The effect of Fenugreek oil (Iraqi fenugreek seeds' extract) on Adult uncoupled rats and mice ovaries Histological and hormonal assay.

Israa M. AL- chalabii * M.B.Ch.B , MSc.

Summary:

Background: *Fenugreek herb is one of the most abundant plants in our country. The dried ripe seeds of this plant are the effective medicinal part the plant.*

Aim of the work: *The aim of this study is to determine the pharmacological effect of a new chemical substance that has been extracted from crude fenugreek seeds which has a hormonal like action and to assess the safety of this experimental material in order to recommend it in future as a stimulator for ovulation or a contraceptive pill.*

Materials and Methods: *Sixty uncoupled female rats and mice were enrolled in this study, categorized into groups as mentioned in the text. Prolactin, estradiol and progesterone serum levels where measured for all groups. Histological and statistical analytical methods were applied to identify the increase in the folliculogenis process within the ovaries of the studied animals.*

Results: *There was an increase in folliculogenesis process in all experimental groups studied when compared to the control (group II, then group III respectively in ascending way). These findings were confirmed histologically as shown in the figures presented in the text showing the mean number of various ovarian components of experimental groups69.2±8.2 in groupli and 103.9 ±14.7 counted as the mean values these componentsand compared to the mean values of the control group which is equal to35.2 t 10.3. Hormonal assay levels, showed increase in the serum levels of hormones studied (prolactin, estradiol & progesterone) in all the experimental groups with percentage in elevation of prolactin in groups II and III were (57% and 44.3% respectively). While the percentage of elevation of estradiol in groups II and III were (76% and 65% respectively). And the percentage of elevation in progesterone hormone in groups II and III were (78% and 73% respectively). The significancy of this elevation was more significant in groups IIR and IIM than. As shown in Figures (12, 13, & 14).*

Conclusion: *It has been concluded that fenugreek oil has a significant effect on folliculogenesis process within the ovary and it increases sex hormones level in the blood due to its wide biochemical effective components.*

Key words: *Fenugreek herb(Trigonella-foenum graecum). Fenugreek oil Rats(Rattus norvegicus). Mice. Ovaries. Folliculogenesis. Crude Fenugreek seeds. a contraceptive pill. Hormonal Assay. mini VIDAS technique. electrone microtome. light microscope. Histological morphometry. H & E stain (Haematoxylin&Eosin stain). Statistical analysis. Photography. Prostaglandins. tocopherol (vitamin E). haematoxylin and eosin stain.*

Introduction:

Fenugreek herb is one of the most abundant plants in our country. Its botanical name is (Trigonella-foenum Qraecum) and its family name is Leguminosae, Known in Iraq as Helba (in Arabic) and Shimli (in Kurdish). The dried ripe seeds of this plant are the effective medicinal part the plant (1), (2), (3) & (4).

Fenugreek oil, which is the extract concerned in this study has foetid odor and bitter taste. Has fatty acid components: 9.6% palmitic, 4.9% stearic, 2% arachidic , 0.9% behenic , 35.1% oleic , 33.7% linoleic and 13.8% linolinic acid (1), (5) & (6).

While the non-saponifiable part of this oil is composed of 35% sterols (1.5% ergosterol), 3% phospholipids, 30% thick oil, 20% tocopherol and 12% a matter that revealed an inflexion in UV region spectrum near 330nm by chromatographic adsorption (7)&(8).

* *Department of Anatomy, College of Medicine, University of Baghdad*

In general all the rats (or mice) ovaries have the same shape and structure. Both have similar inside structures and cells (geometric form and number). Those ovaries have a smooth surface and are friable in touch in addition, to the scanty fatty tissue around them (9), (10) (11) & (12).

A process of folliculogenesis leads to many types of ovarian follicles microscopically. In addition to the appearance of small number of Graafian follicles can be seen within the cortex of the ovary (13) & (14).

This work summarizes the relevant work covering different aspects in the ovarian histology after Crude Fenugreek seeds' and Fenugreek oil feeding to the subjects studied. The aim of this study is to determine the pharmalogical effect of a new chemical substance that has a hormonal like action and to assess the safety of this experimental material in order to recommend it in future as a stimulator for ovulation or a contraceptive pill.

Materials and Methods

Materials

A total of sixty adult uncoupled subjects were used in this study (30 Norway albino female rats (Rattus norvegicus) and 30 female mice). Both had regular estrous cycle, and age range between 7-11 weeks. They were obtained from the animal breeding center of the drugs and biological quality control laboratory/Baghdad. Animals were grouped according to the substances that have been given via oro -gastric tube daily for 14 days in addition to the normal range of tap water and food as shown in table (1).

Table (1): Animal grouping according to the substances they received

	GROUPS	NUMBER	SUNBSTANCE RECIVED	DOSAGES/DAY
RATS	IR	10	Distilled water	4-5 drops
	IIR	10	Crude Fenugreek seeds' powder	1.6 mg/kg B.Wt. suspended in DW
	IIIR	10	Fenugreek oil	4-5 drops
MICE	IM	10	Distilled water	4-5 drops
	IIM	10	Crude Fenugreek seeds' powder	1.6 mg/kg B.Wt. suspended in DW
	HIM	10	Fenugreek oil	4-5 drops

Methods

1. After anaesthetizing each animal by open ether [diethyl ether;], for about 90 seconds , the ovary with its surroundings fatty tissue was removed and immediately fixed for two hours in Carnoy's fluid [6 volumes absolute alcohol: 3 VOLUMES CHLOROFORM : 1 volume Glacial acetic acid].
2. Hormonal Assay: Blood was taken by intra-cardiac aspiration before animal sacrifice for hormonal assay. Hormones included in this study were estradiol, progesterone and prolactin. All these hormones were assayed using mini VIDAS technique. The instrument used is shown in Figure (1).

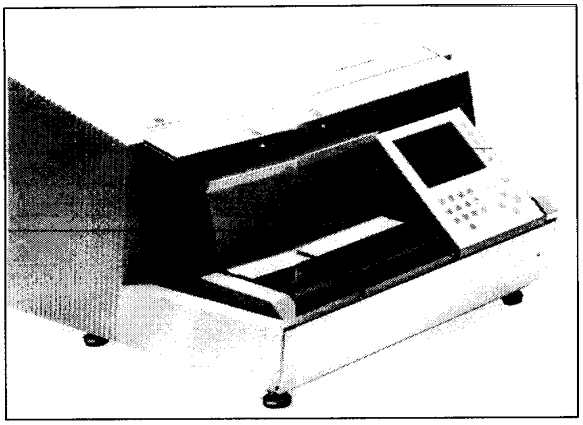


Figure (1): mini VIDAS apparatus

3. Histological morphometry: The fixed tissue specimens were processed for routine paraffin-wax embedding. This includes: Dehydration, Clearing, Infiltration and Embedding. Sections were cut at 4-5 µm thickness, using electrone microtome (Reichert-Jurg, 2030 MOT). Tissues were processed for routine haematoxylin and eosin stain (15). Later on the animals were sacrifices by cutting the abdominal aorta.
4. Statistical analysis: t- Test and histogram representations for the results obtained in this work was done by applying the excel program (16).
5. Photography: The pictures were taken by S.G. 35 Camera attached to the light microscope of Olympus type .

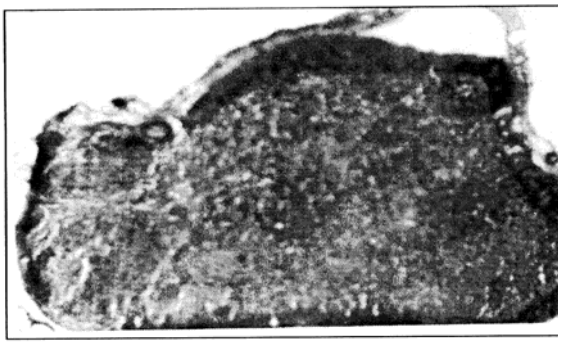
Results

1. Histological morphometry:

a. Control group I (R & M)

Ovaries showed, smooth surface, friable on touch surrounded by small amount of fatty tissue. By light microscope: we can observe different types of follicles (folliculogeneses process). In addition, one or two growing (Graafian) follicles and also one or two corpora lutea may be found (Figure 2).

Figure (2): Growing ovarian follicle (vesicular follicles) stained by H & I stain as can be found in Groups IR an IM (x40)



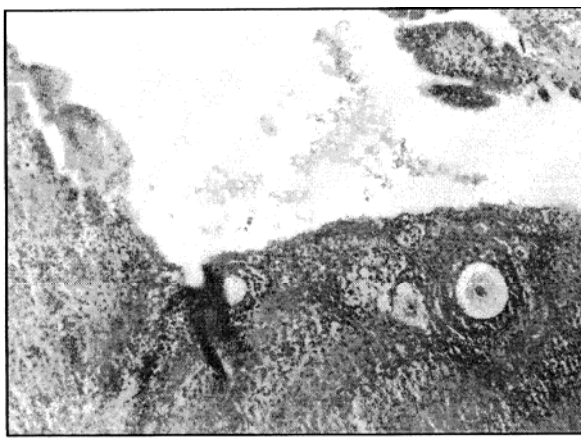


Figure (3): Many primordial follicles and o vesicular follicles can be found in Groups IR and (x400) (H&E stain)

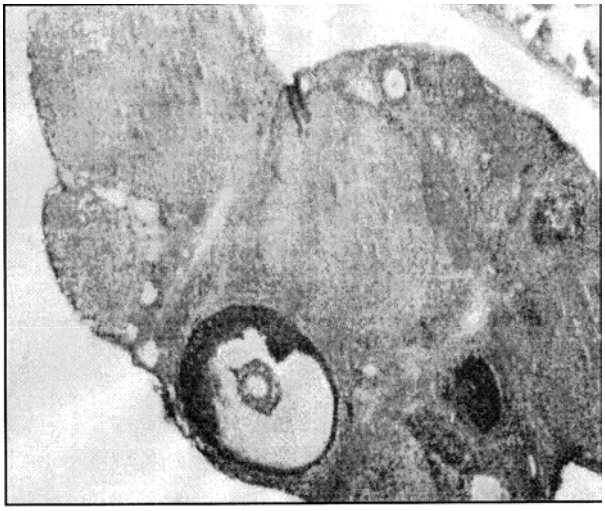


Figure (4): Regressing corpus lutium with many growing ovarian follicles (vesicular follicles) can be found in Groups IR and IM (x40) (H&E stain)

Table (2): The mean number of various ovarian components of control and experimental groups.

	various ovarian components	Control ± SD	C.S. (GII) ± SD	F.O.(GIII) ± SD
1	Prim. Unilam. Foll.	8 ± 4.8	12.3 ± 3.8	18 ±1.2
2	Prim. Multilam. Foll.	10 ± 3.1	14 ± 1.2	20 ±5.1
3	Sec. (antral) Foll.	6 ± 0.1	12± 1.5	23 ±2.3
4	Graafian Matur. Foll.	0.5 ± 2.1	10.1 ± 0.3	11 ±0.6
5	Tot. Foll. (Grow. foll.)	24.5 ± 7.1	48.4 ± 6.8	72 ±8.6
6	Corpora lutea	6.7 ± 2.1	14 ± 0.1	15 ±6.3
7	Atretic Foll.	4 ± 1.1	6.8 ± 1.3	7.9 ±8.2
8	Total	35.2 ± 10.3	69.2 ± 8.2	103.9 ±14.7

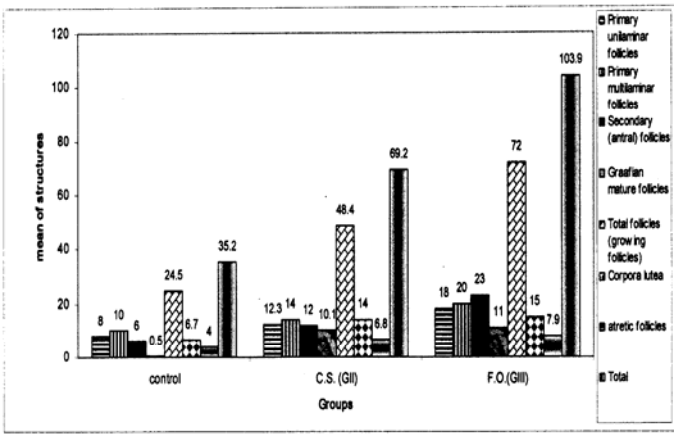


Figure (5): A frequency histogram the mean of different structures in the ovary of groups (IR, IM, IIR, IIM, IIR, IIIM) in relation to the substances given mean obtained from 40 sections from 10 rats and other 40 sections from 10 mice.

b.Crude Fenugreek seeds' treated group II (R & M)
The folliculogenesis process elicited in this group was enhanced more than the group
III by comparison to the control as shown in figures (6, 7, & 8) below:

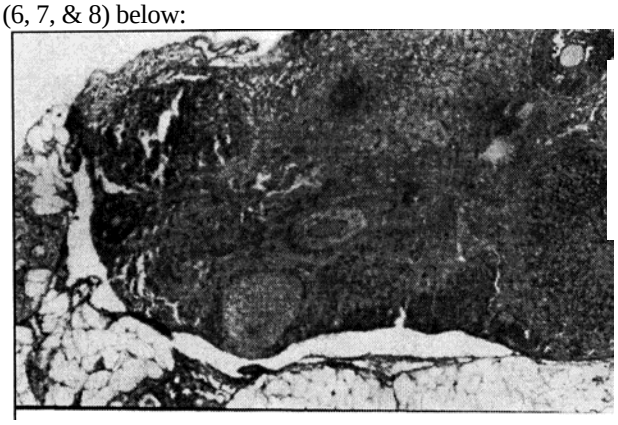


Figure (6): Growing ovarian follicles and Corpus luteum stained by H & E stain as found in Groups IIR and IIM (x100)

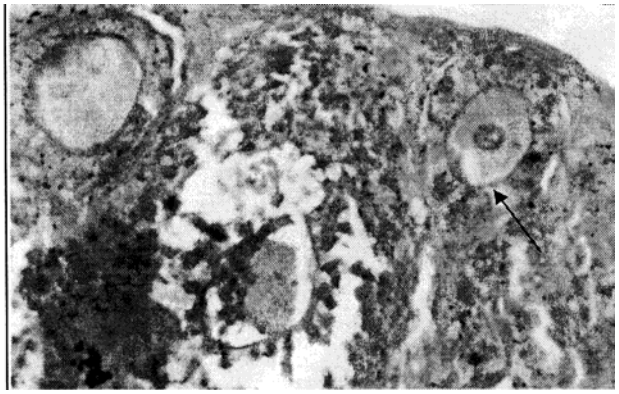


Figure (7): Growing ovarian follicles stained by H & E stain as found in Groups IIR and IIM (x400)

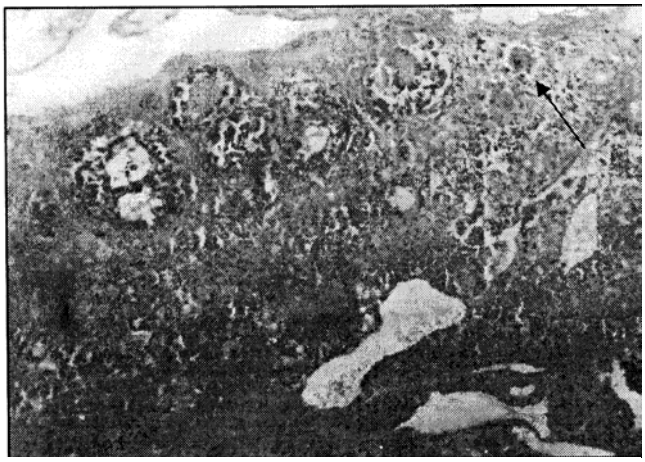
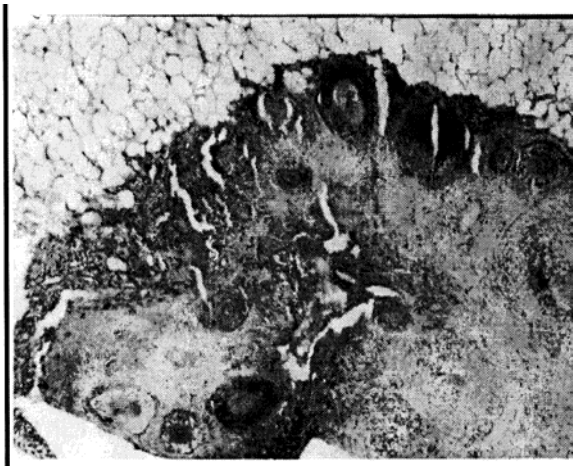


Figure (8): Folliculogenesis process in the ovaries of group IIR and IIM (x100) (H & E stain)

c. Fen ugreek oil treated group III (R & M)
By considering both groups I (R&M) and II (R&M) as a control for group III, the ovaries of group III elicited increase in the fatty tissue around them with a slight increase in their size (Figure 9).

Figure (9): the ovary surrounded by large amount of fatty tissue. The total ovarian components stained by H & E stain as found in Groups IIIR and IIIM (x100)



Microscopically; many growing follicles are found as shown in figure 10 and 11, with increase in granulosa and theca interna cells that surround those follicles. The atretic follicles and corpora lutea were more than the control group, but less than crude fenugreek treated group.

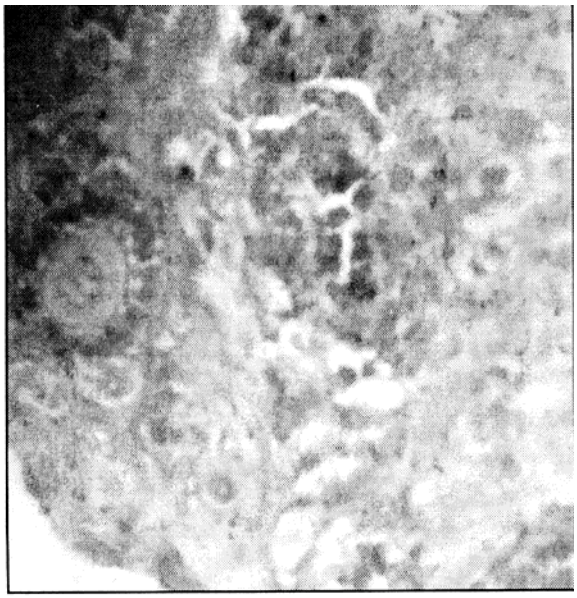


Figure (10): primary follicle growing and surrounded by a layer of granulose cells found within the cortex stained by H & E (x400)

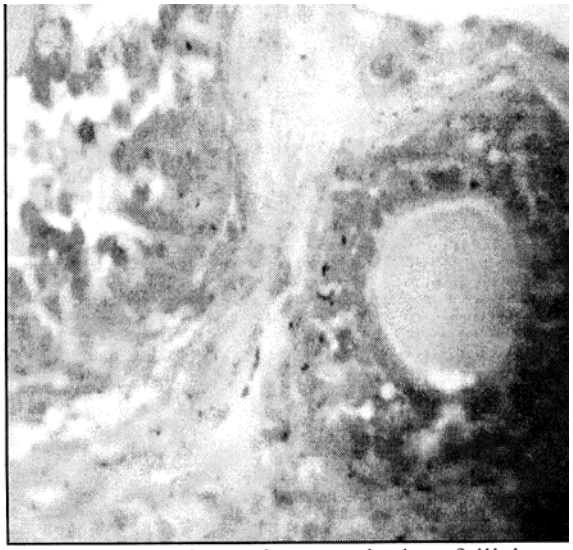


Figure (11): Secondary vesicular follicle surrounded by a multiple layers of granulose cells and theca interna cells stained by H & E (x400)

2. Hormonal Assay:

There is an increase in the serum levels of hormones studied (prolactin, estradiol & progesterone) in all the experimental groups as compared to the control one. The percentage of elevation of prolactin in groups II and III were (57% and 44.3% respectively);while the percentage of elevation of estradiol in groups II and III were (76% and 65% respectively). But the percentage of elevation of progesterone in groups II and III were (78% and 73% respectively). This elevation was significant in both experimental groups, but it was more significant in groups IIR and IIM, as shown in Figures (12, 13, & 14).

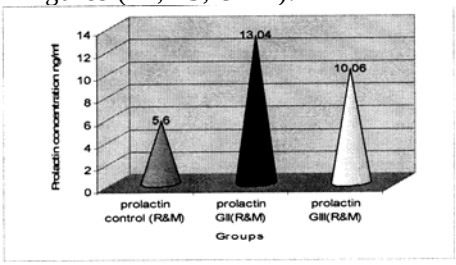


Figure (12): Prolactin concentration in the control and experimental groups

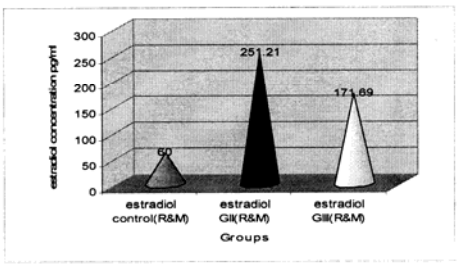


Figure (13): Estradiol concentration in the control and experimental groups

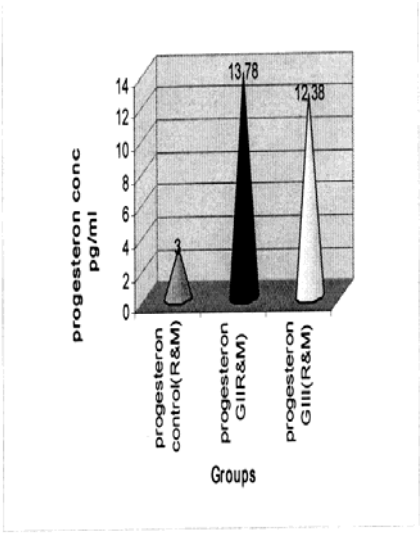


Figure (14): Progesterone concentration in the control and experimental groups

Discusion

1. Histological morphometry:

a. Control group I (R & M)

The results shown in this group, was due to the fact that the animals are young adults uncoupled animals and the folliculogenesis process begins newly at this young age of around 7-11 weeks, so it is difficult to identify large number of Graafian follicles and corpora lutea. While by the end of treatment with distilled water only and even by increasing the time of treatment it can be recognized the increase in the number of growing follicles (folliculogenesis process) and corpora lutea in addition to other ovarian tissue structures which is found normally, as many estrus cycles occur during the time of the experiment (Figures 3, 4, & 5) and table (2).

b. Crude Fenugreek seeds' treated group II (R & M)

The outcome reached is due to the presence of diosgenin which is a saponifiable material that can be considered as a precursor for steroid hormones(28), in addition to the presence of fenugreek oil which is believed to have a vast range of substances that are suspected to have the same effect of diosgenin regarding sex hormone effect resemblances as been documented by several workers since many years ago (7), (17), (18), (19), (20), (21), (22) & (23).

c. Fenugreek oil treated group III (R & M)

Recently the galactogogual role of fenugreek oil was identified by histological and histochemical studies (8). The effect of the whole fenugreek oil component was investigated in this study because of the difficulty of separating these components as pure compounds. It has been concluded that fenugreek oil has a significant effect on folliculogenesis process within the ovary & it increases sex hormones level in the blood due to

its wide biochemical effective components. Precursors are sometimes of considerable advantage, because they also provide access to a natural analogs and derivatives which may exhibit a superior spectrum of properties referring to the demands for a successful drug development and applications (6).

2. Hormonal Assay:

As fenugreek oil components contains different types of lipids saturated and unsaturated.

The latter includes (wt% of total acids): 35.1% olieic acid; 33.7% linoleic acid and 13.8% linolinc acid; while the saturated fatty acids are: 9.6% palmetic acid; 4.9 stearic acid; 2% arachidic acid and 0.9% behnic acid. Each has in addition to the nutritional value, there are other physiological significances specially the unsaturated type for example y -homo linolenic acid (which is a derivative of linolenic acid) is a prostaglandin (24) & (25). Prostaglandins originally discovered in seminal plasma but are now known to exist in virtually every mammalian tissue, acting as local hormones; they have important physiological and pharmacological activities (26).

In the non-saponifiable part of the fenugreek oil there is 35% sterols (1.5 ergo sterols) which acts as precursor for steroid hormones, that can be biosynthesized within the liver or the affecting organ or could be acting on the hormone receptors directly without any metabolism and giving the same hormonal effect as their chemical structures are compatible with the type of hormonal receptors. Also there is 20% tocopherol (vitamin E) within this non-saponifiable part, which has the widest natural distribution and the great biologic activity specially type

D,a-Tocopherol. Its other type's (3, 7, and 6 have dietary significance, and it is known that; vitamin E is the first line of defense against peroxidation of poly unsaturated fatty acids that contained in the cellular and subcellular membrane phospholipids. The phospholipids of mitochondria, endoplasmic reticulum and plasma membranes, possess affinities for aTocopherol, and the vitamin appears to concentrate at these sites (24), (25) & (26).

Although there is no scientific justification for self-medication with vitamin E in the belief that this will increase energy and virility, this substance indicated as dietary supplement and for prophylactic use in haemolitic anemia, intermittent claudication, (3-lipoproteinemia, congenital hematological disorders (G6 P deficiency, thalassemia, sickle cell anemia) as well as to meet the raised requirements (e.g. due to high dietary intake of poly unsaturated fats) (27).

In conclusion:

Fenugreek oil treated group(G-III); showed less in percentage of significance than group(G-II); as this experimental material has various types of fatty acids (saturated & unsaturated) and the non-saponifiable matters, which can be biosynthesized to the above mentioned hormones in different pathways and as mentioned may cross-react each other or negotiate them (28). While the effect of crude fenugreek seeds treated (G- II), was more than group III, as the crude seeds contain both diosgenin which, serves as starting material for the partial synthesis of the medicinal steroids(28), and the fenugreek oil , in addition to many other nutritional materials (7,2 &8).As a result of that, all the histological changes in this work in both(G-II&III) experimental groups regardless to the significance were mostly due to the elevation of the estrogen, progesterone and prolactin hormones with in the blood serum.

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