Asian Journal of Research and Reports in Urology

2(1): 16-26, 2019; Article no.AJRRU.53262

Role of Fenugreek Seeds on both Ovarian and Vaginal Cytology on Rat's Reproductive System: Histological Review

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Author's contribution

The sole author designed, analysed, interpreted and prepared the manuscript.

Article Information

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Complete Peer review History: http://www.sdiarticle4.com/review-history/53262

Review Article

Received 06 October 2019
Accepted 13 December 2019
Published 19 December 2019

ABSTRACT

Background: Reports in the published literature indicate that fenugreek seeds possess significant medicinal effects. The present review showed that fenugreek (Trigonella Foenum-graecum L) seeds contain rich number of steroids and have an estrogen and progestogen like effect.
Objective: Objective of this review was to evaluate the effects of fenugreek seeds on rat's reproductive system.
Conclusion: This review revealed that that fenugreek seeds contain estrogen like action.

Keywords: Fenugreek seeds; vaginal cytology; ovary; histology; hormones.

ABBREVIATIONS

4-OH-Ile : 4-hydroxyisoleucine;
FL : Fenugreek Lactone;
FSH : Follicle-Stimulating hormone;
GnRH : Gonadotropin-releasing hormones;
Hb : Hemoglobin;
HDL : High-density lipoprotein;
IL : Interleukin;
IGFBP4 : Insulin-like Growth Factor Binding Protein 4;
LDL : Low-density lipoprotein;
LH : Luteinizing hormone;
STZ : Streptozotocin;
TG : Triglycerides;
TNF-alpha: Tumor necrosis factor alpha;

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1. INTRODUCTION

1.1 Literature Review

Fenugreek also known as Helba in Arabic, Meth in Hindi, Shamballa in Persian or for its scientific name Trigonella *Foenum-graecum* L. It is aromatic 30-60 cm tall annual herb belonging to the Leguminosae family broadly developed indifferent part of the world such as, India, Mediterranean countries, North Africa, Saudi Arabia and Southern Europe. Their plant produce a small yellowish-white flower containing seeds. Its seeds and leaves used not only for spice used in food or flavoring but also as a medicinal herb [1,2]. The effective therapeutic part of the plant is their dried ripe seeds, which contains similar progesterone and, or estrogen effect [3,4]. Fenugreek seeds contain various possible Active ingredient e.g. Flavonoids, fenugreekine, steroids, tannins and trigonelline [5,6]. It is proven by studies that fenugreek is used in increasing milk supply, decrease serum cholesterol level, improving blood glucose level, treatment of wounds, reduce the severity of dysmenorrhea [7]. inflammation, chronic cough, maintains healthy testosterone Levels and liver disorders [8,9]. Grossly,ovaries lie on each side of uterus (bicornuate in animals) in relation to the lateral abdominal wall. They mostly produce steroid hormones and haploid female gametes (Ova). The Hypothalamus secrete gonadotropin-releasing hormones (GnRH) and its function to stimulate the pituitary glands to secrete Follicle-Stimulating hormone (FSH) and Luteinizing hormone (LH). FSH is responsible for early maturation of ovarian follicles. As the follicle, increase in size and estrogen hormone secreted. When estrogen produced, it inhibits farther secretion of FSH and stimulates the releasing of LH. The ovulation occurs when the level of LH in the blood is high concentrated. Ovulation influence the follicle cells that stays in the ovary and its transformation into corpus luteum. Corpus luteum release a high amount of progesterone and few estrogen hormones, which lead to inhibition of both FSH and LH. Without FSH and LH corpus luteum secretes less progesterone, estrogen and FSH, Also LH is no longer inhibited, and the cycle repeated. This cycle is called Hypothalamo-pituitary ovarian axis [10] (Fig. 1).

1.2 Active Ingredient

Fenugreek contains several active ingredients, fenugreek gum powder is containing Galactomannan, fenugreek oil contains phytic acid, trigonelline and saponing, and seven saponins or more exist in fenugreek leaves which known as graecunins. These compounds are glycosides of diosgenin [11].

Fenugreek seeds contain4-hydroxyisoleucine (4-OH-Ile) control of blood glucose, which is the unique major free amino acid [12]. Also, fenugreek seeds contain various possible active ingredient e.g. alkaloids, amino acids, coumarin, diosgenin, flavonoids, fenugreekine, fenugreek lactone (FL) also called galactomannan, nicotinic acid, phytic acid, polyphenols, saponins, sapogenins, sotolon also called fenugreek lactone (FL), scopoletin, soluble fiber, steroids, tannins and trigonelline [13].

1.3 Mechanism of Action of Fenugreek

1.3.1 Mechanism of action of fenugreek in hyperlipidemia

Triglycerides (TG), low-density lipoprotein (LDL) and total serum cholesterol are decrease due to fenugreek extract (spogenins) that make the biliary increases the cholesterol excretion and decreases serum cholesterol level [14,15]. Galactomannan (Dietary fibers) that found in fenugreek form viscous gel to decrease absorption of lipid and glucose in intestine [16,17].

1.3.2 Mechanism of fenugreek in lowering blood glucose

Fenugreek enhance glucose uptake into cell by stimulating tyrosine phosphorylation [18].

Fenugreek normalize the deranged level of phosphoenol pyruvate,carboxykinase and pyruvate kinase enzymes and inhibit intestine disaccharides by galactomannan [19].

Fenugreek provide a good effect on intestinal and renal disaccharide activity in streptozotocin (STZ) induce diabetic rats through enhance maltase activity reduction during diabetes [20].

In human, steroids and 4-OH-Ile present in fenugreek have a hypoglycemic effect by stimulating glucose-dependent insulin secretion from beta cell [21,22] and by inhibiting two enzymes involve in metabolic of carbohydrates in intestine they are alpha-amylase and sucrose [15,23].

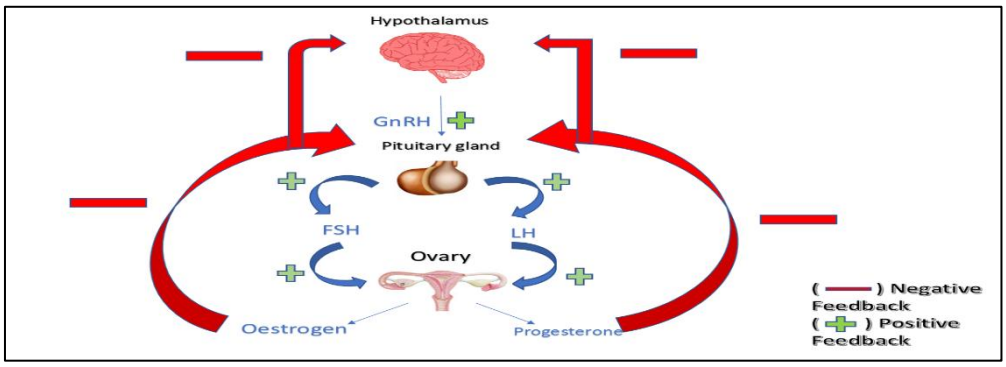

Fig. 1. Hypothalamo-pituitary ovarian axis [10]

1.3.3 Anti-inflammatory activity

In obese rats, the level of tumor necrosis factor alpha (TNF-alpha) protein in plasma and liver are up regulate by orally administration of fenugreek. In vivo study the contrasting result was evident as compared to vitro study. Phorbolmyristate acetate used to produce cytokines as TNF-alpha, Interleukin-1 (IL) and IL-6. Inhibitor and suppression production of TNF-alpha seen in extracted fenugreek with methanol [24]. .Fenugreek seeds and leaves have anti-inflammation and antipyretic activity [25]. Anti-inflammatory activity compared in vivo and in vitro in between fenugreek seed and leaves aqueous extracts. The result found that both of seed and leaves aqueous extracts have an effect against anti-inflammatory because of chloroform [26].

1.3.4 Antitumor activity

Phytoestrogens and saponins are fenugreek chemical that have an anticancer activity [27]. Saponin can inhibit cell division and can do programmed cell death by activating apoptotic program [28]. In vitro studies discover that the ethanolic seed extract have cytotoxic effect on some cancer cell line like breast cancer [29]. Induction of apoptosis of human breast adenocarcinoma cells (MCF-7) human immortalized breast cell that can be kill by extract chloroform seed [30].

1.3.5 The effect of fenugreek seeds on female reproductive system in rat

1.3.5.1 Hormonal activity

Quercitin, trigorelline and gitogenin that extracted from fenugreek have estrogen-making activity. These compounds play an important role in biological activity of increasing estrogen in nitric oxide (NO) toxicity which increase of Insulin-like Growth Factor Binding Protein 4 (IGFBP4) by decrease of the FSH in follicle liquid will be prevent the action of proteases, lead to increase of FSH antagonists and there will be increasing in the follicle evolved with atresia and stop of growth and ripening by preventing the protein (leptin) from dominant follicle [31].

1.3.5.2 Histopathology in rat's vagina

Transverse epithelium septum in young rat's vagina that sealed caudally started to decay at 20-35 days of age, and the lumen have a gradual degeneration of the septum at 40-80 days. The rat's vaginal slit its open is outside separately from urethra and lies on ventral to the anus [32].

The estrogen and progesterone have an effect on the estrous [33] and other studies showed that the first day of the experimental work, in premature normal rats show high concentration of leukocytes with thick mucus, numerous superficial cells, intermediate cells and some parabasal cells (Fig. 2) that showed in daily vaginal smear [34,35] (Figs. 3 & 4).

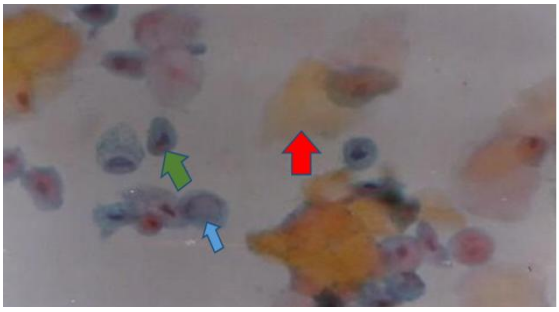

Fig. 2. Showing on vaginal smear in p only the three types of epithelial cells, which are: superficial (Red arrow), intermediate (Green arrow), and parabasal cells (Blue arrow). Magnified at X1320 [4]

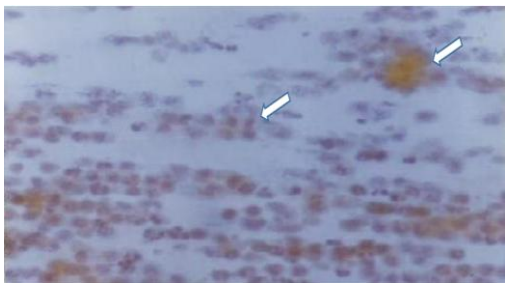

Fig. 3. Premature rats in the first day of experimental work on vaginal smear. Observe thick mucus with high concentration of leucocytes, many superficial cells and some intermediate and parabasal cells. Magnified at X330 [4]

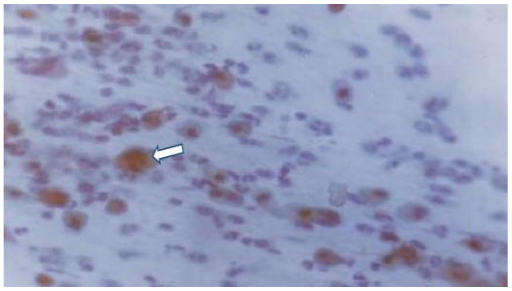

Fig. 4. Premature rats in the fifth day of experimental work on vaginal smear. Observe the number of leukocyte decreased, mucus with higher concentration of intermediate and parabasal cells and superficial cells . Magnified at X330 [4]

However, at 38-40 days of age, most of the cells in pro-estrous (12h) got a cluster of round shape and well-formed nucleated epithelial cells accompanied with decaying leukocytes, relative small epithelial cells and mucous cells (Fig. 5).

The ratio is changed during estrous (12h) medium number of non-nucleated, large cornified squamous cells, leukocytes disappeared and with clear cytoplasm and isolated in a clear background (Fig. 6). During metestrus (21h) there is a lot of small darkly stained polymorphonuclear leukocytes and some cornified squamous epithelial cells with less nucleated cells (Fig. 7). During diestrus (57h) there is a less or rare large cornified squamous epithelial cells. Some mucous cells and leukocytes still command (Fig. 8).

On the other hand, fenugreek seeds- treated premature rats, showed the same picture as the control subgroup. 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 [34,35].

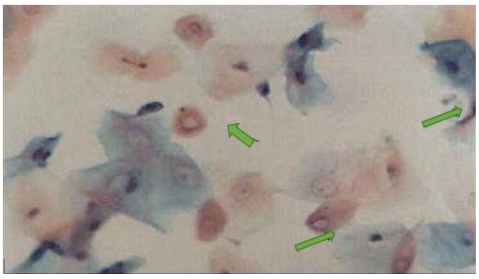

Fig. 5. Vaginal smear during proestrous phase. Observe the nucleated cells, magnified at X1320. (Experimental of 1.6 mg/g body weight) [36]

Fig. 6. Vaginal smear during estrous phase. Observe the transport cytoplasm with the flat cornified cells and leucocyte are absent with clear background. Magnified at X1320 [4]

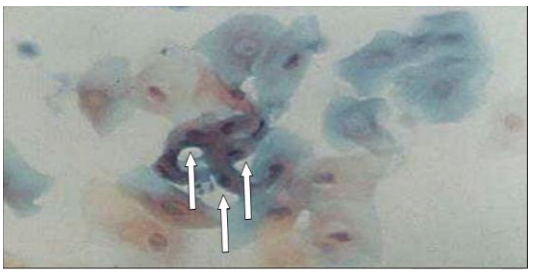

Fig. 7. Showing many leucocytes and some cornified cells, vaginal smear during metestrus phase. Magnified at X330 [36]

1.3.5.3 Histopathology in rat's ovary

Normal immature rat's ovary is small with follicles in various stages of differentiation, but no corpus luteum [3]. (Fig. 9)

The mature ovary has peripheral narrow central medulla and greater cortex. The ovarian got a germinal epithelium and the surface covered by it under which there is tunica albuginea. By many follicles that formed the cortex, collagenous,

reticular fibers and stromal cells (primary interstitial tissue), found in stroma of connective tissue in different stage. The irregular connective tissue rich in vascular bed and the medulla consist of typical dense. Ovarian follicles in [7] are follicle, which are primary oocyte that surrounded by a one layer of flattened follicular, Primary follicle, which are primary oocyte that surrounded by a one layer of cuboidal cells. Secondary follicle, which are primary oocyte surrounded by more than single layer of granulosa cells. [3].

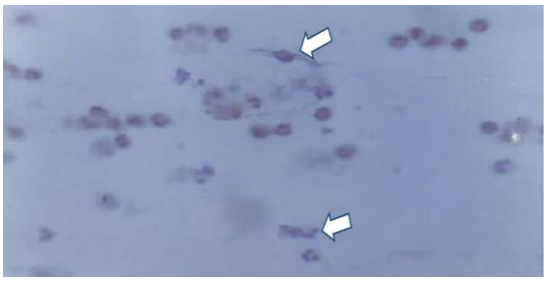

Fig. 8. Vaginal smear during diestrus phase, showing high concentration of leucocytes, the small epithelial cells and some mucus. Magnified at X330 [4]

Tertiary follicle which are known as antral follicle, primary oocyte having antral formation - fluid filled space and surrounded by more than one layer of granulosa cells and Graafian follicle known as ovulatory follicle, have a large antral follicle approximately 0.9–1.0 mm in diameter, which are the primary oocyte is present surrounded by a cumulus oophorus [7].

The stroma immediately around the follicles differentiate to two type of theca, theca interna and theca externa [37].When the first estrous cycle appears, at 6-7 weeks in laboratory rat is sexually mature. The estrous cycle repeat every four or five days, in mature rats. It has no breeding season. Multi-ovulation is the rule in the female rat (Maeda, et al., 2000). The granulosa cells and cells of theca interna, after ovulation it is remain in the corpus luteum that ovary formed a temporary endocrine gland (yellow body). From the time of birth, most ovarian follicles undergo atresia [37].

Hassan (2006) study used an oil fenugreek on mouse in 10 days with different doses (0.05ml/mouse) (0.1 ml/mouse) (0.15 ml/mouse). Mice treated with 0.05 ml/mouse of fenugreek oil showed no histopathological changes and revealed normal developing corpus luteum and follicles. Congestion of mouse interstitial ovarian blood vessels, many mature ovarian follicles and multiple corpus luteum occurred with 0.1ml/mouse fenugreek oil dose (Figs. 11&12). When the mouse treated with 0.15 ml / fenugreek oil dose at the ovary under microscope, show many active primordial follicles and primary and secondary follicles of mouse ovaries (Fig. 13). Mice treated with fenugreek oil show no develop of any toxicity or clinical signs either immediately or after the treatment period even at the highest dose (0.15 ml/mouse) [7].

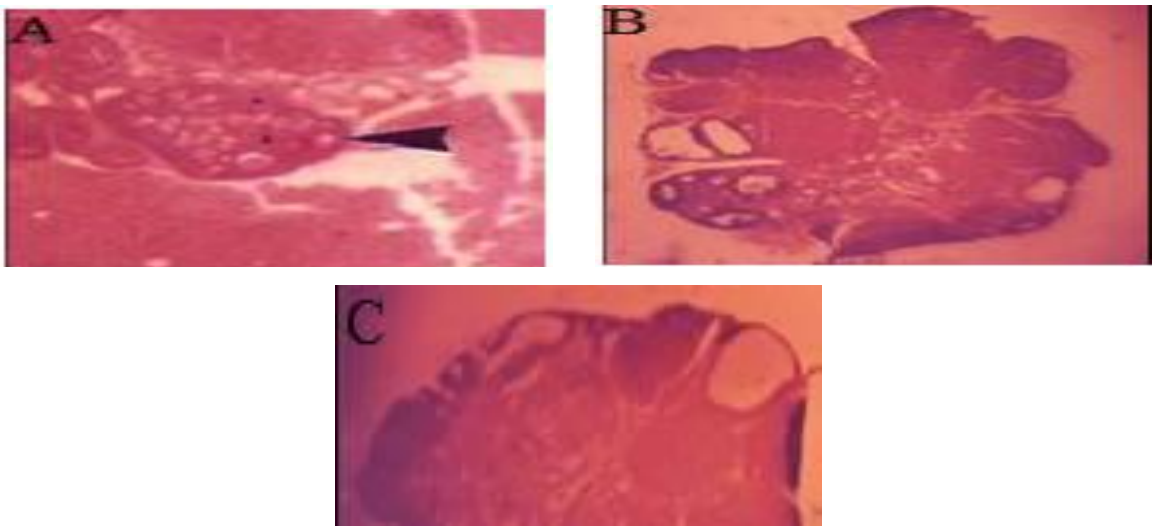

Fig. 9. Showing ovaries sections (six micron thickness), stained by Haematoxylin and eosin. A: Normal immature (five weeks old) rat's ovary with follicles in various stages of differentiation, but no corpus luteun. B: Normal perimmature (six weeks old) rat's ovary with follicles in various stages of differentiation, with corpus lutea in the cortex. C: Normal mature rat's ovary with follicles in various stages of differentiation, with many corpus lutea. (×22) [3]

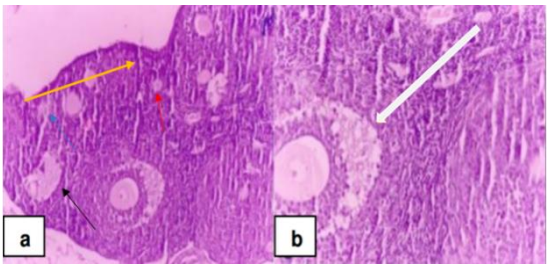

Fig. 10. Photomicrographs of untreated mouse ovaries (a and b) showing normal developing follicles and corpus luteum (H & E X100 and X 200, respectively) [7]

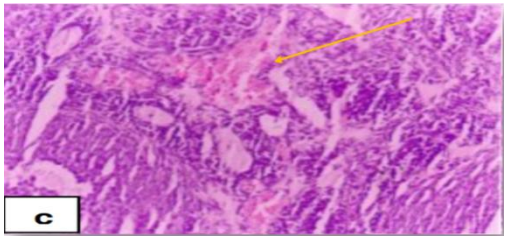

Fig. 11. Photomicrographs of mouse ovaries treated with fenugreek (0.1 ml/mouse) showing: (c) Congestion of interstitial ovarian blood vessels (arrows) (H & E X 200);) [7]

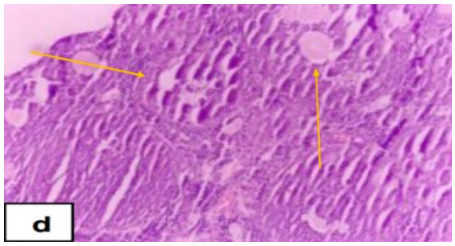

Fig. 12. Photomicrographs of mouse ovaries treated with fenugreek (0.1 ml/mouse) showing:(d) Numerous mature ovarian follicles as well as multiple corpora lutea (H & E X 100). [7]

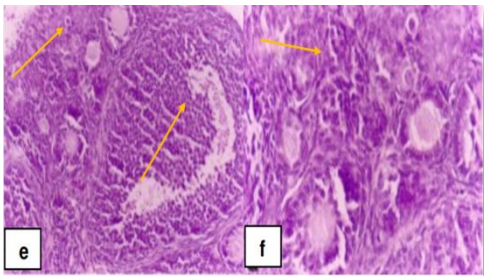

Fig. 13. (e) and (f) Photomicrographs of mouse ovariestreated with fenugreek (0.15 ml/ mouse) showing numerous active primordial follicles, primary and secondary follicles (H & E X 200 and X 400, respectively). [7]

1.4 Fenugreek Drug Interactions

Fenugreek interact with different kind of drugs such as diabetes medications, which make a summation effect because fenugreek can lower blood sugar, anticoagulant drugs (warfarin, heparin) slow blood clotting which can increase the risk of bruising and bleeding and antiplatelet drugs and aspirin [38].

Baquer, et al. (2011) argue that Fenugreek seeds contain 50% fiber and can prevent the absorption of oral drugs [39].

1.5 Fenugreek Doses

Some in vivo studies use cleaned seed powder blending with 4 ml of refined water by the glass bar and given through an oro-gastric tube for enhancing the reproductive activity some rats were given rough squashed fenugreek seeds (1.6 mg/g body weight) suspended in 4 ml refined water every day for 14 day [36].

1.5.1 Methods of Extraction of Fenugreek

In Modaresi, et al. (2012) study, the fenugreek seeds were grinded fully to form a powder and was poured in a sanitize Erlenmeyer flask, a 40 cc of physiological serum was added to it, and was in a cool place. After 1 day, a shaker completely mix the flask contents for 5 minutes. Then, after filtering the solution by filter paper and calculating extract residual in solution, concentration of extract in base solution was determined and doses were prepared. In Sulaiman, et al. (2011) study, said that 2320 g of the fenugreek seeds with 2000 ml of petroleum ether incubation to 16 hours gives 135 ml of fixed oil [36,40] (Fig.14).

Chevassus, et al. (2010) study done on overweight subjects, they were given 1176 mg (approximately 14 mg/kg-1) of dry hydro-alcoholic fenugreek seed extract three times daily as oral coated tablets for 6 weeks, the dose appeared to be the active dose in healthy volunteer to significantly decreased dietary fat consumption in human in this study. Other study done on rat, they given fenugreek oil (0.2 ml/ rat) suspended in 4 ml refined water every day for 14 day [7] (Fig. 13).

In addition, compared with rats given diosgenin extricate (0.024 mg/g body weight) suspended in 4 ml refined water day by day for 14 day [3], [36],

Other study on female mice divided to groups orally administrated with fenugreek oil for 10 days successively at different doses (0.05 ml/ mouse) (0.1 ml/ mouse) (0.15 ml/ mouse) to investigate the effect of fenugreek on kinetics of ovarian and liver tissues [7].

Other in vitro studies evaluated the effect of fenugreek in a dose of 1 µmol/L - 1000 µg/ml (Fig. 15). In addition evaluating its ability to improving oxidation and cell viability at pancreatic β-cells oxidation plate recorded 55% in the human epidermoid cancer cell (HEp-2 cells), 67% in human breast adenocarcinoma (MCF-7) cells, 75% in human amniotic epithelial cells (WISH cells), and 86% in African green monkey kidney cells (Vero cells). Also, used 5 g/day of fenugreek seeds or in diabetes and cholesterol lowering. by 500-600 mg/day for 6-8 weeks of fenugreek seeds will increase testosterone (Fig.16) [41,42].

1.6 Fenugreek Side Effect

Every drug is likely to have a side effects, the same thing goes with herbal the major side effect noticed when consuming fenugreek is that unique smell in urine and sweat (Maple syrup like odor) [2].

Chevassus, et al. (2010) in his study reported 4 cases with mild gastrointestinal symptoms and 1 case with unique urine and sweat smell treated with the double daily dose commonly prescribed in human (1176 mg) [43].

Gupta, et al. (2001), study done on newly diagnosed patients with type 2 diabetes of the 12 participants in the treatment group received 1 gm/day hydro-alcoholic extract of fenugreek seeds, 5 develops mild abdominal and dyspepsia. [42].

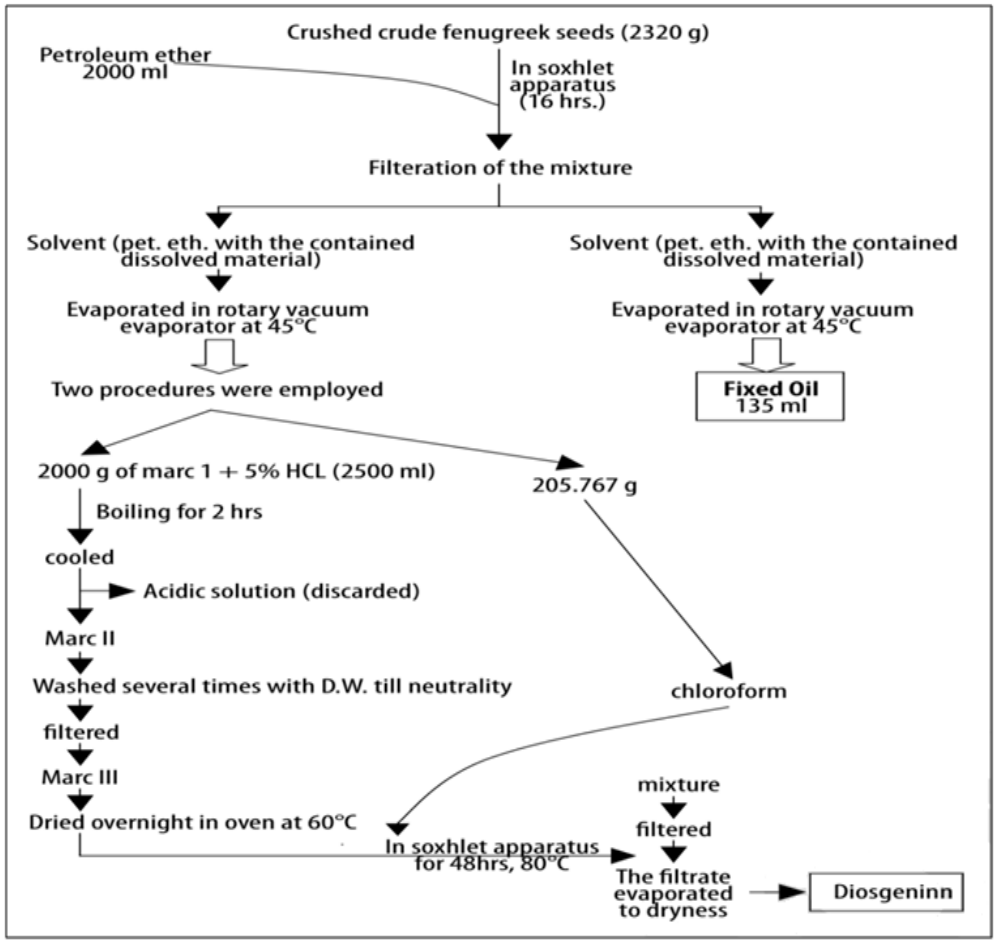

Fig. 14. Schedule showing the steps ofdiosgenin extraction and fenugreek oilfrom fenugreek seeds [36]

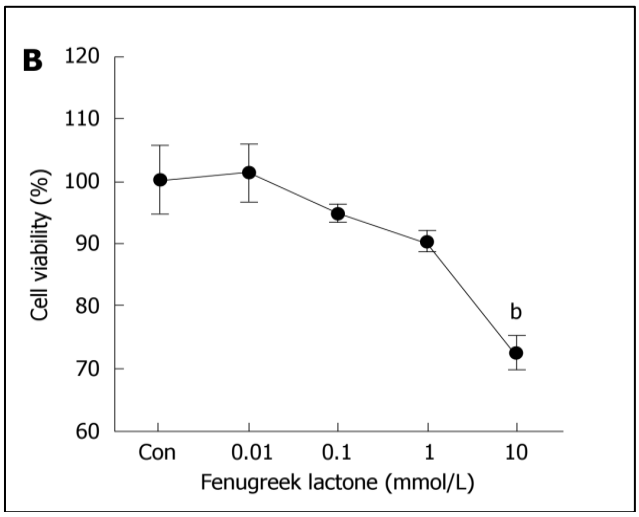

Fig. 15. This diagram shows the percentage of the Viability of pancreatic β-cells when treated with different concentrations (0.01-10 µmol/L) of FL for 24 h. The maximum non-toxic concentration of FL is 1 µmol/L [13]

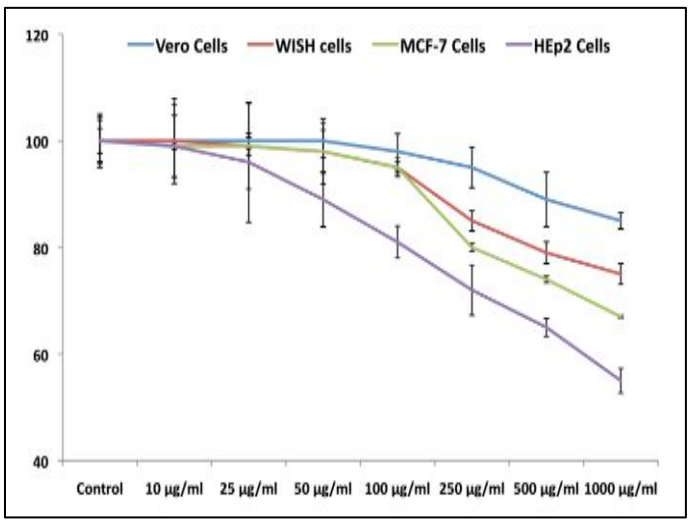

Fig. 16. The effect of different concentrations (10-1000 µg/ml) of seed oil of fenugreek for 24 hon the Cell Viability of Different Cell Lines [41]

Other potential side effect of fenugreek consuming may occur such as bloating, diarrhea or stomach upset [44].

2. CONCLUSION

In this review, it appears that fenugreek seed (*Trigonella Foenum-graecum L*) has an activity against hyperlipidemia, hyperglycemia and it has estrogen and progesterone like effect in female rat's reproductive system and this in the same line with another study *Origanum vulgaris* effect in the rabbit ovary [45,46].

In addition, all, this group of herbs that contain the similar flavonoids have potent effect on reproductive system in male and female [47,48].

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Author has declared that no competing interests exist.

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