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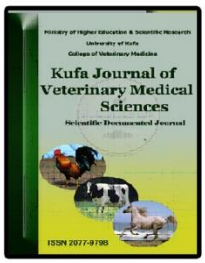

Ameliorative Effect of Fenugreek on sex hormones in Polycystic Ovary Syndrome Female Rats Induced by Letrozole

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Abstract

The present study was carried out in the animal house of the College of Veterinary Medicine/ University of Basrah to investigate the role of the treatment with metformin (Met), ethanolic extract of fenugreek (FN) each alone and their combination on the sex hormones changes in polycystic ovary syndrome induced in female rats. For this purpose, the study included two experiment. First experiment aimed to determine the changes in the hormones concentration of pituitary ovarian axis associated with PCOS, induced by letrozole in rats. Fifty six adult female rats randomly divided in to two group, first group (n=16) administrated 0.5% carboxy Methyl Cellulose (CMC) 0.2ml/rat/orally by gavage and served as control group and second group (n=40) administered Letrozole (1mg/kg B.W/day) dissolved in 1ml of 0.5% carboxymethylcellulose (CMC) orally by gavage for 21 days to induced PCOS. Vaginal smear examination of all female rats was done to ensure the occurrence of PCOS. At the end of the experiment eight rats of each group were sacrificed. Blood samples were collected and serum were separated for hormonal assay. The results revealed a significant increase in serum concentration of luteinizing hormone (LH) and testosterone (T) in PCOS group compared with control group. On other hand a significant decrease in serum concentration of estrogen (E_2), and progesterone (P_4) in PCOS group compared with control.

Second experiment: This experiment was designed to evaluate the role of metformin, fenugreek and their combination therapy in amelioration effect of sex hormones changes of induced PCOS in female rats. The remainder animals from 1st experiment (8 rats from control group and 32 rats from PCOS group) were divided into five equal subgroups including eight rats in each group as follows, First group (Negative control): Rats from the control group of first experiment were administrated of 0.2 ml/rat distilled water (D.W) / daily by oral gavage. Second group (PCOS): PCOS-induced rats administrated 0.2ml/rat D.W daily by oral gavage. Third group (PCOS+Met): PCOS-induced rats were administrated 50mg/kg B.W Met daily by oral gavage. Fourth group,(PCOS+FN): PCOS-induced rats were administrated 15mg/kg B.W FN daily by oral gavage. Fifth group (PCOS+Met+FN): PCOS-induced rats were administrated both Met and FN (50mg and 15 mg)/kg B.W respectively daily by oral gavage. The treatments were extended for 21 days.. Blood samples were collected and serum were separated for

hormonal assay. The results revealed that serum concentration of LH and T still significantly higher and serum concentrations of E2 and P4 significantly lower in -Ve PCOS group compared with control after 21 days of treatment. A significant degrees of improvement were recorded in above cited parameters in all treatment groups compared with -Ve PCOS and control groups.

***key word*: *Fenugreek , sex hormones, PCOS, Letrozole*.**

التأثير المحسن للحلبة على الهرمونات الجنسية في إناث الجرذان المستحدث فيها متلازمة تكيس المبايض المتعدد بوساطة اليتروزول

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الخلاصة

أجريت الدراسة الحالية في البيت الحيواني لكلية الطب البيطري / جامعة البصرة للتحري في دور العلاج بالميتفورمين (Met) والمستخلص الإيثانولي للحلبة (FN) كل بمفرده وكلاهما معا على التغيرات بتركيز الهرمونات الجنسية في متلازمة تكيس المبايض المتعدد المستحدثة في إناث الجرذان. لهذا الغرض، شملت الدراسة تجربتين.

التجربة الأولى: هدفت هذه التجربة للتعرف على بعض التغيرات الكيموحيوية والنسجية المرتبطة بمتلازمة تكيس المبايض المستحدثة بوساطة اليتروزول في إناث الجرذان. استخدم في هذه الدراسة ستة وخمسين من إناث الجرذان البالغة قسمت عشوائيا إلى مجموعتين، المجموعة الأولى (n=16) أعطيت كربوكسي ميثيل السليلوز (CMC) 0.2 ml /جرذ بتركيز 0.5% عن طريق الفم واعدت مجموعة سيطرة والمجموعة الثانية (n=40) أعطيت ليتروزول (1mg/kg B.W/day) مذاب في 1ml من 0.5% كاربوكسي ميثيل السيلولوز (CMC) عن طريق الفم. استمرت المعاملة لمدة 21 يوما لاستحداث متلازمة تكيس المبايض. تم إجراء فحص لمسحات من المهبل لجميع إناث الجرذان خلال هذه الفترة للتأكد من حدوث متلازمة تكيس المبايض. في نهاية التجربة تم التضحية ب ثمانية جرذان من كل مجموعة. جمعت عينات الدم وتم فصل مصل الدم لقياس مستوى الهرمونات. وقد أظهرت النتائج ما يلي:. أظهرت النتائج وجود زيادة معنوية في تركيز مصل الهرمون اللوتيني (LH) وهرمون الشحمون الخصوي (T) في مجموعة تكيس المبايض مقارنة مع مجموعة السيطرة. من ناحية أخرى لوحظ انخفاض معنوي بتركيز هرمون الاستروجين (E2)، والبروجسترون (P4) في مجموعة تكيس المبايض مقارنة مع السيطرة.

التجربة الثانية: تم تصميم هذه التجربة لتقييم دور الميتفورمين (Met) والمستخلص الكحولي للحلبة (FN) كل لوحده أوكلاهما في تحسين التغيرات في تركيز مستوى الهرمونات الجنسية الناتجة من متلازمة تكيس المبايض في إناث الجرذان . تم تقسيم الحيوانات المتبقية من التجربة الأولى 8 جرذان من مجموعة السيطرة و 32 جرذان من مجموعة تكيس المبايض إلى خمس مجموعات فرعية متساوية بواقع ثمانية جرذان في كل مجموعة على النحو التالي، المجموعة الأولى (السيطرة السلبية): (n=8)جرذان من مجموعة السيطرة المتبقية من التجربة الأولى اعطيت 0.2ml/ جرذ ماء مقطر يوميا عن طريق الفم. المجموعة الثانية (تكيس مبايض): ثمانية جرذان أعطيت 0.2ml/ جرذ ماء مقطر يوميا عن طريق الفم. المجموعة الثالثة (تكيس مبايض + ميتفورمين): أعطيت 50mg/kg من وزن الجسم ميتفورمين عن طريق الفم . المجموعة الرابعة، (تكيس مبايض + مستخلص الحلبة): أعطيت 15mg/kg من وزن الجسم مستخلص الحلبة يوميا عن طريق الفم. المجموعة الخامسة (تكيس مبايض + ميتفورمين + مستخلص الحلبة): تم إعطاؤها ميتفورمين(50mg/kg) + مستخلص الحلبة (15mg/kg) من وزن الجسم يوميا عن طريق الفم. واستمرت المعاملة لمدة 21 يوما.

تم جمع عينات الدم وتم فصل مصل الدم لاجراء الفحص الهرموني. أظهرت النتائج أن تركيز الهرمون اللوتيني (LH) وهرمون الشحمون الخصوي (T) لا يزال أعلى معنويا وتراكيز الايستروجين E2 و والبروجسترون P4 أقل معنويا في مجموعة تكيس المبايض السالبة مقارنة مع مجموعة السيطرة بعد 21 يوما من العلاج. وسجلت درجات معنوية من التحسن في مستوى الهرمونات المذكورة أعلاه في جميع مجموعات المعاملة مقارنة بمجموعتي تكيس المبايض السالبة والسيطرة

Introduction

Polycystic ovary syndrome (PCOS) is a common endocrine disorder which causes anovulation in animal and women of reproductive age (1). This imbalance is linked with increased body weight and and high levels of androgens (2). It is now more than 70 years since the PCOS was first

described by Stein and Leventhal in 1935 and we are still searching for the true pathogenesis of this enigmatic syndrome (3). There are several treatments used in PCOS treatments including metformin, cabergoline, Myo-inositol, D-Chiro-inositol, combined oral contraceptives and aromatase inhibitors have been recommended for therapeutic interventions of PCOS (4). However, selected studies demonstrate that phytopharmaceuticals are less invasive, less expensive and equally effective in the management of PCOS. The researchers demonstrated the efficacy of the plants against PCOS induced in rats (5). Fenugreek (Trigonella foenum-graecum of the familyFabaceae) seed extract that effect in treated of PCOS. Fenugreek is enriched infurostanolic saponins (Furocyst), the trial was conducted infemale subjects suffering from PCOS over a period of 90 consecutive days. 94% of patients responded positively to the treatment (2). The seeds of fenugreek have been reported to have anti-diabetic and hypocholesterolemic effects in both animal models and humans 6). The present study, aimed to assesst the efficacy of fenugreek (Trigonellafoenum-graecum) seed ethanolic extract on the treatment of PCOS induced experimentally in Female rats in comparison with metformin.

Materials and Methods

Preparation of ethanolic extracts of Fenugreek seeds

Seeds of Fenugreek was purchased from local markets /Basrah province, Ten gm of Fenugreek seeds. Powdered seeds were taken and extracted with soxlet apparatus by using ethanol 70%.The solvent was removed under reduced pressure in a rotary evaporator until they become completely dry. The residue was stored at 8C0 for further use (7).

First experiment (Induction of polycystic ovary) Animals showing at least two regular estrus cycles were selected for this experiment. This experiment included fifty six female rats divided randomly into two groups as follows:

1- Control group: Sixteen virgin rats were received vehicle only Carboxy Methyl Cellulose (CMC). (0.5%) 0.2ml/rat/ daily by gavage

2-Induced Polycystic ovary group (PCOS-induce female rat): All the experimental animals except control group, were orally administered with Letrozole (Sigma –Aldrich, Germany) at a dose of 1 mg/kg dissolved in (1 ml) 0.5% Carboxy Methyl Cellulose (CMC) daily for 21 days according to (8).Vaginal Smears were collected daily and evaluated microscopically using Giemsa stain to confirm the induction of PCOS (9). Daily vaginal smears examination was done for monitoring the estrus cycle of these animals. After 21 days of treatment, when persistent estrus is present eight rats per group were anesthetized with chloroform and the blood samples were collected for hormonal analysis Estimation of Follicle Stimulating (FSH), LH, E_2, P4, Prolactin (PRL) and T by using ELISA kits..

The Second Experiment The remainder animals from 1st experiment (8 rats from control group and 32 rats from PCOS group) were divided into five equal subgroups including eight rats in each group as follows:-

First group (Nagtive control): Rats from the control group of first experiment were administrated of 0.2 ml/rat D.W daily by oral gavage.

Second group (PCOS): PCOS-induced rats administrated 0.2ml/rat D.W daily by oral gavage.

Third group (PCOS+Met): PCOS-induced rats were administrated 50 mg/kg B.W Met daily by oral gavage .

Fourth group,(PCOS+FN): PCOS-induced rats were administrated 15mg/kg B.W FN daily by oral gavage.

Five group(PCOS-Met+FN: PCOS-induced rats were administrated bothMet and FN (50mg and 15 mg)/kg B.W respectively daily by oral gavage (2).The dose of Met and FN were administrated equivalent to that used in the treatment of women with PCOS.

The treatments were extended for 21 days. Blood samples were collected and serum were separated for hormonal assay (FSH, LH, E2, P4, PRL and T) by using ELISA kits.

Statistical Analysis

The data were expressed as mean ±Standard deviation (SD) and analyzed using studied –t-test in the 1st experiment. In addition to used ANOVA analysis in 2nd experiment. Least significant difference (LSD) was used to test the differences among means for ANOVA indicated a significant ($P<0.05$), using computerized SPSS version 20.

Results and Discussion

First Experiment

Table(1): Effect of induced polycystic ovary syndrome on serum pituitary-gonadal axis hormones concentration in female rats. (Mean ±SD) (n=8).

parameters / Groups	FSH. IÚ/dl	LH. IÚ/L	E2 pg/dl	P4 ng/dl	PRL. ng/dl	Tes ng\dl
Control	39.13±0.09a	33.62±0.57b	151.40±5.29a	49.79±12.59a	37.12±0.69a	124.89±4.84b
PCOS-induced	38.23±0.1.01a	76.9±1.14a	107.57±5.27b	41.55±4.98b	36.84±0.42a	140.27±0.42a

Values expressed in small letters mean significant differences at ($P\leq0.05$) level.

Aforementioned results in table (1) showed that the rats with induced PCOS had a significant increase in serum concentration of LH and T and a significant decrease in serum concentration of E2 and P4 compared with control. This results are in agreement with previous study which found that PCOS patients had significantly higher serum concentration of LH and T (10). The results are in line with those of (11) who found a significant increase in serum LH and T concentrations and a significant decrease in E2 concentration in PCOS patients compared with control, while in `contrast to the present results a significant decrease in serum FSH concentration was recorded compared with control. The results are similar to those recorded by (12) who found that oral administration of letrozole resulted in a significant increase in serum concentration of both LH and T in adult female rats, while E2 concentration reduced greatly. On other hand (2) found a significant increase in serum T level in PCOS rats induced by letrozole compared with control as observed in the present study, while no significant difference in serum E_2 level was observed in PCOS group compared with control. However a very significant decrease in P_4 level was recorded

in PCOS group compared with control. The present results are consistent with (13), who showed that PRL values in PCOS patient within normal limit compared with control. In contrast to the present results (14) revealed that PRL levels increased significantly in PCOS compared with control.

Hyperandrogenism is the key feature of PCOS, resulting primarily from excess androgen production in the ovaries and, to a lesser extent, in the adrenals. The primary mechanisms driving increased ovarian androgen production in PCOS include hypersecretion of LH and increased LH bioactivity, hyperinsulinemia due to insulin resistance and increased volume of theca cells in an expanded ovarian stroma. In the present study, we found a reduction in estrogen level in PCOS group and this coincides with earlier studies (15). High testosterone levels reflected accumulation of androgens because Letrozole as non steroidal aromatatse inhibitor blocks the conversion of androgen substrates to estrogens. The reduction in estrogen weakens the negative feedback on LH production in the pituitary gland, resulting increasing levels of LH (12), which further stimulates theca cells to secrete testosterone.

High LH levels negatively correlation with progesterone level during the luteal phase in PCOS women, possibly implying that low progesterone levels may be associated with LH hypersecretion in this syndrome. It has been hypothesized that high LH concentrations may stimulate the high androgen synthesis observed in PCOS, using progesterone as a precursor, It may also be possible that LH-induced hyperandrogenism suppresses progesterone synthesis, although these possibilities need further confirmation.

Second Experiment

Table (2): Effect of Treatments with Met, FN and Met +FN) on Pituitary-Gonadal Axis Hormones Concentration in Female Rat. (Mean ±SD) (n=8).

Parameter / Groups	FSH. IÚ/dl	LH. IÚ/L	E2 pg/dl	P4 ng/dl	PRL. ng/dl	Tes ng\dl
Control	36.11±4.93a	34.69±3.58d	149.87±6.91a	51.26±3.61a	29.59±2.81c	125.20±9.31c
PCOS	38.57±8.93a	75.13±4.54a	109.94±4.82d	43.91±3.41c	33.83±2.99bc	147.71±4.94a
PCOS+ Met	36.76±7.92a	47.71±10.95bc	130.06±6.81c	46.23±3.37bc	32.58±1.47bc	131.19±3.81bc
PCOS+ FN	32.82±3.27a	52.47±6.26b	124.99±4.62c	44.47±3.75c	39.39±3.95a	132.69±2.72b
PCOS- (Metf+FN)	31.15±5.17a	41.83±6.19cd	141.16±5.29b	49.26±1.56ab	34.53±4.86b	127.59±1.53bc

LSD	**NS**	**8.11**	**6.87**	**3.869**	**4.063**	**6.191**

Values expressed in the small letters mean significant differences at the (P≤0.05) level.

The data illustrated in table (2) revealed a significant increase in serum concentration of LH and T and a significant decrease in the concentration of E_2 and P , while no significant differences were found in FSH and PRL concentration in PCOS group compared with control group. On the other hand a significant degrees of improvement in above cited parameters were observed in PCOS groups treated with FN, Met each alone and their combination.

The present results revealed that administration of Met (50mg/kg BW daily) lead to a significantly decrease in LH and T and this is in agreement with (16) who found that PCOS rats treated with Met resulted in a significant reduction of serum T associated with an increase in sex hormone binding globulin(SHBG) . Elevated testosterone levels in PCOS most probably reflect build-up of androgens because conversion of androgen substrates into estrogens was blocked by aromatase inhibitor. The decrease in testosterone concentration in metformin group reflect diminished androgen biosynthesis by the ovary. The diminution of estrogen production by aromatase inhibition can cause enhanced secretion of LH in hypothalamus and pituitary most likely by negative feedback of estrogens (17).

The favorable effect of metformin on the hyperandrogenism of PCOS patients has been observed in many studies (18). It is possible that reducing circulating insulin levels with metformin may improve hyperandrogenemia, (19). (20). have reported that free testosterone and androstenedione were significantly decreased in metformin PCOS group compared with control group.

Another hypothesis could be elucidating this decline including improvement in hyperandrogenism or to the feedback effect to increased ovarian E_2 production due to resumed folliculogenesis. The favorable effect of Met on hyperandrogenism in PCOS may be due to; (1) reduced pituitary secretion of LH, (2) reduced ovarian secretion of androgens (21). The effect of active ingredients of FN including the Linoleic acid has reducing effect on LH amount via decreasing leptin. Due to quite definite and significant relationship between leptine and nitric oxide in LH releasing from pituitary, Leptin reduction will be led to reduction in nitric oxide and then GnRH releasing (22). According to results, estrogen hormone was increased in all treated groups. Fenugreek seeds extract has Gitogenin, Trigonelline, and Quercitin which have estrogen making activity. It seems that these three compounds play important roles in increasing estrogen by their biological activities. Considering the results, amount of progesterone hormone was increased significantly in FN and Metf group. These increase are similar to increase in corpus luteum of these group. On the other hand, progesterone increase can be ascribed to diosgenin compounds of fenugreek which are progesterone precursor (23).

The present results revealed that FN lead to a significantly decrease in T concentrations and this is in agreement with (24). The improvement of total and free testosterone levels following the supplementation of omega-3 fatty acids and vitamin E (which found in GC/MS of FN appendix 1) might be affected by the changes of insulin sensitivity (25).

Fenugreek increases prolactin via affecting serotonergic system which will be led to prevention in GnRH releasing and then reduction in LH (22). In normal mice treated with different doses of hydro-alcoholic extract of fenugreek seeds cause a significant decrease in serum FSH and LH and a significant increase in serum E_2 and P_4 in all experimental animals compared with control group (23). In the same line of the present results (26) demonstrated a significant decrease in E_2 levels in PCOS women compared with control. in contrast to the results of the current study (27) found a significant increase in serum E_2 and non significant decrease in P_4 concentration in PCOS rats induced by estradiol valerate. The difference in results may be due to the use of different substances in the development of PCOS in both studies.

Similar to our results (6) reported that women treated with combination of Met and FN cause a significant improvement in polycystic appearing ovaries and cycle disorders compared with control. The combination of both remedy (Met and FN) with PCOS demonstrated that the mean serum T and LH concentrations in treatment groups were significantly lower than the concentration in the Met or FN alone which may be attributed to synergistic effects of these combinations.

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