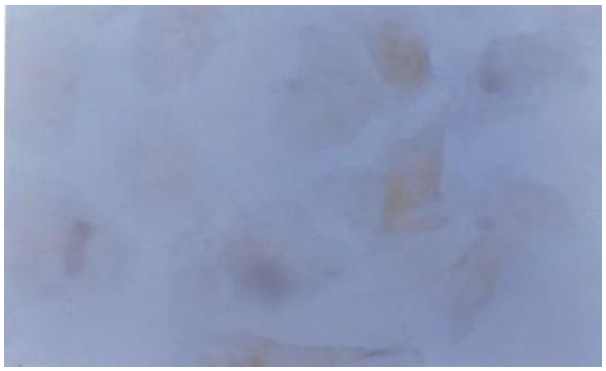

Fig. 6 vaginal smear during estrous phase. Note the flat cornified cells with tansparent cytoplasm, leucocyte are absent with clear background. (x 1320).

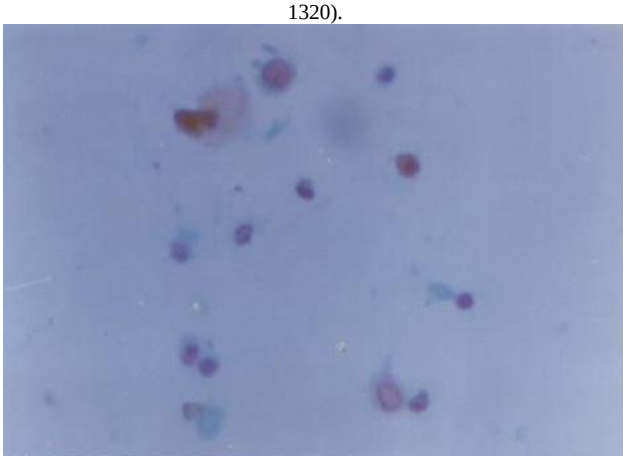

Fig. 7 Vaginal smear during metestrous phase, showing numerous leucocytes and some cornified cells. (x 330).

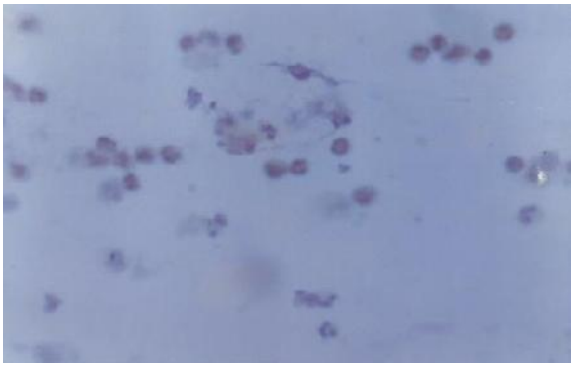

Fig. 8 Vaginal smear during diestrous phase, showing the small epithelial cells, some mucus and high concentration of leucocytes. (x 330).

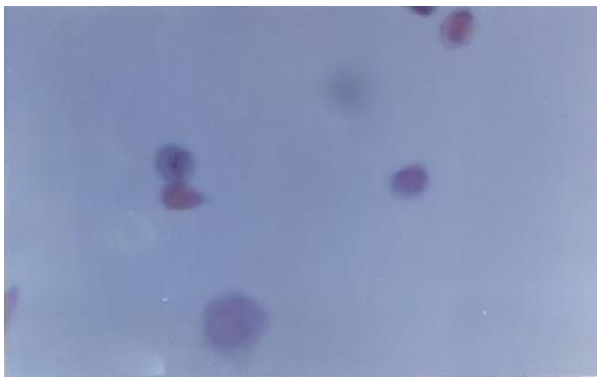

Fig. 9 vaginal smear during proestrous phase. Note the nucleated cells. (x 1320).

DISCUSSION

In experimental perimature subgroups, precocious vaginal opening, appearance of the first estrous phase earlier than the control subgroup, with normal first vaginal cycle and prolong of the second estrous phase (two days), indicate that fenugreek seeds are responsible for precocious maturation of the vaginal epithelium. The disintegration of the vaginal septum and opening of the vagina were used as an index of puberty [17], but perforation of vaginal septum followed by daily vaginal smear in perimature rats, were not found in literature.

The epithelial and connective tissue elements of the vagina are stimulated to full development by oestrogenic hormone [18]. This suggested that fenugreek seeds might have an oestogenic like effect. Under the influence of oestrogen, the vagina epithelium becomes cornified [19]. Thus, prolonged exposure to oestrogen lead to prolongation of estrous phase.

Comparing the cytological results obtained from experimental mature subgroups, with their controls, it is clear that there is shortening of the vaginal cycle, prolongation in estrous and proestrous phases, decrease in the amount of mucus and leukocytes with an increase in the number of cornified and nucleated epithelial cells. Same results were reported by Sulaiman IM, Nimir AR and Ibrahin IA in 2011 [19].

Shortening of vaginal cycle was reported earlier by Van-der-Schoot and Vilenbroek (1983) [20], they suggested that, it is due to prolonged exposure to progesterone.

CONCLUSION

In view of results obtained in this work, it appears that crude Fenugreek seeds have oestrogen and, or progesterone like effect.

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