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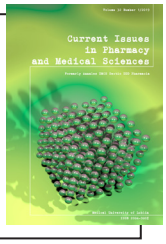

Fenugreek seeds estrogenic activity in ovariectomized female rats

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ABSTRACT

The estrogenic activities of fenugreek seeds (*Trigonella foenum-graecum L.*), widely used in traditional pharmacopoeia, are reflected in the uterus of ovariectomized female rats, with a slight increase in dry and wet weight, a thickening of the stroma and the uterine epithelium and the development of the endometrial glands. In the vagina, the estrogenic action is shown through an increase in the epidermal cell number and a tendency to keratinization, leading to vaginal opening.

Furthermore, this estrogenic potential of fenugreek seeds is confirmed by the over-expression of progesterone receptors in the uterine tissues supporting possible interactions between phytoestrogens and estrogen receptors.

Therefore, Fenugreek seeds may be capable of promoting the development of reproductive tissues of immature ovariectomized rats, and its estrogenic activity may take its action by holding phystoestrogens that interact with estrogen binding sites and activate the same estradiol-mediated cell signaling pathways.

Thus, our results give added scientific support to the popular use of Fenugreek seeds as an alternative for several health problems such as fertility and menopause related disorders.

INTRODUCTION

In Morocco, medicinal plants occupy a crucial place in traditional medicine and play a very important socio-economic role. Among these plants, *Trigonella foenum-graecum L.* (Fenugreek, Halba) is one of the oldest medicinal and culinary plants. It is a small leguminous plant characterized by yellowish brown and angular seeds, known for its nutritional value beside its medicinal effects. Fenugreek seeds contain a substantial amount of mucilaginous fiber, phospholipids, glycolipids, oleic acid, linolenic acid, linoleic acid, choline, vitamins A, B1, B2, C, nicotinic acid, niacin [1]. Many other functional elements in fenugreek are present, among these, alkaloids (about 1%), as well as steroidal saponins (approximately 4 to 8%), phenolic compounds (64.61 mg of gallic acid equivalents/g) and phytoestrogens (mainly flavonoids, 19.4 mg of quercetin equivalents/g) [1-3], which is a compound with similar properties to estrogen.

Scientists have reported several medicinal properties of fenugreek seeds, including appetite stimulant, antidiabetic, hypercholesterolemic, anti-inflammatory effect hepatoprotective against free radicals, gastroprotective and protection against breast and colon cancer [4-7]. Other studies has shown that fenugreek seeds have fertility regulating properties [8-10].

The aim of this study was to evaluate the estrogenic activity of fenugreek seeds in ovariectomized rats, by using uterotrophic assays that combined the analysis of morphological, histological, biochemical and molecular endpoints.

MATERIALS AND METHODS

1. Experimental diets and estimation of total flavonoid content

Trigonella seeds were collected in the Chaouia-Ouardigha, Moroccan region. The fenugreek aqueous extract was prepared in the traditional way through a decoction process. The fenugreek seeds were soaked in drinking water (250 g/l and 500 g/l) and heated up for 5 minutes. Then, the aqueous extract was recovered by filtration. The extracts were kept at 4°C until required for use. In the study, two experimental groups received individually 5 ml of one of the two aqueous extracts, which is equivalent to 450 mg/kg/day (low dose) and 900 mg/kg/day (high dose), respectively. Total flavonoid contents of these aqueous extracts were respectively 16.87 to

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22.91 mg quercetin/g. Total flavonoid content was measured by the aluminum chloride colorimetric assay as described by Zhishen *et al*., 1999 [11].

For the powder treatment, each 5 g of commercial foods were mixed with the fenugreek seed powder (450 mg/kg/day, 900 mg/kg/day). This mixture was both crushed and homogenized in 1ml of olive oil, dried at 100 °C for one hour and stored until used.

The same technique was used for the preparation of estradiol-based cookies: commercial foods mixed with 2 mg *ethinyl estradiol* (Estrofem®).

2. Experimental animals

A total of 36 ovariectomized females *Wistar* rats were used in this study. All experiments were carried out according to the National Institute of Health Guidelines for the care and use of Laboratory animals and the European Council Directive on 24 November 1986 for Care and Use of Laboratory Animals (86/609/EEC), and approved by the Local Ethics Committee.

Ovarietcomy of the rats

Prepubertal female rats, 6 weeks *old* and weighing 90±6 g, were anesthetized with ether and were laid down on their sides. In each side, in the flank region, an incision of 1.5 cm was made on the skin and in the subcutaneous tissue. Through each incision, each corresponding ovary was located. Then, a ligature was made in the uterus-tube junction involving all of the vascularization of the ovary and a section of the tube and other structures between the ligature and the ovary were removed. The uterine horn was replaced in the abdominal cavity and the incision was sutured. The animals were kept under rest for a period of at least 15 days so that they could recover from the surgical trauma and for the uterine involution to happen.

Preparation of test samples

15 days after the spaying, the female rats were housed individually in polypropylene cages and maintained under standard laboratory environmental conditions (temperature 21°C±1°C, 12h light: 12h dark cycle and 50-60% relative humidity). Animals were randomly distributed in six groups (n = 6) and received a daily oral treatment as following:

- *Group I*: used as a negative control received drinking water
- *Group II*: used as a positive control received 2 mg of ethinyl estradiol incorporated into a standard diet.
- *Group III* and *Group IV*: received 5 ml of fenugreek aqueous extracted at doses 450 and 900 mg/kg/day, respectively.
- *Group V* and *Group VI*: consumed powder Fenugreek seeds at doses 450 and 900 mg/kg/day, respectively.

At the end of treatments, within 15 to 30 minutes each morning, the animals had ad libitum access to water and standard pellets enriched with barley and maize.

3. Estrogenic parameters measurement

Various morphological, histological, biochemical and molecular endpoints in the target tissues were used to analyze the estrogenic activity

Measurement of vaginal opening

Vaginal opening is an important secondary sexual character in rats, and can be identified by simple visual inspection. It is used as an external indicator for estrogenic activity and puberty onset [12]. The vaginal opening was observed and noted daily for 7 days after fenugreek seeds oral administration in ovariectomized female *Wistar* rats.

Macroscopic and histological study

At the end of treatment, the animals were weighed and sacrificed under ether anaesthesia and all organs were scrutinized to identify any physical abnormality. The female reproductive organs (uterine horns and vagina) and adrenal glands were immediately removed and weighed. Organ weight was determined and expressed as relative weight (organ mass/body weight × 100).

The organs were fixed with a 10% formaldehyde solution for 72 hours, dehydrated with a crescent series of alcohol (70 to 100%), embedded in paraffin wax and sectioned at a 5 µm thickness. Paraffin sections were stained with hematoxylin-eosin by a standard procedure for histological study.

Immunohistochemical analysis of progesterone receptor expression

Another endpoint for estrogenicity is the expression and regulation of the progesterone receptor (PR). This has been demonstrated to be a sensitive estrogen regulated transcriptional target. An analysis of the PR expression was studied immunohistochemically using specific anti-receptor monoclonal antibodies in uterine tissue samples [13].

Tissue sections 5 µm thick of the uterus were mounted on polylysine-coated slides, deparaffinated, rehydrated, and then heated with 10 mM citrate buffer (pH 6.0). After being washed with phosphate buffered saline (PBS), slides were incubated with 3% hydrogen peroxide in methanol for 30 min to quench endogenous peroxidase activity. After washing with PBS, tissues were incubated with a blocking buffer (Thermo Scientific) at room temperature for 5 min. Sections were then overlaid for 1h with a rabbit anti-progesterone antibody. Sections incubated in PBS without antibody served as controls.

Subsequently, slides were washed two times for 5 min each with PBS and incubated for 15 min with goat anti-rabbit horseradish peroxidase-conjugated secondary antibody (HRP Polymer Quanto, Kit UltraVision Quanto, Thermo Scientific). The sections were subsequently stained with 3,3'-diaminobenzidine (DAB quanto chromogen, Thermo Scientific) and incubated for 5 minutes. The reaction was stopped by exhaustive washing with distilled water. The tissues were then counterstained with hematoxylin and dehydrated with ethanol and toluene to prepare for mounting.

4. Statistical analysis

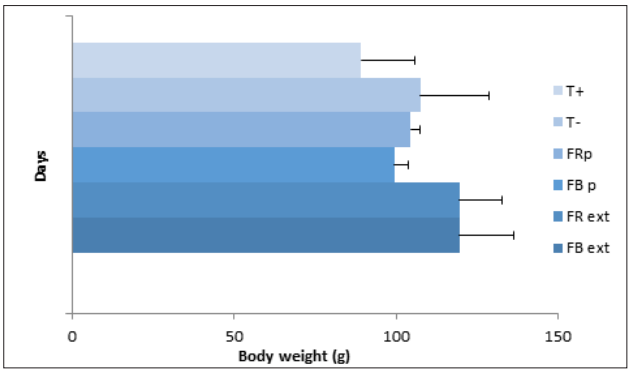
Statistical analysis was carried using one-way analysis of variance (ANOVA) followed by Tukey's test for multiple comparisons. Herein, p-values less than 0.05 were considered to be statistically significant. All results are expressed as mean ± SEM.

3. RESULTS

Effect of treatments on total body weight

The administration of estradiol slightly significantly reduced the body weight in comparison with the untreated control group ($F = 2.19$, $p<0.1$).

After 7 days of treatment with aqueous extract of fenugreek seeds, no dose significantly affects Total body weight of the female rats compared to untreated controls ($F = 2.19$, $p>0.05$). However, the animals consuming powdered fenugreek seeds showed a slight but still not significant increase in their body weight, at the two doses 450 mg/kg/day and 900 mg/kg/day ($F = 2.19$, $p>0.05$).



T+ – controls treated with ethinyl estradiol; T- – untreated controls; FRp – High dose of fenugreek seed powder 900 mg/kg/day; FB p – low-dose of fenugreek seed powder 450 mg/kg/day; FR ex – high-dose of aqueous extract 900 mg/kg/day; FB ex – Low-dose of aqueous extract 450 mg/kg/day Results are expressed as the mean±SEM of 6 rats per each group; *** – represents significant differences at ($p<0.001$) between means (Data were compared using Student's t test and ANOVA, differences with p-value <0.05 were considered as significant)

Figure 1. Effect of fenugreek seeds on body weight gain

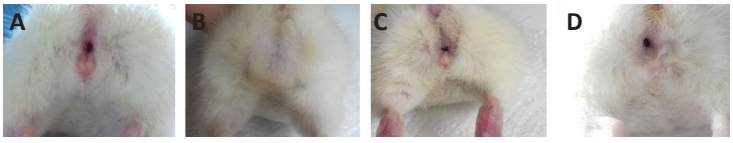
Effect of treatment on vaginal opening

All female rats treated with ethinyl estradiol have a well-observable vaginal opening (Figure 2). At the same time, treatment with aqueous fenugreek extract at doses of 450 mg/kg/day induced vaginal opening in only 84% of the female rats (Table 1).

Table 1. Percentage of vaginal opening in control and treatment groups

Groups	Group I: Control (Untreated animals)	Group II: Estradiol-treated rats	Aqueous extract		Seeds Powder	
			Group III	Group IV	Group V	Group VI
			450 mg/Kg/J	900 mg/Kg/J	450 mg/Kg/J	900 mg/Kg/J
Vaginal opening	0/6	6/6 (100%)	5/6 (84%)	6/6 (100%)	6/6 (100%)	6/6 (100%)

In addition, vaginal opening occurred in 100% of the rats treated with a high dose of aqueous extract (900 mg/kg/day) and in all female rats treated with fenugreek seed powder (Table 1).



A - Positive controls treated with ethinyl estradiol, B - untreated controls, C - Treated with low dose of fenugreek (450 mg/kg/day), D - Treated with high dose of fenugreek (900 mg/kg/day)

Figure 2. Examples of vaginal opening observed in female rats of the 4 groups

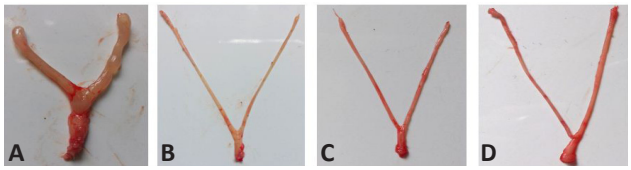
Effect of treatments on the female genital tract: macroscopic and microscopic observations

No treatment-related macroscopic alterations were observed in any of the dissected female rats.

1. Changes in genital tract morphology

Morphological analysis of the reproductive system of the different groups of treated and control rats showed significant differences (Figure 3). The uterus and vagina of estradiol-treated animals are very large and rich in fluid secretions, whereas those of untreated controls are very thin and devoid of secretions.

Comparatively, in the treated groups, a slightly thicker genital tract compared to untreated control rats was noted. However, the uterus of these rats is less distended and contains reduced secretions compared to those observed in animals treated with estradiol.



A – controls treated with ethinyl estradiol, B – untreated controls, C – Treated with low dose of fenugreek (450 mg/kg/day), D – Treated with high dose of fenugreek (900 mg/kg/day)

Figure 3. View of the reproductive system in the four groups of rats

2. Uterine weight

Estradiol administered to ovariectomized rats resulted in a significant increase in relative uterine dry ($F = 32.04$, $p<0.001$) and wet weight ($F = 18.4$, $p<0.001$) as compared to the untreated control group.

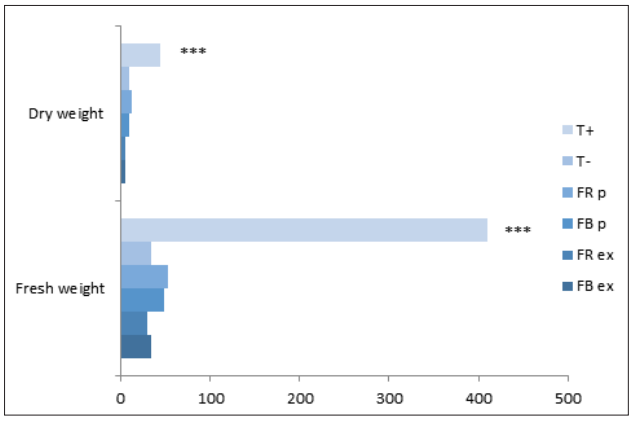
Meanwhile, there was a slight but not statistically significant increase ($p>0.05$) in fenugreek-treated rats, as compared with the control, especially at a high dose (900 mg/kg/day) of fenugreek powder (Figure 4), but this increase is still much lower than that recorded in female rats treated with estradiol.

3. Vagina weight

Estradiol treatment resulted in a very highly significant increase in the relative vagina weight in ovariectomized female rats ($F = 11.94$, $p<0.05$), compared to the control group. However, daily consumption of fenugreek seeds as powdered or aqueous extract did not cause any significant changes in this parameter ($F = 11.94$, $p>0.05$) as compared to the untreated control (Figure 5).

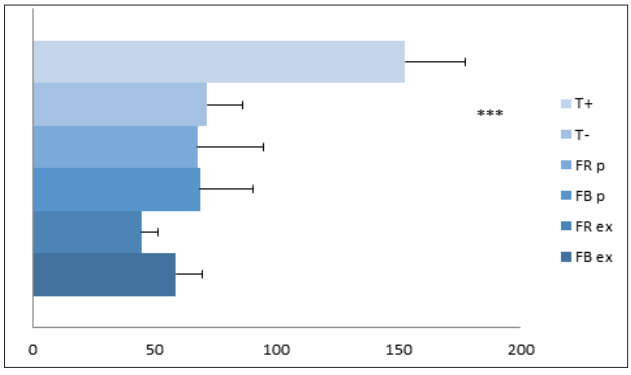
EFFECT OF TREATMENTS ON THE WEIGHT CHANGES OF THE ADRENAL GLANDS

The administration of fenugreek seed powder or aqueous extract did not cause any significant variation in the relative weight of the adrenal glands ($F = 1.12$, $p>0.05$), compared with the control group and estradiol-treated female rats (Figure 6).



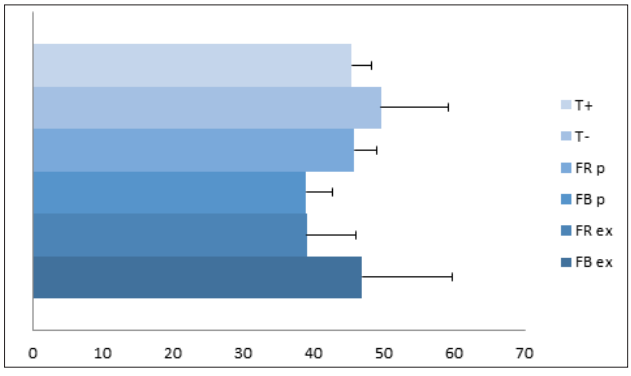
T+ – controls treated with ethinyl estradiol; T- – untreated controls; FR p – High dose of fenugreek seed powder 900 mg/kg/day; FB p – low-dose of fenugreek seed powder 450 mg/kg/day; FR ex – high-dose of aqueous extract 900 mg/kg/day; FB ex – Low-dose of aqueous extract 450 mg/kg/day;
Results are expressed as the mean±SEM of 6 rats per each group;
*** – represents significant differences at ($p<0.001$) between means;
(Data were compared using Student's t test and ANOVA, differences with p-value < 0.05 were considered as significant)

Figure 4. Effect of fenugreek seeds on the fresh and dry weight of the uterus



T+ – controls treated with ethinyl estradiol; T- – untreated controls; FR p – High dose of fenugreek seed powder 900 mg/kg/day; FB p – low-dose of fenugreek seed powder 450 mg/kg/day; FR ex – high-dose of aqueous extract 900 mg/kg/day; FB ex – Low-dose of aqueous extract 450 mg/kg/day;
Results are expressed as the mean±SEM of 6 rats per each group;
*** – represents significant differences at ($p<0.001$) between means
(Data were compared using Student's t test and ANOVA, differences with p-value <0.05 were considered as significant)

Figure 5. Effect of fenugreek seeds on the weight of the vagina



T+ – controls treated with ethinyl estradiol; T- – untreated controls; FRp – High dose of fenugreek seed powder 900 mg/kg/day; FB p – low-dose of fenugreek seed powder 450 mg/kg/day; FR ex – high-dose of aqueous extract 900 mg/kg/day; FB ex – Low-dose of aqueous extract 450 mg/kg/day
Results are expressed as the mean±SEM of 6 rats per each group;
(Data were compared using Student's t test and ANOVA, differences with p-value <0.05 were considered as significant)

Figure 6. Effect of fenugreek seeds on adrenal glands weight

HISTOLOGICAL ANALYSIS OF THE FEMALE GENITAL TRACT

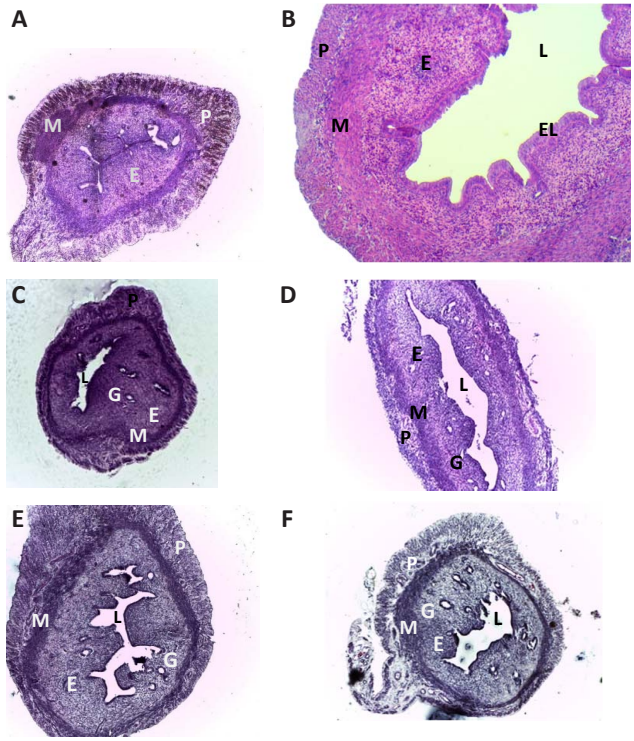
Histopathological examination showed some changes in the morphology of the reproductive organs of treated animals.

Uterine histology

Estradiol treatment caused a large increase in circumference and thickness of the uterus, compared with the control group. Histologically, a perimetrium is composed of a thin layer of connective tissue. The myometrium, consists of inner circular and outer longitudinal smooth muscle cells (Figure 7). Moreover, the endometrium consists of a stromal containing rare uterine glands, lined with a luminal unistratified epithelium, the thickness of which is more than 300% compared with uteri of untreated female rats (Figure 7).

At the same time, fenugreek seeds administered to ovariectomized rats induced a slight increase in uterine size and thickness, with a reduced myometrial layer. Endometrial stroma is relatively thick, but its development is modest compared to the estradiol-treated rats; it is particularly rich in many well-developed uterine glands (Figure 7).

In addition, the administration of fenugreek seeds to ovariectomized rats increases the luminal epithelial height and thickness from 20 to 200%, compared to unstimulated control rats. This epithelium consists of a layer of cubic cells facing a very small uterine lumen (Figure 7).



A – Group of rat controls, B – Group of estradiol-treated rats, C – Group of female rats treated with low-dose fenugreek aqueous extract (450 mg/kg/day), D – Group of female rats treated with aqueous extract of fenugreek at high dose (900 mg/Kg/day), E – Group of female rats treated with fenugreek powder at dose (450 mg/kg/day), F – female group fenugreek powder at dose (900 mg/kg/day), (G X 10)

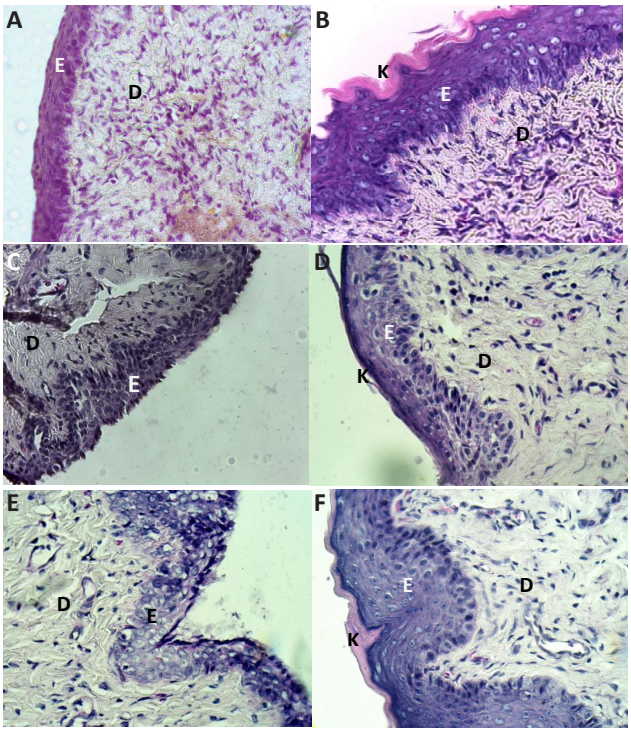
P: Perimeter; M: Myometrium; E: Endometrium; L: Light; EL: Luminal epithelium; G: Uterine gland

Figure 7. Representative histological analysis of the uterus in rats treated with fenugreek seeds (G X 10)

Vagina histology

Figures 3F-3J show microscopic preparations of representative vagina from one animal per treatment group. Compared with untreated immature rat, the estradiol-treated female rats (Figure 8) displayed a typical squamous multilayered epithelium layers with cornification.

Treatment with fenugreek seeds at two doses increased epithelial thickness, the number of cell layers and the cornification of the vagina, particularly in animals treated with a high dose of fenugreek seed powder. These histological features are comparable to those seen in estradiol-treated rats.



A – Untreated female rats, B – Ethinyl-estradiol treated rats, C – Ovariectomized rats treated at low dose aqueous fenugreek extract (450 mg/kg/day), D – female rats treated at high dose aqueous extract of fenugreek (900 mg/kg/day), E – Rats treated at low dose fenugreek powder (450 mg/kg/day), F – Rats treated with high dose fenugreek powder (900 mg/kg/day)

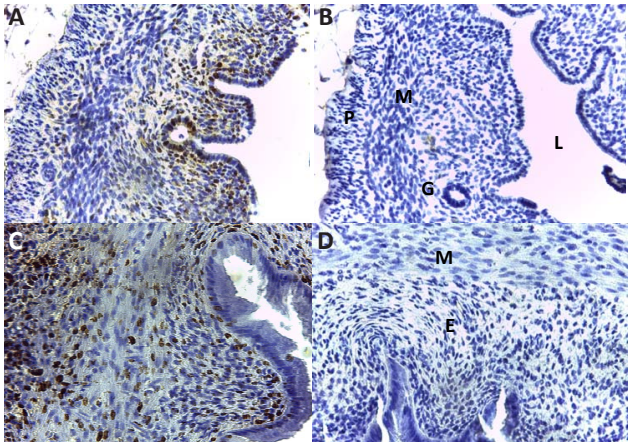
E: Epidermis; D: Derme; K: Corneal cells

Figure 8. Representative histological analysis of the vagina in rats treated with fenugreek seeds (G X 100)

EFFECT OF TREATMENTS ON PROGESTERONE RECEPTOR (PR) EXPRESSION BY IMMUNOHISTOCHEMICAL ANALYSIS

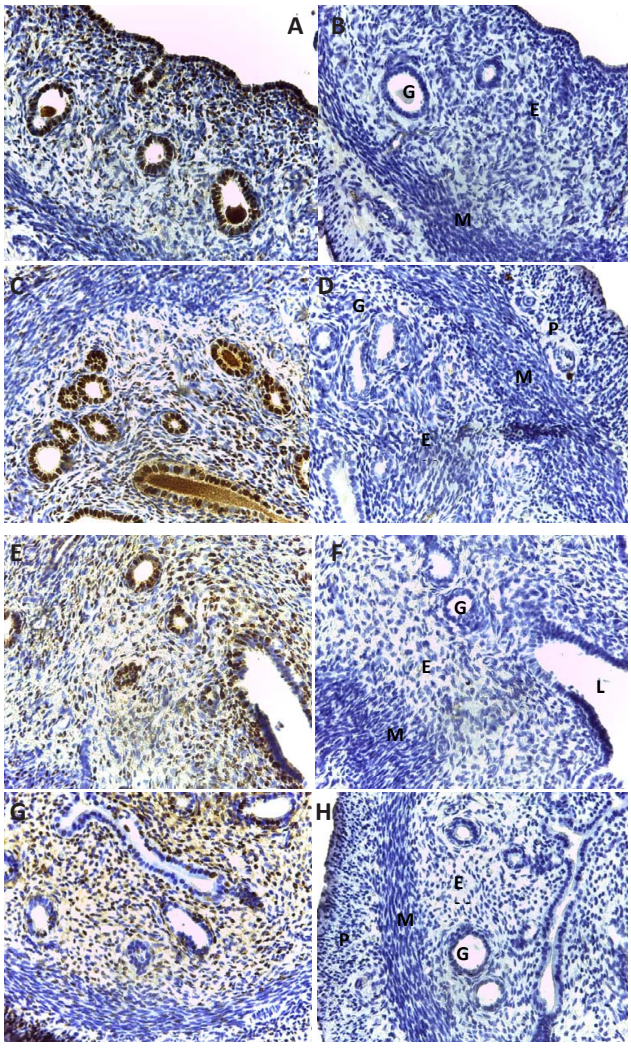
The expressions of PR in the uterus from each group are shown in Figure 9. Treatment with ethinyl estradiol induced clear expression of PR in the chorion and smooth muscle cells; no staining is observed in the uterine epithelium.

In ovariectomized animals treated with fenugreek seeds, a very intense endometrial staining is observed. Overexpression of PR is noted particularly in the epithelium and extensively developed endometrial glands (Figure 9). These data further support the indication that fenugreek seeds had very potent estrogenic activity.



Expression of progesterone receptors in the uterus of untreated ovariectomized control rats (A) and ovariectomized rats treated with ethinyl estradiol (C). B and D are negative controls
P: Perimeter; M: Myometrium; E: Endometrium; L: Light; EL: Luminal epithelium; G: Uterine gland

Figure 9. Effects of ethinyl estradiol treatment on uterine expression of progesterone receptors (G X 40)



Expression of progesterone receptors in the uterus of female rats treated with fenugreek seed aqueous extracts at dose 450 mg/kg/day (A) or 900 mg/kg/day (C) and rats treated with fenugreek seed powder at dose 450 mg/kg/day (E) or 900 mg/kg/day (G). *In the figure B, D, F and H are negative controls*

P: Perimeter; M: Myometrium; E: Endometrium; L: Light; EL: Luminal epithelium; G: Uterine gland

Figure 10. Effects of fenugreek seed treatments on uterine expression of progesterone receptors (G X 40)

DISCUSSION

The aim of the present study was to evaluate the estrogenic effects of fenugreek seeds, aqueous extract or powdered form, in ovariectomized rats, using uterotrophic bioassay standardized by the OECD (Organisation for Economic Co-operation and Development) [14]. This evaluation is carried out for the first time in this work by assays consisting of morphological observations, histological analysis of uterine and vaginal tissues, combined with immunohistological study to evaluate the expression of progesterone receptors and the transcriptional target of estrogens.

The study presented here shows that Fenugreek seeds possess real uterotrophic-like activities at the doses commonly used, and that the aqueous extract used in traditional Moroccan medicine has similar activity to fenugreek powder. Fenugreek seeds contain phytoestrogens with estrogenic activity that would allow an increase in the uterine weight, epithelial cell proliferation and vaginal cornification. Phytoestrogens may elicit estrogen-like effects through estrogen receptors that abound in target tissues. A critical action of estrogen activity is to induce intracellular synthesis of progesterone receptors that would lead to a rapid extension of stromal and glandular cell proliferation [15,16].

After 7 days of administration of fenugreek seeds, no dose significantly affects the body weight gain in female rats. This result is in agreement with those of Petit *et al*. (1995) and Harchane *et al.* (2012) [5,17] who have shown that a body weight gain related to the increase in food intake is effective only after two weeks of fenugreek-treatment. On the contrary, estradiol causes a significant decrease in food intake and meal size, and a concomitant decrease in body weight, compared to the control group. A similar reduction in body weight was obtained in ovariectomized rats exposed to ethinyl estradiol [18]. In ovariectomized rats, estradiol made them lose appetite [19]. This decrease in meal size is explained by the anorexigenic effect of estradiol, by acting on appetite control centers, and by potentiating the positive effect of cholecystokinin on satiety [19,20].

Vaginal opening is the first visible signs of the estrogenic effect of fenugreek seeds [12]. It was observed in 84-100% of the ovariectomized rats exposed to fenugreek for 7 days and in all female rats treated with estradiol. This causal link between the estrogenic effect of plant extracts and vaginal opening in ovariectomized female rats has been established in other studies, such impact evaluation of *macerated aqueous* extracts of the leaves of *Holarrhena floribunda* L.[21], leaves of *Sarcocephalus latifolius* [18] or those of petroleum ether extract of *Citrus medica* seeds [22].

Vaginal opening is an apoptosis-mediated event used as an external index of puberty onset [12,23]. It occurs as a result of increasing estradiol secretion and can be stimulated with an injection of estradiol into immature rats [24], whereas vaginal opening in the rat occurs simultaneously with vaginal cornification [25]. This finding was confirmed by an increase in the number of epidermal cells with a tendency to keratinization of the vaginal epithelium in fenugreek-treated rats, compared with control and estradiol-treated rats.

In addition, fenugreek seeds produced a trend towards an increase in uterine horn size and in the wet and dry weight of the uterus, but without statistical significance. However, these changes remain modest compared with the effects observed in the estradiol-treated female group.

Similar estrogenic activities have been noted in other studies, notably the effects of macerated aqueous extracts leaves of *Holarrhena floribunda* L. [21], and of the methanolic and aqueous extracts of *Ficus asperifolia* L. [26] or treatment with different extracts of *Glycine max* L. [27]. The estrogenic properties of these plants might be due to the common presence of flavonoids (12.68 to 17.6 mg quercetin/g, 39.90 mg Rutin equivalent/g and 0.68 to 2.13 mg quercetin/g, respectively) [21,26-30]. Concentrations of total phytoestrogens in our tested fenugreek extracts (450 and 900 mg/kg/day) were 16.87 to 22.91 mg eq quercetin equivalents/g, respectively.

Histologically, it is well known that ovariectomy in rats is usually followed by uterus atrophy, reduction in cell proliferation and an increase in cell apoptosis [31,32]. Conversely, administration of estradiol induces endometrial thickness, mainly due to intense stromal cell proliferation accompanied by an increase in uterine weight and in the epithelial cells height secreting a luminal fluid, as described by Aguilar and Reyley (2005) and Jefferson (2006) [33-35].

Comparatively, the uterus of a fenugreek seed treated animals is relatively larger than that of the control group. These changes are revealed mainly by the development of the endometrium, with an increase in luminal epithelium height from 20 to 200% compared to the untreated control rats, and in numbers of uterine glands. Comparable results were obtained with methanolic extracts of *Millettia conraui* bark, leaves of *Bridelia ferruginea* [32], and also onion extract [36]. More pronounced uterotrophic effects, mainly resulting in proliferation of endometrial and myometrial cells, were obtained by treating ovariectomized female rats with different soy extracts [27,37]. This uterotrophic effect of estrogens or compounds with estrogen-like activity has been described as biphasic. Water infiltration into epithelial cells is the earlier marker of estrogen action in uterus. This uterine water imbibition, due to enhanced microvascular permeability, increases the uterine weight without being necessary followed by cell proliferation [32,38,39].

Otherwise, fenugreek seeds significantly increased vaginal epithelial height and vaginal cornification after 7 days of treatment compared to untreated ovariectomized rats. A same result was obtained in rats treated orally with other plant extracts, *Nauclea latifolia*, *Millettia drastica*, *Bridelia ferruginea*, and *Millettia conraui* [32].

Furthermore, the estrogenic action of fenugreek seeds has been confirmed immunohistochemically by an overexpression of progesterone receptors in fenugreek treated animals as in controls given to ethinyl-estradiol. Indeed, it is well known that progesterone receptors are critical effectors of estrogen receptor signaling required for uterine development and thus prepare the secretory phase of the menstrual cycle [40].

In untreated controls and in the absence of the ovaries, which are the main source of estrogen, the week expression of endometrial progesterone receptors is certainly due

to the action of steroids secreted by peripheral conversion of androgens circulating in the adrenal glands and adipose tissue.

Considering all findings, the result of the present study support the estrogenic activity of the fenugreek seeds aqueous extract that may be attributed to the phytoestrogens and it may be a therapeutic agent for treating some fertility problems and certain metabolic disturbances, such as those related to menopause. However, further study is warranted to understand the exact cellular and molecular mechanism involved in Fenugreek's phytoestrogen mediated health benefits.

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