

Pharmacological Activity of *Trigonella foenum-graecum* Seed Extract on Reproduction of Virgin Rabbits

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Abstract: Fenugreek has been used in folk medicine to treat many disorders and possesses phytoestrogenic properties which appear to have both estrogenic and anti-estrogenic effects. The study aimed to investigate the pharmacological effects of fenugreek on reproductive activity of virgin rabbits. Alcoholic extract of the fenugreek seeds (*Trigonella foenum-graecum*) was prepared and dissolved in tween 80 and distilled water to obtain 20% suspension for use. Biological assays screening were done for detection of estrogen-like activity of the extract in immature female mice. *In vivo* study was carried out on virgin rabbits. Thirty immature rabbits (1-1.2 kg b. wt.) were divided into three groups (10 rabbits/group). The first group was given 0.1 ml/ kg. b.wt. olive oil subcutaneously daily for 30 days (a control group). The second group (standard group) was given 0.4 µg / kg. b.wt. oestradiolmonobenzoate daily by s/c administration for 30 days. The third group was given 800mg/kg. b.wt. fenugreek ethanol extract orally for the same period. The blood samples were used for determination of hemogram and serum samples for hormonal assay. The animals were slaughtered at 30th days of experiment and the ovaries and uteri were dissected out and weighed. The oocytes were collected from the ovarian follicles and cultured *in vitro* for maturation in TCM-199. The oocytes were cultured in maturation medium for 24 hrs under conditioned environment (95% relative humidity, 5% CO₂ at 38°C). Phytochemical screening of active constituents of fenugreek ethanol extract contain carbohydrates and / or glycosides, flavonoids, tannins, steroids and / or triterpenoids, alkaloids, resins and saponins. All the tested extracts produced significant oestrogen-like activities with various degrees according to the given dose ($p < 0.05$, 5mg/10g b.wt. and $p < 0.01$, 10mg/10g b.wt.) in rats. In rabbits, fenugreek (800mg/kg.b.wt.) produced significant oestrogen-like activities ($p < 0.001$) increased significantly both uterine and ovarian weights as compared to the control group. In immature rabbits given fenugreek seed extracts, the percentages of large follicular oocytes were 20%, 51% and 50% for olive oil, oestradiolmonobenzoate and fenugreek extract, respectively. Also, improved the quality of oocytes (78% vs. 65%). The maturation rate of rabbit oocytes *in vitro* elevated greatly in the group given oestradiolmonobenzoate (82%); or fenugreek extract (80%). In addition, the fertilization rates were increased to 45% and 44% in groups given oestradiolmonobenzoate and fenugreek ethanol extracts, respectively, as compared to the control group given olive oil (25%).

Key words: Fenugreek-Estrogenic Activity • Biological Assay • IVM-Oocytes

INTRODUCTION

Fenugreek (*Trigonella foenum-graecum* L.) is an old annual medicinal plant from the family of Papilionaceae-Leguminosae and is extensively cultivated in various regions of the world like small Asia, Iran, Egypt, Algeria,

India, Morocco, Italy, some parts of England and Spain [1].

Most of the medicinal properties of fenugreek are found in the seeds, which have been used for thousands of years in Greco-Arab and Islamic medicine as well as in Indian and Chinese medicine [2].

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It was used in old Egypt as incense, for mummifying corpses, for easy confinement and increase in milk yield. Even recently Egyptian women use this plant for curing menstrual pain, as a tea for stomach problems of tourists and also as a complement matter for wheat and corn flours for baking breads and confectionaries [3].

In ancient Chinese drugs, seeds of fenugreek were used as strengthen drug [4]; as sterilizer, mild laxative, diuretic, in treatments of bronchial inflammation, leprosy, hemorrhoids and mouth deodorant [5] and as joint pain reliever, pulmonary and bone tuberculosis and it is used for increase in weight [1].

Fenugreek posses a phytoestrogenic properties which appear to have both estrogenic and anti-estrogenic effects[5, 6]. Therefore, these have been considered as a part of selective estrogen receptor modulators (SERMs) and studied as an alternative for hormone replacement therapy [7]. Fenugreek is used as an abortifacient [8], inducelabour, promote the lactation for breast feeding, reduce menstrual discomfort, minimize symptoms of menopause [9-11] and as aphrodisiac [12].

The deflated seeds are rich source of steroids [7]. However, studies on fenugreek seeds [13] and its extract [14].have been reported to affect the fertility in male and female rabbits.

In addition, Mehrdad *et al.* [15] found that the extract of fenugreek seed stopped folliculogenesis and destroyed ovarian tissue in female mice.

Fenugreek seeds have been shown to possess estrogenic activity that disturbs the endometrial lining system and interferes with fetal development in female rabbits [13, 16] prominent congenital disorders including hydrocephalus, anencephaly, cleft palate and spina bifida were reported among women who consumed fenugreek seeds during pregnancy [17]. Khalki *et al.* [18] reported that fenugreek supplementation 1 g/kg/day to pregnant females during gestation period, decreased litter size, increase pup mortality, reduced body weights and formation of cleft palate and a bump on head in newborns. Another research group, Mozaffari *et al.* [19] highlighted that, fenugreek treatment (3.2 g/kg) to pregnant rats caused severe adverse alterations in rat fetus such as disorder in developing hind limb long bone. However, Aswar *et al.* [20] have reported that furostanol glycosides fraction of fenugreek did not change testosterone level and androgenic activity in rats.

Therefore the present study aimed to investigate the pharmacological effects of fenugreek on reproduction of female virgin rabbits.

MATERIALS AND METHODS

Plant: The fenugreek seeds (*Trigonellafoenumgraecum*, Family Fabacea), was purchased from a local market of Medicinal Plants and Herbs, Cairo, Egypt. The plant seeds were air-dried, pulverized and kept in tightly closed glass containers at room temperature till subjected to preliminary phytochemical screening.

Animals:

Mice: Immature female mice (10-15 g. body weight) were used for revealing the oestrogenic like effect of the tested plant extract. Mice were purchased from the laboratory unit, National Research Center, Dokki, Egypt.

Rabbits: Immature female rabbits weighing 1-1.2 kg body weight were used for revealing the effect of the tested extract on ovarian activity, serum levels of estrogen and progesterone, blood picture and histopathology of the uterus and ovaries.

Methods:

Preparation of Plant Alcoholic Extract: To each 250 grams of dried plant powder, one litter of ethyl alcohol 90 % was added in a wide mouth plastic container and kept for a night. On the next morning filtration of the container content was carried out using double layer gauze. The liquid alcoholic extract was evaporated in a Rota vapor apparatus (manufactured in GDR). The temperature required for evaporation of the solvent was 40 °C under reduced pressure for drying the ethanolic extracts till complete evaporation of alcohol. The obtained extracts were dissolved in Tween 80 and distilled water to obtain 20% suspension for use.

Phytochemical Screening of Active Principles of Fenugreek Seeds: The tested plant seeds (*Trigonellafoenumgraecum*) were subjected to the following phytochemical tests as explained by Claus [21].

- Tests for carbohydrates and / or glycosides (Molish's test, Fehling's test and Benedict's test).
- Tests for tannins.
- Tests for alkaloids and/ or nitrogenous bases (Mayer's reagent Dragendorff's reagent &Wagner's reagent) [22].
- Tests for Flavonoids (Shinoda's test).
- Tests for saponins (Froth test &Haemolysis test) [23]
- Tests for unsaturated sterols and / or triterpenes (Liebermann-Burchard test & Salkowisk's test).
- Tests for Resins:

***In vivo* Studies**

Estimation of the Biological Activity of Plant Extract on Genital Tract of Experimental Animals:

In Mice (Uterine Weight Test): Twenty eight immature female mice weighing 10-13 grams body weight were divided into four groups (n = 7). The first group was given olive oil (control group) by subcutaneous administration in a dose of 0.1 ml/ kg.b.wt. daily for 7 days. The second group (standard group) was given oestradiolmonobenzoate daily by subcutaneous administration in a dose of 0.4 µg / kg.b.wt. for 7 days. The third and the fourth groups were treated with fenugreek ethanol extract orally in a dose of 5 and 10mg/10 g, b.wt., respectively, daily for 7days. At the end of experiment the mice in each group were weighed on digital balance then they were sacrificed and their uteri were dissected and weighed.

In Rabbits: Thirty immature New Zealand White female rabbits were purchased from a herd in a commercial farm, for the purpose of this study. Rabbits were weighed 1-1.2 kg initial weight. Rabbits were individually housed in metal wire mesh cages provided with separate facilities for feeding and water supply. All rabbits offered a commercial ration pellets (Atmida Co.). Each rabbit was fed an amount of pellet ration (60gm/kg BW/day) that provides normal growth and maintains life. Fresh tap water was supplied *ad libitum*. Chemical analyses of pellets were performed according to A.O.A.C. [24].

The experiment was carried out at the experimental rabbit try of animal house lab., National Research Center, Dokki, Giza-Egypt. Rabbits were left two weeks for acclimatization before treatment. Daily observation of the experimental animals was conducted in order to record any change in the clinical signs.

Rabbits were divided into three groups (10 rabbits in each group). The first group was given olive oil by subcutaneous administration in a dose of 0.1 ml/ kg. b.wt. daily for 30 days and kept as a control group. The second group (standard group) was given oestradiolmonobenzoate daily by subcutaneous administration in a dose of 0.4 µg / kg. b.wt. for 30 days. The third group was given fenugreek ethanol extract orally in a dose of 800mg/kg. b.wt. daily for the same period.

The animals were slaughtered at 30th days of experiment and the ovaries and uteri were dissected out and weighed on an electric digital balance. The dissected ovaries from each group were divided into two halves, ten ovaries were preserved in 10% neutral formalin solution

for histopathological studies (for further studies) and other ten ovaries were used for *in vitro* maturation (IVM) and fertilization (IVF) of oocytes.

***In vitro* maturation of rabbit oocytes**

Material:

The Media:

Aspiration Medium: Modified Phosphate Buffer Saline (m.PBS): Modified Dulbecco's phosphate buffer saline solution (m.PBS) was used for aspiration and searching of oocytes. It prepared in the lab., from pure ingredients and stored as stock solution, preserved in refrigerator up to 3 months. For preparing the work solution, a suitable volume of the stock solution is supplemented with sodium pyruvate (0.036 g/l), glucose (1.0 g/l), bovine serum albumin (3.0-4.0 g/l) and antibiotic antimycotics (penicillin & streptomycin- CID Co. Pharm. Cairo).

Maturation Medium (TCM-199): Tissue culture medium-199 with Earl's salt, L-glutamine and 25mM Hepes (Sigma, USA) obtained in liquid form and stored in refrigerator at 5°C till used. TCM-199 was supplemented with 10% FCS (Sigma, lot 33K3396, U.S.A.) and gentamycin 20mg (Memphis Co. for pharm. and Chem. Ind-Cairo) at a dose level (1ul/1ml) at the time of use. Medium was used for oocytes washing and maturation.

Preparation of solutions (capacitating medium) according to the method of Brackett and Oliphant [25].

Method

Ovaries: At slaughtering time the rabbits were weighed, their weights ranged from 2.20 to 2.50 kg. The ovaries were collected within 20 minutes after slaughtering of the rabbits and kept in physiological saline (0.9% NaCl) containing antibiotics (100 µg/ml Streptomycin and 100 i.u./ml Penicillin) at 32-35°C. The ovaries were transported from Animal House to the laboratory within 30 minutes after slaughtering [26]. In the Lab. the ovaries were dissected from surrounding tissues, to eliminate adherent blood, tissues and surface organisms, The ovaries were washed three times in warm normal saline solution then dipped in ethyl alcohol (70%) for 30 seconds before re-rinsed in normal saline, rinsed several times in normal saline then transferred to a glass Petridishes containing 5 ml of modified phosphate buffer saline (M-PBS)

Oocytes Collection: The oocytes were harvested from the ovaries through puncturing the visible ovarian follicles using sterilized syringe needle, then the ovaries were sliced into small pieces to recover more oocytes from

invisible follicles. The ovarian tissues were removed from the Petridishes to facilitate oocytes searching under stereomicroscope (Correct, Tokyo, Seiwa Optica, Japan). The oocytes were picked up using prepared sterilized glass Pasteur pipette with suitable pore. The collected oocytes were washed three times in M-PBS, examined under stereomicroscope for classification and evaluation.

Oocyte Classification (Grades): According to Leibfried and First [27], the oocytes were classified under the stereomicroscope according to the appearance of the cumulus surrounding the oocyte into three categories,

Grade 1: Oocytes with complete compact dense cumulus oophorus more than three layers.

Grade 2: Oocytes with compact cumulus layer not completely surrounding the oocyte.

Grade 3: Oocytes enclosed only the zonapellucida without cellular investment.

Only the oocytes surrounded by compact layers of cumulus cells (Grade 1) were used for maturation and fertilization.

In vitro Maturation of Oocytes: The selected oocytes were washed three times in maturation medium TCM-199 (pH 7.2-7.4) supplemented with fetal calf serum and antibiotics. The oocytes (10-20 oocytes) were placed in 50µl-100µl droplet of maturation medium in a four- well culture plate (Nunc, Denmark). Covered with sterilized mineral oil (Sigma, USA). The culture dishes were placed in a CO₂ incubator (95% relative humidity, 5% CO₂ at 38°C) for 24 hrs. (NBS CO₂ incubator, New Brunswick Scientific- Edison, N.J., U.S.A.).

Assessment of Maturation: After 24 hrs, maturation rate was assessed according to the degree of cumulus cells expansion [28].

Sperm Capacitation:

Brackett and Oliphant Medium (BO): Spermatozoa were capacitated using either BO medium. Freshly collected rabbit semen ejaculate were in water bath at 37 °C until used for capacitation. Spermatozoa were washed twice at 2500 r.p.m. for 5 min using semen washing solution of BO medium Brackett and Oliphant, [25] Supplemented with 20µg/ml heparin plus 3.383mg/ml caffeine [29], then, spermatozoa were maintained in CO₂ incubator at 5% CO₂ for 1 hr for capacitation [30]. The

spermatozoa were re-suspended in 1 ml of semen diluents solution of BO medium and the sperm number was counted using haemocytometer and adjusted to be 2x10⁶ sperm/ml.

In vitro Fertilization Procedure:

Preparation of Semen for in vitro Fertilization

- Fresh diluted semen were diluted with 6ml of SWS in a centrifuge tube, mixed gently, centrifuged at 1850 rpm (485xg) for 5 min (in warm water).
- Supernatant was decanted by sterilized pipette, the washing was repeated two times.
- 0.5-0.8 ml of semen washing solution (SWS) was added to the sediment with pipette and the volume was measured (initial volume).
- The concentration of sperm cell was counted by adding 50µl of sperm suspension to 4.95 ml of 3 % NaCl, mixed and the sperm cell concentration was counted by haemocytometer as follow:
- Sperm cell concentration = 50000 x N x D ml
- Where: N = number of counted sperm, D = rate of dilution.
- Adjust sperm cell concentration first to 2 x 10⁶ sperm/ml by adding SWS and SDS (1: 1 v/v).
- Rate of dilution = sperm cell concentration x initial volume x motility %
- 2 x 10⁶
- Once established the insemination dose (2 x10⁶ sperm/ml) 4 drops (100 ul/drop) was prepared, covered with sterilized and equilibrated paraffin oil, kept in CO₂ incubator for 30 minutes[31].

The *in vitro* matured oocytes were co-cultured with the sperm suspension (BO-medium supplemented with heparin and caffeine). Every five to ten matured rabbit oocytes were washed three times with oocyte washing solution and transferred to the sperm micro droplet which was already prepared. Spermatozoa and oocytes were co-cultured for five hours in CO₂ at 38.5 °C under 5 % CO₂ and 90% relative humidity. The oocytes were washed three times with culture media (TCM-199) supplemented with 10% FCS and 1% antibiotic-antimycotic (Gentamycin), then transferred to 50 µl droplets of the same medium and cultured for 6 days at 38.5 °C in CO₂ incubator (5% CO₂ and 90% relative humidity). After 48 h of insemination, the oocytes were then examined for fertilization as indicated by cleavage rate. The criterion for fertilization was cleavage to two-to four- cell stage 48 hours after insemination [32].

Statistical Analysis: Statistical analysis was carried out by using one way ANOVA followed by Duncan test and SPSSversion 9.0. the difference of means at $p < 0.05$ considered significantly [33].

RESULTS

Preliminary phytochemicals screening of active constituents of fenugreek ethanol extract contain carbohydrates and / or glycosides, flavonoids, tannins, steroids and / or triterpenoids, alkaloids and resins. Concerning saponins and Saponinsas recorded in Table (1).

The effect of fenugreek ethanol extracts on body weight, uterine weight in immature mice was recorded in Table (2). The results revealed that subcutaneous administration of oestradiol mono benzoate (standard group) in a dose of $0.1 \mu\text{g} / 10\text{gm.b.wt.}$ caused significant ($p < 0.01$) increase in uterine weight of immature mice. This increase reached to 108.75 ± 7.88 (mg) compared to 65.00 ± 5.00 and (mg) of the control group. All the tested extracts produced significant oestrogen-like activities with various degrees according to the given dose. With a significant ($p < 0.05$) in a dose of $5\text{mg}/10\text{gm b.wt.}$ and higher significant ($p < 0.01$) in a dose of $10\text{mg}/10\text{gm b.wt.}$ Oral administration of fenugreek ethanol extracts in a dose of $5\text{mg}/\text{kg.b.wt.}$ to immature mice resulted in significant ($p < 0.05$) increase in uterine weight that reached to 88.63 ± 4.75 mg compared to 65.00 ± 5.00 mg of the control group (olive oil). fenugreek ethanol extract when given orally in a dose of $10\text{mg}/10\text{gm.b.wt.}$ to immature mice showed significant ($p < 0.01$) increase in uterine weight to 90.65 ± 3.15 mg as compared to 65.00 ± 5.00 mg of the control group.

The effect of tested ethanol extract on body weight, uterine weight and ovarian weight in immature rabbits was recorded in Table (3). The results revealed that subcutaneous administration of oestradiol mono benzoate (standard group) in a dose of $0.4 \mu\text{g} / \text{kg.b.wt.}$ caused significant ($p < 0.001$) increases in both uterine and ovarian weights of immature rabbits. These increases reached to 7.02 ± 0.65 and 0.30 ± 0.01 (g) compared to 3.16 ± 0.09 and 0.14 ± 0.04 (g) of the control group, respectively. All the tested extracts produced significant oestrogen-like activities with various degrees. Fenugreek ethanol extract when given orally in a dose of $800\text{mg}/\text{kg.b.wt.}$ to immature rabbitsshowed significant ($p < 0.001$) increases in both uterine and ovarian weights to 6.95 ± 0.37 and 0.26 ± 0.01 (g) as compared to the control group, respectively (3).

Table 1: Preliminary phytochemical screening of active constituents of fenugreek ethanol extracts.

Active constituents	Extracts Tests	Fenugreek extract
Carbohydrate and / or glycosides	Molish's test	+ve
	Fehling's test	+ve
	Benedict's test	+ve
Flavonoids	Shinoda's test	+ve
Tannins	Ferric chloride test	+ve
	Phenazone test	+ve
Sterols and / or triterpenes	Liebermann- Burchard test	+ve
	Salkowisk test	
Alkaloids	Mayer's R.	+ve
	Dragendorff's R.	+ve
	Wagner's R.	+ve
Saponins	Froth test	+ve
	Haemolysis test	+ve
Resins	Turbidity test	+ve

R= Reagent +ve: means positive-ve :means negative

Table 2: Effect of oral administration of fenugreek ethanol extract at two dosage levels on the uterine weight in immature mice. (n = 7 mice)

Groups and doses	Body weight (gm)	Uterine weight (mg)
Olive oil (0.1 ml/ kg. b.wt.)	12.11 ± 0.18	65.00 ± 5.00
Oestradiol (0.4 μg / kg.b.wt.)	11.73 ± 0.12	$108.75 \pm 7.88^{**}$
Fenugreek (5mg / 10gm b.wt.)	11.75 ± 0.13	$88.63 \pm 4.75^{*}$
Fenugreek (10mg / 10gm b.wt.)	11.55 ± 0.16	$90.65 \pm 3.15^{**}$

* Significant at $p < 0.05$ ** Significant at $p < 0.01$

As shown in Table (4) the recovery rate of oocytes / ovary of immature rabbits given either olive oil (control group) or oestradiolmonobenzoate (standard group) for 30 days was 60% or 96%, respectively. In immature rabbits given orally fenugreek ethanol extracts, the recovery rate was 103.2%. Concerning the follicular size the percentages of large follicular oocytes were 20%, 51% and 50% for olive oil, oestradiolmonobenzoate and fenugreek extract, respectively. The percentages of medium follicular oocytes were 69%, 42.3% and 39.7% for olive oil, oestradiolmonobenzoate, fenugreek extract, respectively. The percentages of small follicular oocytes were 11%, 6.7% and 10.3% for olive oil, Oestradiolmon obenzoate and fenugreek extract, respectively.

The quality, maturation and fertilization rates of rabbit oocytes *in vitro* of different groups are shown in Table (5). The quality of oocytes varied according to the treated group in comparison with control one. Subcutaneous administration with oestradiol mono benzoate ($0.4 \mu\text{g}/\text{kg b.wt.}$), and fenugreek ethanol extract ($800 \text{ mg}/\text{kg b.wt.}$) increased (80, and 78%) the grade I oocytes compared to 65% of the control group (olive oil), respectively. Grade II oocytes not greatly varied among treated groups, it averaged from 15, 15 and 20% in all

Table 3: Effect of oral administration of fenugreek seeds ethanol extracts to immature rabbits for 30 days on body weight, uterine weight and ovarian weight. n = (5 rabbits)

Groups	Body weight (kg)	Uterine weight (g)	Ovarian weight (g)
Control (olive oil, 0.1 ml/ kg. b.wt.)	2.36 ± 0.25	3.16 ± 0.09	0.14 ± 0.04
Standard (Oestradiol, 0.4 µg / kg.b.wt.)	2.50 ± 0.14	7.02 ± 0.65***	0.30 ± 0.01***
Fenugreek (800 mg / kg.b.wt.)	2.50 ± 0.28	6.95 ± 0.37***	0.26 ± 0.01***

* Significant at $p < 0.05$; ** Significant at $p < 0.01$, *** Significant at $p < 0.001$

Table 4: Effect of oral administration of fenugreek ethanol extracts to rabbits for 30 days on follicular dynamics and recovery rate in immature rabbits. (N = 5 rabbits)

Groups	No. of ovaries	T. No. of oocytes	R. rate / ovary	Follicular size					
				Large		Medium		Small	
				No.	%	No.	%	No.	%
Olive oil (0.1ml/kg. b. wt.)	10	600	60.0	120	20.0	414	69.0	66	11.0
Oestradiol (0.4 ig /kg. b. wt.)	10	960	96.0	490	51.0	406	42.3	64	6.7
Fenugreek (800mg/ kg. b. wt.)	10	1032	103.2	516	50.0	410	39.7	106	10.3

Table 5: Effect of oral administration of fenugreek seed ethanol extract to immature rabbits for 30 days on quality of recovered oocytes, maturation and fertilization rates *in vitro*.

Groups	Quality of oocytes (%)			Maturation rate (%)	Fertilization rate (%)
	Grade I	Grade II	Grade III		
Olive oil (0.1ml /kg. b. wt.)	65	20	15	65	25
Oestradiol (0.4 ig /kg. b. wt.)	80	15	5	82	45
Fenugreek (800mg/ kg. b. wt.)	78	15	7	80	44

treated groups given oestradiolmonobenzoate, fenugreek ethanol extract, respectively in comparison with control group (20%). The maturation rate of rabbit oocytes *in vitro* elevated greatly in group given oestradiolmonobenzoate (82%); or fenugreek extract (80%). In addition, the fertilization rates were increased to 45% and 44% in groups given oestradiolmonobenzoate and fenugreek ethanol extracts, respectively, as compared to the control group given olive oil (25%).

DISCUSSION

Phytochemical Constituents: In the present study the phytochemical screening of fenugreek seed extract revealed that fenugreek ethanol extract contains carbohydrates and/or glycosides, flavonoids, tannins, sterols and/or triterpens, alkaloids and saponins. These findings are in accordance with those of Mishkinsky *et al.* [34], Skibola and Smith, [35]. Flammang *et al.*, [36], they mentioned that trigonelline is an important alkaloidal component of the seeds of fenugreek. These results also confirms previous finding Sharma [37] Petit *et al.*, [38], Skibola and Smith, [35] who said that fenugreek seeds are rich in saponins. Hemavathy and Prabhakar [39] mentioned that the total lipid content (7.5 per cent) of the fenugreek seeds consists of neutral

lipids, glycolipids and phospholipids. Varshney and Sharma [65] 40 reported that the fenugreek seeds were also known to contain flavonoids, carotenoids, coumarins and other components with very low LD₅₀ values.

Biological Activity of Fenugreek Extract: Our study revealed that fenugreek ethanol extracts in a dose of 5mg/10 gm.b.wt. increased significantly the uterine weight of immature female mice compared with the control group (olive oil). This finding is in accordance with the finding of Al-Hamood and Al-Bayatti [41] who mentioned that the effect of fenugreek on the uterus was attributed to a possible estrogenic activity. This finding is opposed to previous finding of Sharma and Bhinda [42] who revealed that the uterine weight was declined in adult female albino rats which were fed steroidal extract of *Trigonella foenum-graecum* (100 mg/day/rat for 15 days). The vaginal smears were also examined daily during the treatment and the female rats were mostly either in metoestrus or diestrus stage.

Uterine and Ovarian Weights in Rabbits: Fenugreek ethanol extract given in a dose of 800mg/kg.b.wt. to immature rabbits for 30 days increased both uterine and ovarian weights significantly, other researcher found

similar results, they reported that Fenugreek possesses phytoestrogenic properties which appear to have both estrogenic and anti-estrogenic effects [5, 6]. This finding is opposed to the previous finding of Sharma and Bhinda [42] who revealed that the uterine and ovarian weights were declined in adult female albino rats which were fed steroidal extract of *Trigonella foenum-graecum* (100 mg/day/rat for 15 days). In addition, Kassem *et al.*, [13]; Sreeja *et al.*, [16] reported that Fenugreek seeds have been shown to possess estrogenic activity that disturbs the endometrial lining system which may affect on uterine condition and weight as recorded in the present work.

In vivo and in vitro Studies of Fenugreek: Fenugreek ethanol extract given in a dose of 800mg/kg.b.wt. to immature rabbits for 30 days increased significantly the total number of oocytes/ovary and quality of oocytes (as it increased oocytes of grade I and grade II percents). It also improved oocytes maturation and fertilization rates compared to the control group (olive oil). These findings are in accordance with those of Aziza *et al.* [43] who reported that administration of fenugreek oil to Swiss albino female mice at 0.1 and 0.15 ml/mouse increased the total number of cumulus-oocyte complexes as well as improved their quality. Other investigators [12,13] have been reported that fenugreek extract affects the fertility in male and female rabbits, due to fenugreek possessing phytoestrogenic properties and as a rich source of steroids [7]. Others Mehrdad *et al.* [14] found that the fenugreek seed extract stopped folliculogenesis and destroyed ovarian tissue in female mice.

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