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GENETICAL AND PATHOLOGICAL STUDY ON EFFECT OF FENUGREEK ON KINETICS OF THE MICE TISSUES

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SUMMARY

In the present investigation, the effects of fenugreek (*T. foenum graecum*) administration on the liver and ovarian activity were studied. Seventy two Swiss albino female mice were orally administrated with different doses of fenugreek oil for 10 days. The mode and magnitude of effect was found to be depending on the dose of fenugreek oil and type of tissue. Administration of fenugreek oil at 0.1 and 0.15 ml/ mouse increased the total number of cumulus-oocyte complexes (COCs) as well as improved their quality. Cytogenetically, fenugreek oil was able to stimulate the oocytes collected from treated mice at different doses to progress in meiosis. Half of the oocytes number collected from female mice treated with fenugreek oil were arrived at GVBD and M I stages. However, most oocytes collected from untreated female mice were still in GV stage. Levels of nucleic acids content in all groups did not significantly change neither in the DNA nor RNA in ovarian- or liver-tissues. Histopathological examination of the ovaries collected from untreated mice as well as from mice treated with 0.05 ml/ mouse of fenugreek oil showed no histopathological alterations. However, ovaries of mice treated with 0.1 or 0.15 ml/ mouse of fenugreek oil showed numerous mature ovarian follicles as well as multiple corpora lutea. According to the available literatures, this is the first study that suggests significant stimulating effects of fenugreek oil on the ovarian activity in mice.

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INTRODUCTION

Fenugreek (*Trigonella foenum graecum*) is an annual herb belonging to the family Leguminosea, widely grown in India, Egypt, and Middle Eastern countries (Alarcon-Aguilara et al., 1998). Its seeds are commonly used for flavoring and as a spice in curries due to their strong flavor and aroma. Fenugreek contains 4,5-dimethyl-3-hydroxy-2 [5H]-furanone, known as sotolon, which is frequently used as a flavoring agent for artificial maple syrup. Fenugreek natural extractives, oleoresins, and essential oils are generally recognized as safe (GRAS) approved (21 CFR 182.20), included by the Council of Europe in the list of substances granted Approval (COE No.460), and GRAS by the Flavor and Extract Manufacturerës Association (FEMA No.2485) (Flammang et al., 2004). Trigonella foenum graecum is one such plant whose seeds and leaves are used not only as food but also as an ingredient in traditional medicine. Its leaves are consumed widely in India as a green leafy vegetable and are a rich source of calcium, iron, β-carotene and other vitamins (Sharma et al., 1996). Trigonella foenum graecum seeds contain lysine and L-tryptophan rich proteins, mucilaginous fibre and other rare chemical constituents such as saponins, coumarin, fenugreekine, nicotinic acid, sapogenins, phytic acid, scopoletin and trigonelline, which are thought to account for many of its presumed therapeutic effects (Billaud, 2001). Various components of its seeds have varying activities such as fenugreekine, a steroidal sapogenin peptide ester has hypoglycemic properties (Jellin et al., 1999). It is shown to lower blood glucose level and partially restore the activities of key enzymes of carbohydrate and lipid metabolism close to normal values in various animal model systems (Amin et al., 2005). It can increase the erythrocyte insulin receptors and peripheral glucose utilization, thus showing improved pancreatic function (Raghuram et al., 1994). Therefore, fenugreek seeds are used as a traditional remedy for the treatment of diabetes and hypercholesterolemia in Indian and Chinese medicine (Basch et al., 2003; Miraldi et al., 2001). In Saudi Arabia, fenugreek was found to be the most common herb used among people with diabetes (Al-Rowais, 2002). Fenugreek have also been reported to exhibit pharmacological properties such as antitumor, antiviral, antimicrobial, anti-inflammatory, hypotensive and antioxidant activity (Cowan, 1999; Shetty, 1997). Fenugreek powder failed to induce any signs of toxicity or mortality in mice and rats who received acute and subchronic regimens (Muralidhara et al., 1999). Moreover, there were no significant histopathological changes in weanling rats fed fenugreek seed for 90 days (Rao et al., 1996).

As it is well known, ovary of the mammals produces of steroidal hormones. One of the steroidal hormones in the ovary is estrogens (α-estradiol, β-estradiol, estriol, and estrone), which play an important role in the estrous cycle (Ying and Zhang, 1999). During the mammalian estrous

cycle, 17 β-estradiol induces a sudden increase in gonadotropin-releasing hormone (GnRH) secretion from the neurosecretory cells of the hypothalamus, which in turn brings about a surge in luteinizing hormone (LH) secretion from the pituitary gland. This LH surge initiates final maturation of oocytes and the ovulatory process (Espey, 1999). Seeds of *T. foenum graecum* contain steroidal saponin such as gitogenin and traces of trigogenin and vitamin A (Jayaweera, 1981; Petit et al., 1995). This report evaluate the effect of *T. foenum graecum* oil at different doses on kinetics of ovarian activity, (oocyte quality and meiotic progression), changes in DNA and RNA content in liver and ovarian tissues as well as the histopathological alterations to determine the toxicity probabilities of fenugreek.

MATERIALS AND METHODS

Chemicals

Fenugreek oil was purchased from Elcaptain Company (CAP. PHARM., Egypt).

Animal and maintenance

Seventy two Swiss albino adult female mice (8 weeks old) were drawn from animal house of the National Research Center. The animals housed in several groups in rectangular polypropylene cages with dust-free paddy husk as bedding material. Prior to the experiments, they were acclimatized for one week by feeding on commercial pellet diet and water ad libidum.

Experimental design

The experiment was conducted to investigate the genetical and pathological effects of fenugreek. Seventy two animals, were divided into 4 groups (18 animals for each), as following: First group was served as control group (untreated); the other three groups were treated orally with fenugreek oil for 10 days successively at 0.05, 0.1 and 0.15 ml/ mouse. In all groups the nucleic acid content, meiotic progression test and histopathological examination were evaluated (6 animals in each analysis).

Cytogenetical analysis

Body weight and determination of nucleic acids

After the acclimatization period and prior to fenugreek treatment, animals were weighed. After oral administration of fenugreek oil for a continuous period of 10 days at different doses, all animals were weighed again then killed. The ovaries and liver of each mouse were excised, blotted, weighed and processed for determination of nucleic acid content. Contents of the nucleic acids (DNA and RNA) in the tissues of liver and ovary were determined according to the method of Dische (1955) and Schneider (1957). The tissues were homogenized using standard procedures (Peares, 1985) and were initially precipitated in 10 % cold trichloroacetic acid (TCA) followed by centrifugation for 10 min at 2500 rpm. The pellet was washed once with cold TCA and twice with

95% ethanol then digested in boiled mixture of 95% ethyl alcohol and diethyl ether (3:1). The pellet was centrifuged for 10 min at 2500 rpm and resuspended in 5% TCA. The supernatant was used for assessment of DNA and RNA content. DNA content was determined using diphenylamine method (Dische, 1955). The optical density of the resulting colour was read at wave length 600 nm. RNA contents were determined using orcinol method according to Schneider (1957). Optical density of the resulting color was read at 660 nm.

Collection of oocytes

After the treatment period female mice were killed by cervical dislocation. Mice ovaries were collected and transported in a warm (32-35°C) saline solution in small Petri cixhdx 935 x 10mm). Ovaries were washed three to four times usuing ogisogate byffered sakubne (PBS)> Oocytes were retrieved from the ovaries by slicing. In this method, ovaries were kept in a sterile Petri dish containing PBS. Ovaries were sliced with a sterile surgical scalpel blade into small pieces. The Petri dishes containing the ovarian pieces were screened under a stereo zoom microscope (WILD Heerbrugg, Switzerland) for oocytes. Oocytes recovered from ovaries were examined under a stereo microscope at x 35 to 45 and classified into one of three categories based upon the appearance of the surrounding cumulus cells as follows: Good: Oocytes with more than three layers of compact cumulus cell masses and a homogenous ooplasm; Fair: Oocytes with a homogenous evenly granulated ooplasm surrounded by fewer than three layers of granulosa cells; Poor: Oocytes with a homogenous evenly granulated cytoplasm surrounded by less than one layer of granulosa cells or loosely attached granulosa cells (partially denuded or naked oocytes) (Leibfried and First, 1979; Kolbe, 1998).

Staining and assessment of nuclear maturation

Cumulus cells were removed by incubating COC's in mDPBS (supplemented with 1 mg/ml polyvinyl alcohol, 0.1 % {w/v} porcine trypsin and 0,2 % EDTA {w/v}) for 20 min at 37°C. Oocytes were denuded mechanically by repeatedly pipetting them with a fine Pasteur pipette. Denuded oocytes of good and fair categories were subject to cytogenetical analysis to determine the nuclear maturation state. Immediately after removal of cumulus cells, the denuded oocytes were fixed in Carnoyís solution (25 % {v/v} acetic acid in ethanol) for at least 48 h at 4 °C and stained with 1 % (w/v) orcein in 45 % (v/v) acetic acid. For classification of different meiotic stages, the system described by Polanski and Kubiak (1999) was adopted.

Histopathological studies

Samples from mouse ovaries and liver in all experimental groups were collected and fixed in neutral buffered formalin 10%, washed in tap water overnight and exposed to ascending concentrations of ethanol (70, 80, 90 and 100%), cleared in

xylene and embedded in paraffin. Sections of the tissues (4-5 μ thick) were prepared and stained with Hematoxylin and Eosin for subsequent histopathological examination (Bancroft et al., 1996).

Statistical analysis

Analysis of differences between treatments was carried out according to the chi-square test (Snedecor and Cochran, 1982).

RESULTS

Cytogenetical analysis

Clinical signs, body weight and alterations in DNA and RNA content

Mice treated with fenugreek oil did not develop any clinical signs of toxicity either immediately or during the post-treatment period even at the highest dose (0.15 ml/ mouse). Oral administration of fenugreek oil did not cause any appreciable alterations in the feed intake (data not shown) during 10 days. Furthermore, there is no significant difference between the body weight gain during the observation period and the relative ovaries weights among the treated animals with low and highest doses (0.05 ml and 0.15 ml), comparable to their respective control (Table 1). However, there is a significant increase in the body weight of mice treated with fenugreek oil (0.1 ml/ mouse) compared to control group (Table 1).

Changes in DNA and RNA content in the liver and ovarian tissues of the albino female mice treated with fenugreek oil are shown in Table 2. Content of nucleic acid in the liver was significantly higher than that in the ovary. Whereas the levels of the DNA and RNA content in all treated groups did not significantly change compared with control group, neither in the liver nor in the ovary (Table 2).

Oocytes collection and meiotic progression analysis.

Numbers of mouse ovaries and oocytes per ovary were counted and classified according to the appearance of cumulus cells. A comparison of the recovery rate of the three different types of COCs is shown in table (2). Administration with fenugreek oil at different doses increased the total number of COCs as well as improved the quality of COCs (Table 2). At doses 0.1 and 0.15 ml/ mouse a significant increase in total number of the COCs (from 170 in control group to 219 and 265 in previous doses respectively) as well as a significantly improving in the percentage of good COCs (from 58.2 % in control group to 67%, 65% and 80% at doses 0.05, 0.1 and 0.15 ml/ mouse, respectively) was found (Table 3). There is no significantly different in fair COCs between treated groups and control. While, poor mice COCs were significantly decreased in group of 0.15 ml/ mouse in comparison to other groups (Table 3).

Table (1): Effect of fenugreek oil treatment on the body and ovary weights.

Fenugreek treatment (ml/ mouse)	Body weight		Weight of ovary
	Initial	*Final*	
Control (0)	15.8 ± 0.94	19.1 ± 0.44	0.17 ± 0.01
0.05	15.0 ± 0.86	19..3 ± 1.4	0.18 ± 0.01
0.1	15.8 ± 0.68	25..3 ± 1.6*	0.19 ± 0.01
0.15	16.0 ± 0.57	21.6 ± 1.2	0.19 ± 0.0

Table (2): Effect of fenugreek oil on the DNA and RNA content in liver and ovarian tissues of albino mice.

Fenugreek treatment (ml/ mouse)	DNA level (mg/g tissues)		RNA level (mg/g tissues)	
	Liver	Ovary	Liver	Ovary
Control (0)	0.45 ± 0.02	0.09 ± 0.01	0.18 ± 0.04	0.05 + 0.00
0.05	0.47 ± 0.02	0.10 ± 0.01	0.19 ± 0.03	0.05 ± 0.00
0.1	0.51 ± 0.02	0.10 ± 0.00	0.20 ± 0.01	0.05 ± 0.00
0.15	0.40 ± 0.01	0.09 ± 0.04	0.19 ± 0.01	0.05 ± 0.01

Table (3): Quality of cumulus-oocyte complexes (COCs) recovered from mice treated with fenugreek oil.

Fenugreek treatment (ml/ mouse)	No. of ovaries	Total No of COCs	Quality of COCs								
			Good			Fair			Poor		
			Total No.	%	No/ ovary	Total No.	%	No/ ovary	Total No.	%	No/ ovary
Control (0)	12	170	99	58	16.5±4.1	30	18	5.0±0.8	41	24	6.8±0.7
0.05	12	181	122	67	20.3±0.9	34	19	5.6±0.9	25	14	4.2±0.7
0.1	12	219	142	65	23.6+0.9**	48	22	7.8±0.9	29	13	4.8±0.7
0.15	12	265	221	83	36.8+4.0**	31	12	5.1±0.7	13	8	2.3±0.7

Table (4): Meiotic progression of the albino mouse oocytes after treatment with fenugreek oil.

Fenugreek treatment (ml/ mouse)	No. of ovaries	No. of oocytes	State of nucleus									
			GV		GVBD		MI		AI/TI		MII	
			No.	%	No.	%	No.	%	No.	%	No.	%
Control (0)	12	129	97	75.2	22	17.1	10	7.7	--	--	--	--
0.05	12	156	83	53.2	42	26.9	31	19.9	--	--	--	--
0.1	12	190	98	51.5	54	28.4	34	17.9	2	1.1	2	1.1
0.15	12	252	129	51.2	71	28.2	45	17.8	2	0.8	5	2.0

GV= Germinal vesicle, GVBD= Germinal vesicle breakdown, M I= Metaphase I,
A I/T I= Anaphase I/ Telophase I, M II= Metaphase II.

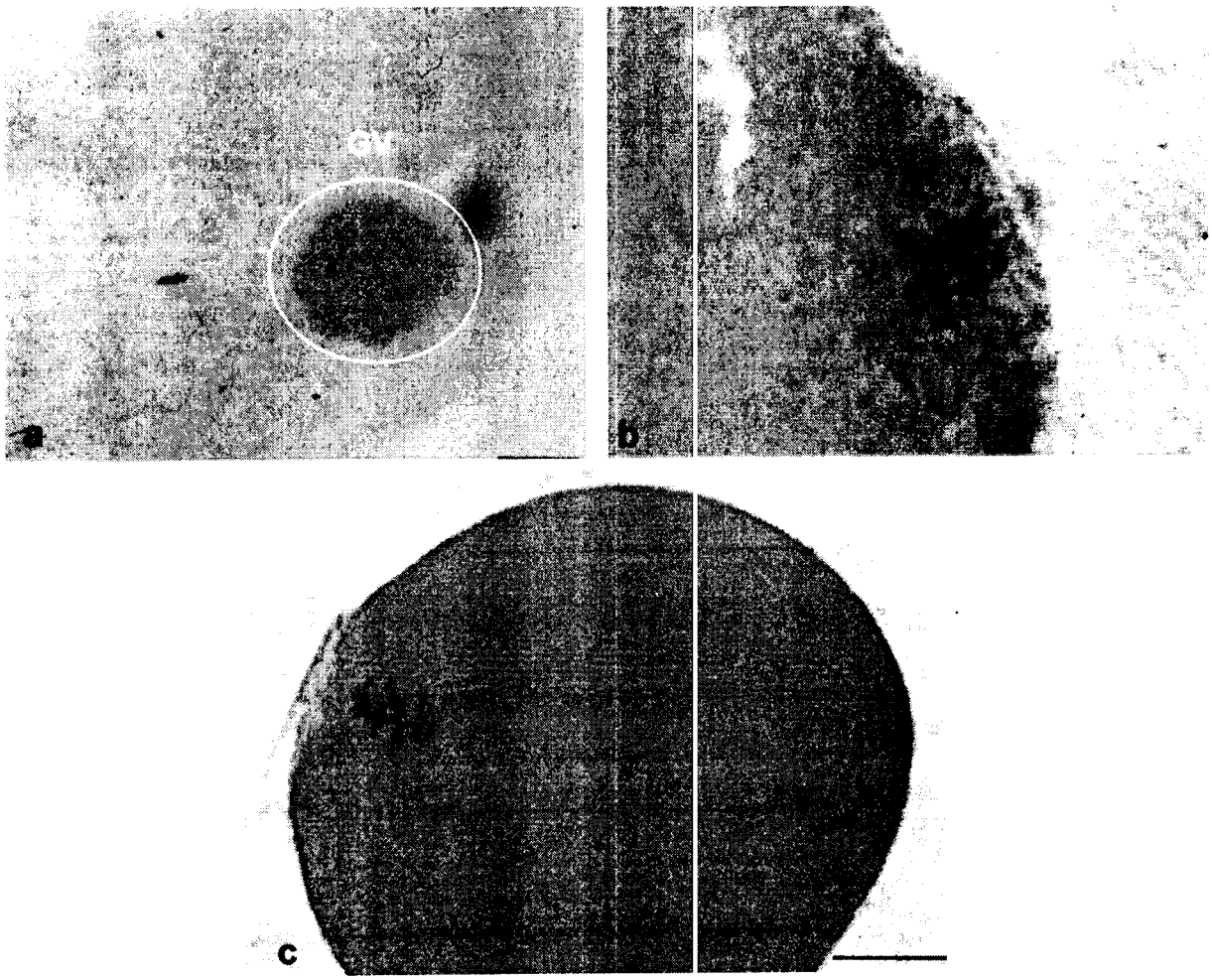

Fig. (1): Photomicrographs of stained whole mounted mouse oocytes representing various nuclear stages during maturat on. **(a)** Germinal vesicle stage (GV (NU) (bar = 20 um). **(b)** Germinal vesicle breakdown stage (bar = 20 um). **(c)** Metaphase I (bar = 15 um).

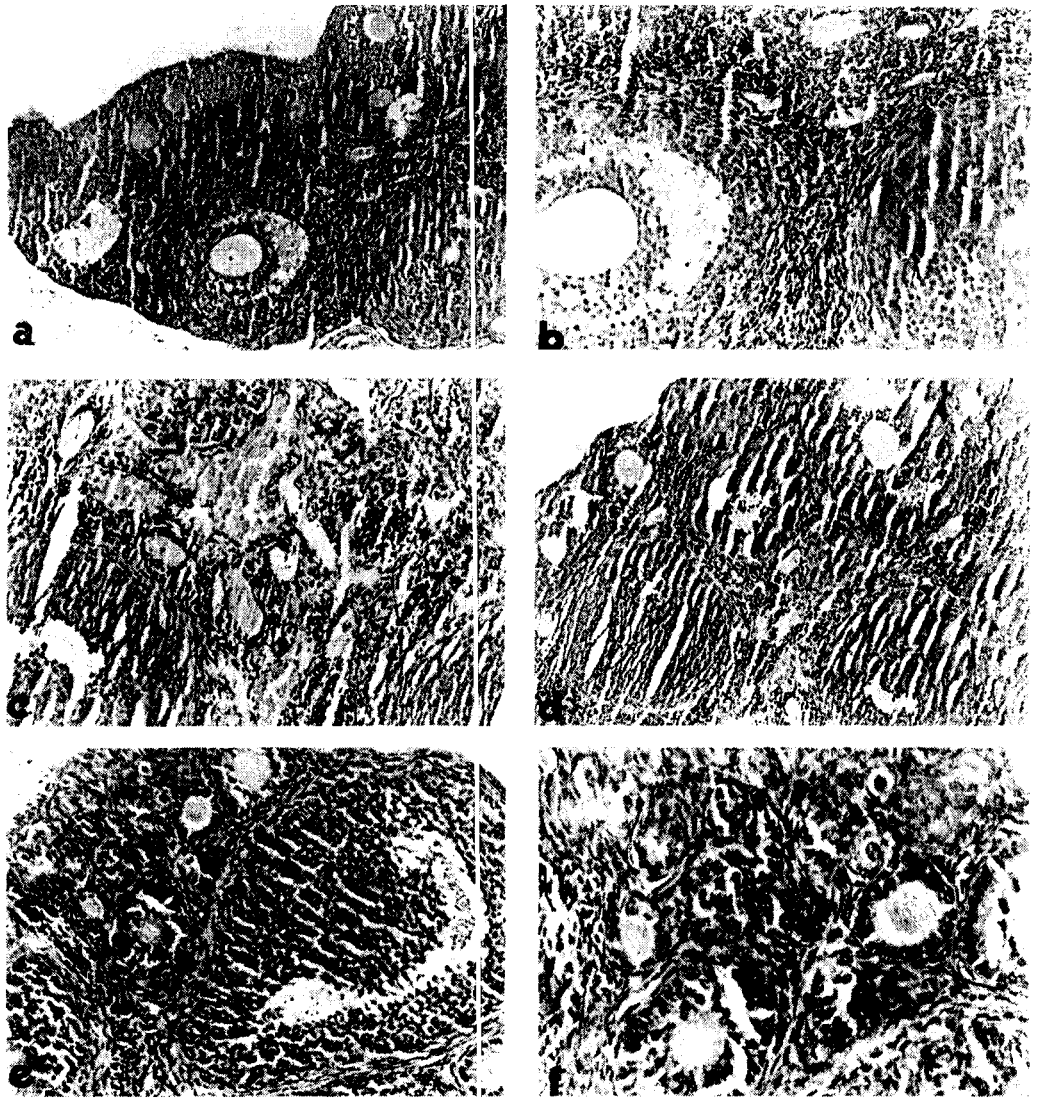

Fig. (2): Photomicrographs of: **a)** and **b)** C vary of control, untreated mouse showing normal developing follicles and corpus luteum. (H & E X 100 and X 200 respectively). **c)** and **d)** Ovary of mouse treated with fenugreek (0.1 ml/ mouse) showing **c)** congestion of interstitial ovarian blood vessels (arrows) (H & E X 200).**d)** Numerous mature ovarian follicles as well as multiple corpora lutea (H & E X 100)). **e)** and **f)** ovary of mouse treated with fenugreek (.15 ml/mouse) showing numerous active primordial follicles, primary and secondary follicles (H & E X 200 and X 400 respectively).

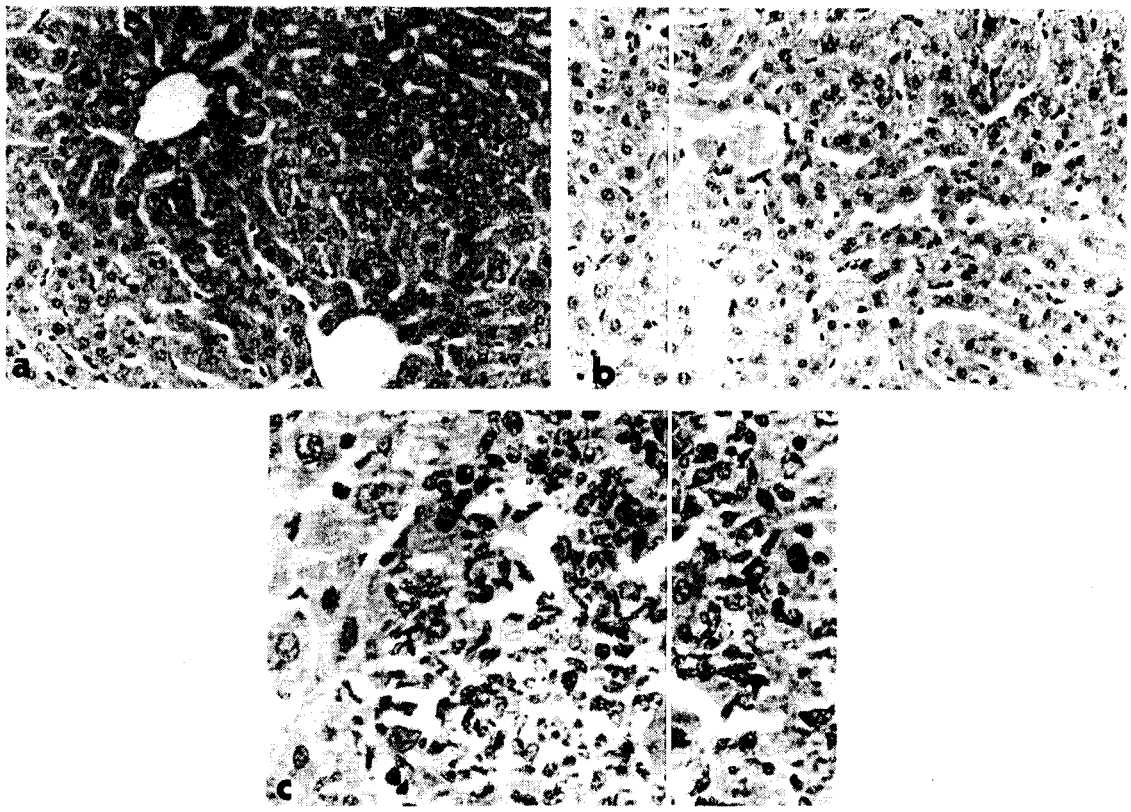

Fig. (3): Photomicrographs of liver of: **a)** control untreated mouse showing non histopatholigcal changes (H & E X200. **b)** Mouse treated with 0.1 ml/ mouse fenugreek showing slight activation of kupffer cells and slight congestion of central veins as well as hepatic sinusoids (H & E x200). **c)** Mouse treated with 0.15/mouse fenugreek showing foca area of hepatic necrosis replaced by mononuclear leucocytic cells infiltration (H & E X 400).

Table 4 shows state of meiotic progression of the oocytes collected from albino mice treated with different doses of fenugreek oil. Meiotic progression in most oocytes (75.2 %) collected from untreated female mice was still in GV stage. While, only half of the oocytes number collected from female mice treated with different doses of fenugreek oil were arrested in GV stage (Table 3). Furthermore, the treatment with fenugreek oil stimulated the nucleus of the oocytes to arrive at GVBD and M I stages. Where, half of the oocytes number collected from female mice treated with fenugreek oil was arrived at GVBD and M I stages (Table 3). However, a few number of the oocytes collected from control mice were arrested in GVBD and M I stages (17.1 % and 7.7 %, respectively) (Table 3).

Pathological findings:

The macroscopic appearance of ovary showed no significant changes conserning the size, colour and shape. Microscopically, the ovaries collected from untreated mice as well as from mice treated with 0.05 ml/ mouse of fenugreek oil showed no

alterations. They revealed normal developing follicles and corpora lutea (Fig. 2a and Fig. 2b). However, ovaries of mice treated with 0.1 or 0.15 ml/ mouse fenugreek oil showed marked congestion of interstitial ovarian blood vessels (Fig. 2c) associated with presence of numerous mature ovarian follicles as well as multiple corpora lutea (Fig. 2d). Numerous active primordial follicles, primary or secondary follicles were also noticed in the examined sections (Fig. 2e and Fig. 2f).

Examined liver of untreated mice as well as liver of mice treated with 0.05 ml/ mouse fenugreek revealed no histopathological changes (Fig. 3a). Moreover, liver of mice treated with medium dose of fenugreek showed no histopathological changes except slight activation of kupffer cells and slight congestion of central veins and hepatic sinusoids (Fig. 3b). Apoptosis as well as small focal areas of hepatic necrosis replaced by mononuclear leucocytic cells infiltration was the only histopathological findings observed in the liver of mice treated with high dose (0.15 ml/ mouse) of fenugreek (Fig. 3c).

DISCUSSION

To date, study of the ovarian activity induced by fenugreek oil has been not investigated. Therefore, our present work aims to investigate the effect of *T. foenum* graecum oil on the biological roles of the mice ovaries. As known Seeds of *T. foenum* graecum contain steroidal components (Jayaweera, 1981; Petit et al., 1995), in which they are precursor to form estrogens (Ying and Zhang, 1999). Estrogens, in which they are one of steroidal hormones in the ovary, play an important role in the estrous cycle (Ying and Zhang, 1999). 17 ß-estradiol induces a sudden increase in GnRH secretion from the hypothalamus. GnRH is carried to its target cells in the anterior hypophysis (pituitary) where it stimulates secretion of FSH (follicle-stimulating hormone) and LH. FSH induces follicles to grow and increase in size (Espey, 1999). In the current work, administration with fenugreek oil at different doses increased significantly the total number of the oocytes as well as improved the quality of oocytes compared with oocytes collected from control mice. Dedieu et al. (1998) and Dekel (1999) reported that LH surge initiates final maturation of mammalian oocytes. Where, LH surge induces GV breakdown (GVBD), chromosome condensation, metaphase I spindle formation, extrusion of the first polar body and arrest at metaphase II. We have found that fenugreek oil was relatively effective to stimulate the mouse oocytes to progress in meiosis. Where, half of the oocytes number collected from female mice treated with fenugreek oil were arrived only at GVBD and M I stages. However, most oocytes collected from untreated female mice were still in GV stage. From these observations, we can suggest that fenugreek may be able to stimulate the pituitary-ovarian axes to secret FSH more than LH. Because most oocytes collected from treated mice

had not the efficiency to complete their meiotic progression up to M II stage. Therefore, the mechanism of action of fenugreek on the ovarian activity in mice may attribute to the endocrine influence or/ and the chemicals components of fenugreek.

It is not possible to identify the most effective constituent of fenugreek at ovarian kinetics in mice. However, essential amino acids like 4-hydroxyisoleucine and lysine may seem to be eliciting the improving effects.

Nucleic acids and proteins could be considered the most important compounds in the cell, since they are responsible for information, storage and usage (Elser et al., 1996). DNA concentration provides a good estimate of total number of cells. Similarly, RNA concentration also provide useful information about a sample and the ratio of DNA to RNA varies widely between different animals and tissue types because it reflects the metabolic activity of the constituent cells (Bregman, 1990). DNA content of tissues, provide an information about genotoxicity by replication or mutation, and DNA content of many tumors provides a good prognosis for the progression of the disease (Silvestrini, 2000). Our present study established that fenugreek has the efficiency to improve the ovarian activity including number and quality of the oocytes without any influence on the total content of DNA or RNA in both liver and ovarian tissues. This indicated that fenugreek oil have no genotoxic effects on female albino mice that agrees the observations of Muralidhara et al. (1999), who concluded that debitterized fenugreek powder dose not produce any significant acute and cumulative toxicity at the doses administered (2 and 5 g/ kg body weight). Furthermore, Basch et al. (2003) postulated that simultaneous administration of aqueous extract of fenugreek seeds with ethanol prevent enzymatic leakage and the rise in lipid peroxidation and enhanced antioxidant potential.

In the current study we have attempt to investigate the histopathological effect of fenugreek oil on liver and ovarian tissues of female mice. We have found that fenugreek oil did not affect significantly on liver and ovary weights in females mice. This is further supported by the lack of any histopathological changes. The histopathological findings in the liver of our present work were in agreement with Abdel-Barry and Al-Hakiem (2000), who described that the main target organ affected among the four different organs studied (liver, kidney, stomach, small and large intestine) was the liver, where early degeneration with infiltration of mononuclear and mild hepatitis was found in some animals treated with toxic doses of glycosidic extract. They concluded that the glycosidic extract of *T. foenum-graecum* leaves is considered to be safe and have minimal adverse effect. Our histopathological observations indicated that the most vital biological effects of fenugreek were observed only in the ovaries of mice

treated with 0.1 and 0.15 ml/ mouse of fenugreek, which confined as marked congestion of interstitial ovarian blood vessels associated with presence of numerous mature ovarian follicles as well as multiple corpora lutea. Numerous active primordial follicles, primary or secondary follicles were also described. None of the available literatures described the histopathological effects of fenugreek on the rodent ovaries. The histopathological results were confirmed with the results of the cytogenetic study described before. Rao et al. (1996) reported that there were no significant histopathological changes in weanling rats fed fenugreek seeds for 90 days. As well as histopathological examination of various tissues including ovaries in rats revealed no significant changes attributable to the consumption of fenugreek seeds at 5, 10 and 20 g/ kg over a 90 day period (Udayasekhara et al., 1996).

To understand the mechanism of action how fenugreek can improve the ovarian activity genetically and histopathologically further investigations are underway to unravel the molecular mechanism that mediates the improving effects. In addition, further studies are underway to isolate and characterize the fenugreek's active ingredients that contribute to its effects.

In conclusion: The beneficial effect of fenugreek oil in improving number and quality of oocytes as well as stimulation of oocyte maturation did not affect neither weight of the ovaries nor the content of DNA and RNA in liver and ovarian tissues of the albino mice. In addition, administration of fenugreek oil up to 0.15 ml/ mouse for 10 days period did not produce any toxicity effects as evidenced by histopathological studies employing on ovary and liver of the albino female mice.

REFERENCES

Abdel-Barry J. A ., Al-Hakiem M. H . (2000): Acute intraperitoneal and oral toxicity of the leaf glycosidic extract of Trigonella foenum-graecum in mice. J Ethnopharmacol Apr; 70 (1) : 65- 8

Alarcon-Aguilara, F. J., Roman-Ramos, R., Perez-Gutierrez, S., Aguilar-Contreras, A., Contreras-Weber, C. C. and Flores-Saenz, J. L. (1998): Study of the antihyperglycemic effects of plants used as antidiabetics. J. Ethnopharmacol. 61, 101-110.

Al-Rowais, N. A. (2002): Herbal medicine in the treatment of diabetes mellitus. Saudi Med. J.; 23: 1327-1331.

Amin, A., Alkaabi, A., Al-Falasi, S. and Daoud, S. A. (2005): Chemopreventive activities of Trigonella foenum graecum (Fenugreek) against breast cancer. Cell Biology International; 29: 687-694.

Bancroft, D., Stevens, A. and Turner, R. (1996): Theory and practice of histological techniques. Fourth edition, Churchill Livingstone, Edinburgh, London, Melbourne.

Basch, E., Ulbricht, C., Kuo, G., Szapary, P. and Smith, M. (2003): Therapeutic applications of fenugreek. Alternative Med. Rev.; 8: 20-27.

Billaud, C. (2001): Composition, nutritional value and

physiological properties. Adrian J. Fenugreek. Sciences-des-ailments: 21 (1) 3-26.

Bregman, A. (1990): Laboratory investigations in cell and molecular biology. 3rd edition. John Wiley and Sons, Inc., New York. 315.

Cowan, M.M. (1999): Plant products as antimicrobial agents. Clinical Microbiology Reviews. 12, 564-582.

Dedieu, T., Gall, L., Hue, I., Ledan, E., Crozet, N., Ruffini, S. and Sevellec, C. (1998): p34cdc2 expression and meiotic competence in growing goat oocytes. Mol Reprod Dev; 50: 251-262.

Dekel, N. (1999): Meiotic cell cycle, Oocytes. In: Encyclopedia of Reproduction. Knobil, E. (eds.), San Diego, California, Academic Press; Vol, III: 168-176.

Dische, Z. (1955): Colour reactions of nucleic acid components. In: The nucleic acids. Chargall, E. and Davidson, J. M. (eds.), Academic Press, New York, Vol. I, pp. 270-284.

Elser, J. J., Dobberfuhl, D. R., MacKay, N. A. and Schampel, J. H. (1996): Organism size, life history, and N: P. stoichiometry. BioScience 46 (9), 674-684.

Espey, L. L. (1999): Ovulation. In: Encyclopedia of Reproduction. Knobil, E. (eds.), San Diego, California, Academic Press; Vol, III: 605-614.

Flammang, A.M., Cifone, M.A., Erexson, G.L. and Stankowski Jr, L. F. (2004): Genotoxicity testing of a fenugreek extract. Food and Chemical Toxicology; 42, 1769-1775.

Jayaweera, D. M. A. (1981): Medicinal plant: Part III. Peradeniya, Sri Lanka: Royal Botanic Garden; p. 255.

Jellin, J.M., Batz, F. And Hitchens, K. (1999): Pharmacists Letter/Prescribers Letter Natural Medicines Comprehensive Data base.

Kolbe, T. (1998): Befruchtung von Schweineeizellen in vitro (IVF), mittels intrazytoplasmatischer Spermatozoeninjektion (ICSI) und im Eileiter (GIFT). Diss, Göttingen, Germany.

Leibfried, L. and First, N. L. (1979): Characterization of bovine follicular oocytes and their ability to mature in vitro. J Anim Sci; 48: 76-86.

Miraldi, E., Ferri, S., Mostaghimi, V. (2001): Botanical drugs and preparations in the traditional medicine of West Azerbaijan (Iran). J. Ethnopharmacol.; 75: 77ñ87.

Muralidhara, Narasimhamurthy, K., Viswanatha, S., Ramesh, B. S. (1999): Acute and subchronic toxicity assessments of debitterized fenugreek powder in the mouse and rat. Food Chem Toxicol.; 37: 831-838.

Peares, A. G. E. (1985): Histochemistry theoretical and Applied volume two: Analytical technology Churchill Livingston, 4th Edition, Edinburg, London, Melbourne and New York.

Petit, P. R., Sauviaire, Y. D., Hillaire-Buys, D. M., Leconte, O. M., Baissac, Y. G., et al. (1995): Steroid saponins from fenugreek seeds: extraction, purification and pharmacological investigation on feeding behavior and plasma cholesterol. Steroids;10: 674-680.

Polanski, Z. and Kubiak, J.Z. (1999): Meiosis. In: Encyclopedia of Reprodction. Knobil, E. (eds.), San Diego, California, Academic Press, Vol, III: 160-167.

Raghuram, T.C. Sharma, R.D. Sivakumar, S.H. and Sahay, B.K. (194): Effect of fenugreek seeds on intravenous glucose disposition in non-insulin dependent diabetic patients. Phytother. Res., 8 83-86.

Rao, P. U., Sesikeran, B., Rao, P. S. (1996): Short term nutritional and safety evaluation of fenugreek. Nutr. Res.; 16: 1495-1505.

Schneider, W. C. (1957): Determination of acid in tissues by pentose analysis. In: Method enzymology. Colowick, S. P. and Kaplan, N. O. (eds.), Academic Press, New York, pp. 680-684.

Sharma, R. D., Sarkar, A., Hazra, D. K. (1996): Hypolipidaemic effect of fenugreek seeds: a chronic study in non-insulin dependent diabetic patient. Phytother Res; 10: 332- 334.

Shetty, K. (1997): Biotechnology to harness the benefits of dietary phenolics. Asia Pac J. Clin. Nutr. 21, 79-102.

Silvestrini, R. (2000): Relevance of DNA-ploidy as a prognostic instrument for solid tumors. Ann Oncol. 11, 259-261.

Snedecor, C.W. and Cochran, W. G. (1982) Statistical Methods. 7th Edition, Iowa University Press. Ames, USA, pp.593.

Udayasekhara, R. P.; Sesikeran, B. and Srinivasa, R. S. (1995): Short term nutritional and safety evaluation of fenugreek. Nut Res 16, 1495-1505.

Ying, S. Y. and Zhang, Z. (1999): Ovarian hormones, overview. In: Encyclopedia of Reproduction. Knobil, E. (eds.), San Diego, California, Academic Press; Vol, III: 578-582.

دراسة وراثية وباثولوجية على التأثير الحركى للحلبة فى أنسجة الفئران

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فى الدراسة الحالية يدرس تأثير إعطاء زيت الحلبة على الكبد وعلى نشاط المبايض . لقد تم إعطاء جرعات مختلفة من زيت الحلبة عن طريق الفم لعدد ٧٢ من إناث الفئران . وقد أثبتت النتائج أن تناول الجرعات ٠،١ و ٠،١٥ ملى/فأر من زيت الحلبة أدت إلى زيادة فى العدد الكلى للبويضات مع تحسين نوعيتها. من ناحية الوراثة الخلوية فقد وجد أن زيت الحلبة أحدث زيادة فى عدد البويضات المتجمعة من الفئران المعاملة بالجرعات المختلفة لكى تصل إلى الإنقسام الميوزى حيث إنها وصلت إلى بعض مراحل نشاط البويضة GVBD< MI بينما البويضات المتجمعة من إناث الفئران الغير معاملة بزيت الحلبة لاتظل فى مرحلة GV. كما وجد مستوى الحمض النووى فى أنسجة البويضات والكبد لايتغير فى جميع المجموعات. أما من ناحية الهستوباثولوچى فإنه لاتوجد تغيرات هستوباثولوچية فى أنسجة المبايض فى الفئران الضابطة السالبة والفئران المعاملة بجرعة ٠،٠٥ ملى/فأر من زيت الحلبة. بينما لوحظ زيادة فى الحويصلات البويضية الناضجة فى أنسجة المبايض للفئران التى أخذت جرعات ٠،١ و ٠،١٥ ملى/ فأر من زيت الحلبة. طبقاً للأبحاث المتاحة تعتبر هذه هى الدراسة الأولى التى تبحث عن التأثير الإيجابى لزيت الحلبة على النشاط البويضى للفئران