

Treatment of polycystic ovary syndrome in rats by kisspeptin

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Abstract

The study designed to evaluate influence of kisspeptin on polycystic ovary syndrome in female rats, **First experiment (Induction of polycystic ovary):** thirtieth virgin female rats divided randomly into two groups first group via used ten rats serves as control group . While the second group twenty female rat (PCOS-induce group) were orally administered with letrozole at a dose of 1 mg/kg/21 days. **The Second Experiment Effect of treatments with kisspeptin on PCOS :** Animals were divided as follows: first group (negative control) .Second group (Positive control) .While animals in third and fourth groups (PCOS+Kisspeptin): five animal in each group PCOS-induced rats administrated of 20 and 40 nmol/rat S/C 21 days Kisspeptin daily respectively .The result show in first experiment a significant increase in serum activity of LH, FSH, estrogen and testosterone and significant decrease progesteron concentration in serum of female rats treated with letrozole. While in the second experiment there was significant decrease in FSH in the C+ compared with C and PCOS treated group. A significant increase in the LH in C+ compared with C and treated group 20 and 40 nmol/rat Kisspeptin . Also the results showed that there was a significant decrease in serum estrogen concentration was observed in PCOS nontreated group compared with control. On other hand a significant increase in serum estrogen in T1(20 nmol/kg B.W) compared with T2(40 nmol/kg B.W). The results of the histopathological study of ovary after treatment PCOS with kisspeptin 20 nmol/kg show partial return of follicular cyst to normal ovarian tissue, but the treated with 40 nmol/kg show semicomplete treated and return follicular cyst to normal ovarian tissue. On conclusions, the present study confirmed that PCOS affect the female reproductive hormonal balance and kisspeptin 40 nmol/rat daily S/C injection show effect in normal restoring of female reproductive hormonal and histological balance of PCOS rats.

Key word: kisspeptin, PCOS, rats.

علاج متلازمة تكيس المبايض في الجرذان بالكيسبيتين

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الخلاصة:

صممت الدراسة لتقييم تأثير كيسبيتين على متلازمة تكيس المبايض في اناث الجرذان البالغة حيث نتم تقسيم ثلاثون من اناث الجرذان الى تجربتين التجربة الاولى (استحداث تكيس المبايض المتعدد) شملت هذه التجربة استعمال ثلاثون من اناث الجرذان حيث قسمت بشكل عشوائي إلى مجموعتين المجموعة الاولى تم علاجها عشرة من اناث الجرذان بكاربوكسي ميثيل سيليلوز (1%) عن طريقة التجريع الفموي واعدت كمجموعة سيطرة. في حين المجموعة الثانية تم استعمال عشرون من اناث الجرذان لاستحداث الاصابة بمتلازمة تكيس المبايض المتعددة عن طريق التجريع الفموي لمادة الليترازول المذاب بكاربوكسي ميثيل سيليلوز (1%) بجرعة 1مغ/كغم/ يوميا لمدة ٢١ يوم و تم ايضا جمع المسحات المهبلية يوميا و فحصها مجهريا لغرض

مراقبة وتحديد الدورة التناسلية للجرذان. وفي نهاية التجربة تم جمع عينات الدم من خمسة حيوانات من كل مجموعة لغرض لتقييم هرونات (الايستروجين، البروجيستيرون، LH و FSH) اضافة الى جمع المبايض لغرض دراسة التقطيع النسيجي. التجربة الثانية (تأثير العلاجات بالكيسيبيتين على متلازمة تكيس المبايض) تم تقسيم الحيوانات من التجربة الأولى (5 حيوانات من مجموعة سيطرة و 15 حيوان مجموعة متلازمة تكيس المبايض) حيث تم تقسيمها الى أربع مجاميع متساوية (خمسة فئران في كل مجموعة) على النحو التالي: المجموعة الأولى (السيطرة السلبية): خمسة جرذان حققت تحت الجلد 0.1 مل / جرذ من المحاول المغذي العادية يوميا. المجموعة الثانية (السيطرة الإيجابية): خمسة جرذان من مجموعة متلازمة تكيس المبايض حققت تحت الجلد 0.1 مل / جرذ من المحاول المغذي العادية يوميا. بينما الحيوانات في المجموعتين الثالثة والرابعة (خمس جرذان كل مجموعة) حققت ٢٠ و ٤٠ نانومول/ جرذ من علاج الكيسيبيتين على التوالي لمدة ٢١ يوم. وفي نهاية التجربة تم جمع عينات الدم من خمسة حيوانات من كل مجموعة لغرض لتقييم هرونات (الايستروجين، البروجيستيرون، LH و FSH والتستوستيرون) اضافة الى جمع المبايض لغرض دراسة التقطيع النسيجي.

أظهرت نتائج التجربة الأولى زيادة معنوية في معدلات هرمون التستوستيرون وهرمون LH وانخفاض في معدلات الايستروجين و FSH و عدم تغير بهرمون البروجيستيرون اما نتائج التقطيع النسيجي اظهر وجود احجام مختلفة من الاكياس مع تتخن بجدارها مقارنة مع نتائج مجموعة السيطرة.

اما نتائج التجربة الثانية اظهرت في المجموعة الثالثة والرابعة مقارنة السيطرة الموجبة زيادة مستوى الايستروجين و FSH و انخفاض بمعدل هرمون التستوستيرون وهرمون LH و عدم تاثر البرجيستيرون مع ملاحظة كون تأثير علاج المجموعة ٤٠ نانومول/ جرذ اكثر فعالية بينما اظهرت دراسة المقاطع النسيجية تأثير العلاج بعودة النسيج الطبيعي للمبيض و قلة الاكياس و شبه اختفاء في مجموعة علاج ٤٠ نانومول/ جرذ اما استخدام الصبغة الخاصة للمقاطع النسيجية تبين وجود النسيج الليفي في المجموعة المصابة بمتلازمة تكيس المبايض و قلة كمية النسيج الليفي مقارنة بمجموعة العلاج الكيسيبيتين. الاستنتاجات: وُثر متلازمة تكيس المبايض على مقدرة الجسم على افراز الهرمونات الانثوية بشكل كبير بينما او ضحت نتائج التجربة فعالية و قدرة العلاج ب الكيسيبيتين ٤٠ نانومول/ جرذ تحت الجلد على استعادة الفعالية الطبيعية للهرمونات الانثوية و النسيجة لاناث الجرذان المصابة بمتلازمة تكيس المبايض .

الكلمات الافتتاحية: تكيس المبايض ، الكيسيبيتين ، الجرذان

Introduction

The kisspeptins are peptide hormones (Kumar *et al.*, 2018). Kisspeptin was called metastatin , which represents its capacity to repress melanoma metastasis (Lee *et al.*, 1996). Only in 2003 did Kisspeptin reveal its biochemical role in the central nervous system. KISS1 and Kiss1 represent human and non-human markers for kisspeptins, while KISS1R and Kiss1r suggest human and non-human receptors, consecutively. Kisspeptins are all gene products of KISS1 and Kiss1. (Donald and Clifton, 2009). Because the gene was located on chromosome-1 the gene was named, the original nomenclature of KiSS-1 gene and the product (kisspeptine) was discovered at the Pennsylvania State College of Medicine in Hershey to connect the findings to their home towns and their most famous product, the "Hershey chocolate kiss."and addition the letters „SS" (suppressor sequence); the scientists named the gene, "KiSS-1(Smith *et al.*, 2006). There have been evidence of the existence, in placental mammals (human, oposium) and reptiles, of a single ligand (Kiss) and receiver (Gpr54 or Kissr) thus mammalian mammals (platypus) have two types of KISS and KISSR. In other

tetrapods, such as amphibians, that have three KISS and kissr gene while the kisspeptin feature is missing among birds (Pasquier *et al.*, 2014).

Inactivating and activating mutations in both KISS1 or GPR54 genes were associated with hypogonadotropic hypogonadism and precocious puberty (Trevisan *et al.*, 2018). Kisspeptins are neuropeptides essential to puberty in females that develop sexually through central control of hypothalamic / pituitary – gonadal axis and regulate ovulation (Hu *et al.*, 2018). The synthetic kiss neurons are mainly found in the anteroventral periventricular hypothalamic (AVPV / PeN) and arcuate (AC) nuclei. (Stephens *et al.*, 2017) Kisspeptin (KP) in human synthesizing neurons of the hypothalamic infundibular region (Takács *et al.*, 2018).

Polycystic ova syndrome (PCOS) in premenopausal patients are serious endocrine and metabolic disorders. (Escobar-Morreale, 2018). 6%-15% of females affected by PCOS. It's the most common cause and the main source of feminine infertility due to anovulation (Barbosa *et al.*, 2016). Hirsutism, anovulation and polycystic ovaries are characteristic of PCOS. Often comorbid with

tolerance to insulin, dyslipidemia and obesity, with a significant possibility of cardiovascular and metabolic sequelae development including diabetes and metabolic syndrome (Meier, 2018). PCOS is frequently associated with abdominal adiposity, insulin resistance, obesity, metabolic disorders and cardiovascular risk factors (Escobar-Morreale, 2018). The association of PCOS with the higher levels of expression to kisspeptin and LH in such patients suggests that kisspeptin is linked to the excess release of LH that is involved in the pathophysiology of the syndrome (Matsuzaki *et al.*, 2017 and Albalawi *et al.*, 2018). The aim of this study is to find the effect of kisspeptin as a treatment of polycystic ovary syndrome in female rats induce by letrozole and identified the best concentration of treatment for these syndrome.

Materials And Methods

Animals treatment protocol: A total of thirty virgin female rats divided for two experiment study: First experiment (Induction of polycystic ovary) This experiment included thirtieth virgin female rats divided randomly into two groups as follows: first group Control group: ten virgin rats were received vehicle only Carboxy Methyl Cellulose (CMC) (1%) 1ml/kg daily. Second group (20 rats PCOS-induce): were orally administered with letrozole/ (Accord healthcare limited, united kingdom) at a dose of 1 mg/kg dissolved in 1% Carboxy Methyl Cellulose (CMC) /(HiMedia, India) daily for 21 days. Vaginal Smears were collected daily and evaluated microscopically using crystal violet to confirm the induction of PCOS. Daily vaginal smears examination was done for monitoring the estrus cycle of these animals. After 21 days, when persistent estrus is present five rats per group were anesthetized with Ketamine\ Xylazine and sacrificed. The blood collected from heart puncture for biochemical analysis and some organs (ovaries, uterus) removed for histopathology study.

The Second Experiment : The remnant animals from 1st experiment (five control group and fifteen from PCOS group) were divided into four equal subgroups including

five rats in each group as follows: First group (C-): were administrated of 0.1 ml/rat normal saline daily by sub cutaneous injection.

Second group PCOS geoup (C+): Administrated of 0.1 ml/rat normal saline daily by sub cutaneous injection. Third group (PCOS+Kisspeptin): PCOS-induced rats administrated of 20 nmol/rat Kisspeptin daily by S/C injection. Fourth group (PCOS+Kisspeptin): PCOS-induced rats administrated of 40 nmol/rat Kisspeptin daily by sub S/C injection

The treatments were extended for 21 days. At the end of the treatment blood samples were collected from heart puncture of each rats and serum were separated for measured the following parameters, serum progesterone, testosterone, Luteinizing Hormone (LH), Follicle Stimulating Hormone (FSH) and Estradiol (E2) concentration: (ELIZA Kit Monobind- Inc. USA. Product Code: 4825-300, 3725-300, 625-300, 425-300 and 4925-300 respectively) .and the organs (ovaries, uterus) were taken for histological study.

Ethical consideration

The consent was taken from the Central Committee for Bioethics University of Kufa.

Statistic Analysis

Statistical analysis of the experimental results was conducted according to SPSS version 23 were T-test and one way (ANOVA) was used to assess the significance of differences between groups and within times. The data were expressed as mean $\pm$ standard errors (SE) and P value < 0.05 was considered statistically significant LSD was carried out to test the significant level among means of treatment (SPSS, 2002).

Results

Vaginal smear cytology for the determination of the rat estrus cycle phases Figure 1-(A, B, C, D) show nucleated epithelial cells in proestrus cycle phase (A), while increase in cornified epithelial cells is found in estrus phase compared with other cycle phases. (B), and, cornified cells and leukocytes in metestrus (C), whereas, few nucleated cells and leukocytes appeared in diestrus cycle phase (D).

First experiment the effect of induced polycystic ovary syndrome on serum LH and FSH concentration in table (1) showed a significant increase ($P \leq 0.05$) in serum concentrations of LH and FSH in serum of female rats treated with letrozole (1 mg/kg letrozole dissolved in 1% Carboxy Methyl Cellulose (CMC) daily for 21 days) to induce PCOS in rats compared with control group normal female rats.

Effect of induced polycystic ovary syndrome on serum estrogen, progesterone and testosterone hormone in female rats in the table (2) showed a significant increase ($P \leq 0.05$) in serum concentrations of estrogen and testosterone in serum of female rats treated with letrozole (1 mg/kg letrozole dissolved in 1% Carboxy Methyl Cellulose (CMC) daily for 21 days) to induce PCOS in rats compared with control group normal female rats, whereas, the results indicated that there was a significant decrease ($P \geq 0.05$) in serum progesterone concentration in PCOS group as compared with control group. The histological examination results of induction of PCOS in the ovary Figures (2). Showed normal ovarian cellular tissue with normal follicles (F) and corpus luteum (CL), while, figure (3-A and B), histopathological features of ovary in letrozole induced PCOS in rats the section of ovary showing multi follicular cyst (FC) in group treated with letrozole for induce PCOS lined with a thin layer of granulosa cells and hyperplasia of thica cell the follicular cyst different in size.

Second experiment: The effect of treatments with kisspeptin on serum LH and FSH in PCOS-induced clarified in table (3) The results showed that there was significant decrease ($P \geq 0.05$) differences in the concentration of serum FSH in the C+ group (PCOS non-treated) compared with C group (control) and PCOS treated group T1(20 nmol/kg B.W SC injection for 21 day) and T2(40 nmol/kg B.W SC injection for 21 day). On other hand in LH there was a significant increase ($P \leq 0.05$) in the serum LH in C+ group compared with C group and treated group (T1 and T2).

The effect of treatments with kisspeptin on serum estrogen, progesterone and

testosterone hormone in PCOS-induced rats table (4) the results showed that there was a significant ($P \geq 0.05$) decrease in serum estrogen was observed in PCOS non treated group compared with control. On other hand a significant ($P \leq 0.05$) increase in serum estrogen in T2 group compared with T1, but both of them more than C+ group (PCOS non treated), while the values of estrogen still significantly ($P \leq 0.05$) lower in all treated groups compared with C group (control). While in Progesterone there was no significant ($P \geq 0.05$) differences in all group. The results of the histopathological study of ovary in female rats with induced PCOS treated for 21 days with 20 and 40 nmol/kg B.W of kisspeptin. In figure (4) show normal ovarian cellular tissue with normal follicles (F) and corpus luteum (CL). While, figure (5), Histopathological features of ovary in letrozole induced PCOS in rats. The section of ovary showing multi follicular cyst (FC) in group treated with letrozole for induce PCOS after 21 day from first experiment, the cyst lined with a thin layer of granulosa cells and hyperplasia of thicacell. In figure (6) show ovary after treatment PCOS induced rat with kisspeptin for 21 day (A) treated with 20 nmol/kg B.W of kisspeptin show partial return of follicular cyst to normal ovarian tissue, while in (B) treated with 40 nmol/kg B.W of kisspeptin show semi complete treated and return follicular cyst to normal ovarian tissue.

Discussion

Female rodents are known to be spontaneous ovulation and have regular and successive estrous cycles which may differ with age and species. Such processes are also influenced by light the seasons of the year and the conditions of life. In comparison, estrous cycles can take place without seasonal impact in rodents under laboratory conditions if their effect is regulated by the climate (Lohmiller and Swing, 2006). In several reproductive studies the rodent was selected as the main animal model. However, the data relating to its estrous cycle are rare and conflicting. It has phases that are characterized by varying types of cells in the vaginal tissue (diestrus,

metestrus, estrus and proestrus) (Paccola *et al.*, 2018).

The reproductive axis is involved in life from major changes in puberty to the reproductive health required. Being fertile to the symptoms that sex hormones are taken out of age. The hypothalamic-pituitary-gonadal network and hormone secretions, including GnRH, gonadotrophins (FSH, and LH), and sex steroids hormones (testosterone, progesterone and estrogen), per process depend on the sensitively balanced system (Boehm *et al.*, 2015). The basic control of the reproductive response in mammals is the hypothalamic-pituitary-gonadal (HPG) axis. GnRH neurons in females play an axial position in the coordination of a number of major hormonal events for ovarian maturation, estrogen synthesis and daily reproductive function (Couse *et al.*, 2003). In the mammalian brain, estrogen plays a major role in the control of activity in kisspeptin neurons, GnRH neurons and gonadotropes, with a double impact on the hypothalamus with inhibitory and activating GnRH secretions. (estrogen positive and negative feedback) (Handa *et al.*, 2012 and McEwen *et al.*, 2012). Studies of knockout mice (KO) for neurons showed that ER α is necessary for negative estrogen feedback in the neuroendocrine gonadal system (Cheong *et al.*, 2014). While α estrogen receptor dominates in mediating estrogen feedback, this process probably is not the only mediators because β estrogen receptor is expressed in GnRH neurons and seems to play an important part in controlling the activity of the HPG axis. the α estrogen receptor is a dominant mediator in mediating estrogens feeding feedback. (Krsmanovic *et al.*, 2009). The most importance of estrogen receptors in the regulation of GnRH neurons has been a controversial subject (Chu *et al.*, 2009).

The Letrozole mediated PCOS model is the appropriate method because many of human PCOS features, like abnormal follicles, have been established by animals. (Kafali *et al.*, 2004), hyperglycemia (Zhu *et al.*, 2013) and change in sex hormones levels (estrogens, testosterone, LH and FSH) (Kafali *et al.*, 2004). Hormonal changes negatively

influence or stop the follicle maturation, which cause anovulation (Gopal *et al.*, 2002). In the current study we observed that letrozole-induced PCOS easily in rats after 21 days of continuous administration. These results are similar to other reports (Lee *et al.*, 2018; Yang *et al.*, 2018).

Depending on what mention above in these research use letrozol to induced PCOS in rat for 21 day and result was hormonal increase in testosterone, LH and decrease in estrogen, FSH while Progesterone showed no significant change. Control group (normal rats received CMC) showed normal histological ovarian tissues. While, letrozole induced PCOS group (rats received letrozole 1 mg/kg) multiple cystic follicles have been shown with reduction of granulosa cells layer, and hyperplasia of theca layer as show in many studies (Atef *et al.*, 2019; Khaled *et al.*, 2019).

Letrozole-inducing PCOS recorded high levels of LH and testosterone, but small estrogen and FSH concentrations in compare with control case. These findings are consistent with researchers' findings (Orio *et al.*, 2008). Data showed also that testosterone level in the Letrozole group has increased significantly. Letrozole blocks the transfer of testosterone to estrogen, which results in hormonal disequilibrium (Baravalle *et al.*, 2006). Polycystic ovary syndrome (PCOS) increased the frequency of gonadotropin (GnRH) pulses, which favored the production of (LH) over follicle-stimulating hormone (FSH) (Burt *et al.*, 2012). This rise in LH concentrations improves androgen production in the cells of theca, while the relative FSH deficit decreases the cells of granulosa ability to convert androgens into estrogen (McCartney *et al.*, 2002), LH activates the production of androgen in theca cells, while FSH activates androgens to estrogen through granular cells and follicle maturation. Intra-ovarian androgens enhance the development of prenatal and early antral follicle stages, which encourage the recruitment of primary follicles, whereas elevated androgen rates induce atresia in later antral phases. (Pan *et al.*, 2015). Catteau-Jonard and Dewailly, (2012). The FSH rates for women with PCOS

are slightly lower than those for the follicular phase, therefore the aromatization of excessively androgenic compounds is not necessary, nor are the follicles fully matured according to FSH. Dominant follicle leading. Although several requirements exist for PCOS evaluation and classification by various specialist populations, All of them accept that certain causes including ovulation dysfunction, such as recurrent oligo-ovulation or anovulation and biochemical or pathological hyperandrogenism (Fauser *et al.*, 2004)

Kisspeptin Since its discovery several studies have been conducted based on cell level, animal model and even human beings have reported a major role for the kisspeptin in regulating the pituitary-gonadal axis, and the LH / FSH ratio after taking kisspeptin in those previous reports (Oakley *et al.*, 2009). Kisspeptin plays a major role in the initiation of puberty and control of ovulation in sexually mature women by central control of the hypothalamus-pituitary axis. Recent experimental data show the expected involvement of the kisspeptin signal in the direct control of ovarian function, including follicular growth, oocyte maturation, steroidogenesis and ovulation. (Hu *et al.*, 2018). Recently appeared additionally effect to kisspeptin beside endocrine, with a major role in glucose level control, Secretion of insulin, consumption of food and body structure, and the missing signal of kisspeptin led to reduced loco-motor activity and increased adiposity (Hussain *et al.*, 2015). Kisspeptin also performs roles in multiple male reproductive systems, like sperm capacitation and spermatogenesis, and tests in preclinical animal models are also considered to be active in renal physiology have reported that the kisspeptin and/or KISS1R activity in dysfunctional kidneys has been improved. The greater significance of kisspeptin in the urogenital system has been highlighted by the fact that urogenital carcinoma metastatic behavior is inhibited. In relation to this discovery's therapeutic potential, modifications of tissue and tumor at a certain stage of kisspeptin and KISS1 / KISS1R expression can be used to generate

biomarkers diagnose or diagnose urogenital carcinomas. (Wahab *et al.*, 2016). Peripheral administration of kisspeptin can be used as a new and promising way to stimulate the maturation of an oocyte in women undergoing IVF treatment because of its estimated effectiveness through pregnancy rates when compared to currently used pharmacological agents (Kasum *et al.*, 2017). Increased LH and FSH by subcutaneous injection of kisspeptin-54. LH reaction to a SC infusion was directly related to the levels of baseline estrogen ($P < 0.001$) of kisspeptin-54 (0.3 and 1.0 nmol / kg / h). More statistical examines found that an improvement of 100 pmol / l in baseline estrogen was linked to a rise of 1 IU / L in LH in a population handled with 1 nmol / kg / h. Kisspeptin provided through SC infusion which could provide a viable future route of care for Infertility patients. The rates of estrogen baseline can be important factors for leptospin reaction in women, and should be taken into account in the assessment of gonadal reaction (Lippincott *et al.*, 2017).

In this study show effect of treatment of rat letrozole induced PCOS with kisspeptin in two dose kisspeptide (20 nmol/kg B.W.) and with kisspeptide (40 nmol/kg B.W.) ,for the hormonal result was improve and increase in FSH and estrogen level, while, decrease in LH and testosterone level compared with the hormonal A level in PCOS rat, even so estrogen level still not reach to normal level compared with normal rat estrogen level, as show histopathological section effect of kisspeptin as treatment in PCOS return to normal ovary tissue as degree where 40 nmol/kg B.W group more effect in kisspeptin than 20 nmol/kg B.W group. Therefore for future studies must take consider increase dose of kisspeptin for more effected result. On conclusions, the present study confirmed that PCOS affect the female reproductive hormonal balance and kisspeptin 40 nmol/rat daily by sub cutaneous injection show effect in normal restoring of female reproductive hormonal and histological balance of PCOS rats.

Conflict of interest

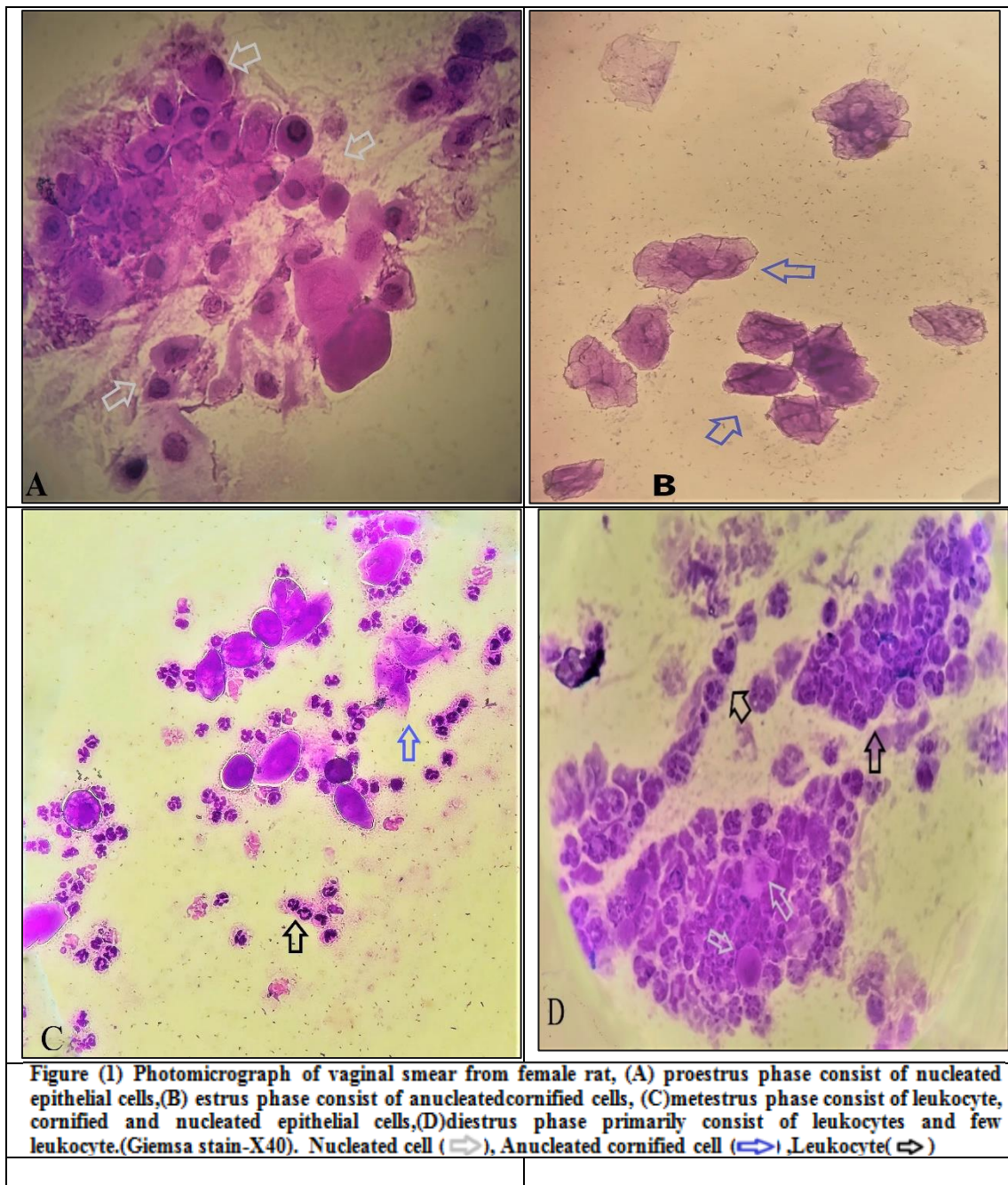
Authors declare that they do not have any conflict of interest.

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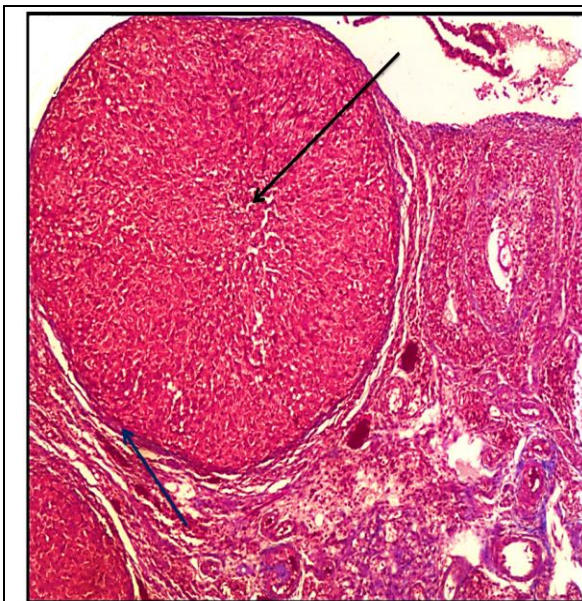
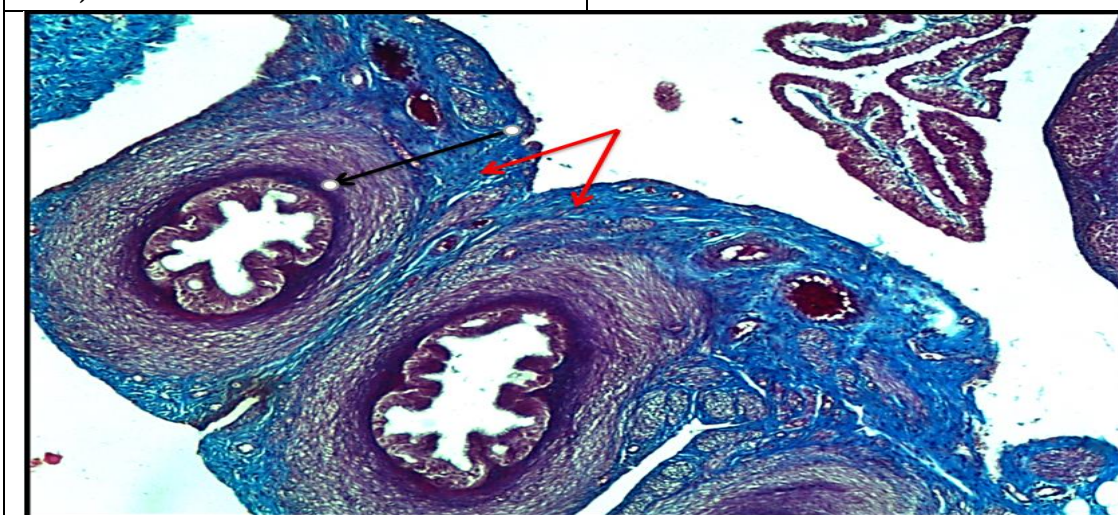


Figure (2) histological section showing the mature ovarian follicles containing granulosa cells (black arrow) surrounded by the theca interna cells (blue arrow). The section stains with H&E stain.



Figure(3-A) histopathological section showed the multiple ovarian cysts (black arrows) appear at the tissue parenchyma. The section stained with H&E stain.



Figure(3-B) histopathological section showed the multiple ovarian cysts (black arrows) appear at the tissue parenchyma. The section stained with H&E stain. The histopathological sections showed the ovarian follicles (black arrow) surrounding by fibrous connective tissues (typical ovarian cyst) (red arrow). The section stained with (Masson's trichrome stain).

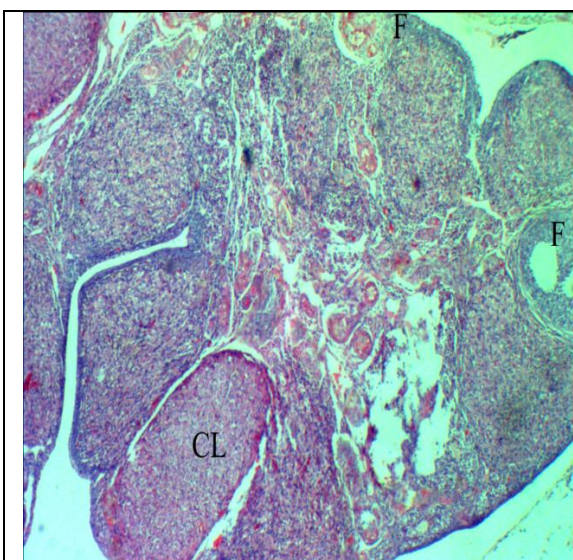


Figure (4) Photomicrograph of normal ovary rat (control) in second experiment show normal ovarian cellular tissue with corpus luteum (CL) and normal developing follicles (F). Stain (H&E) 4X

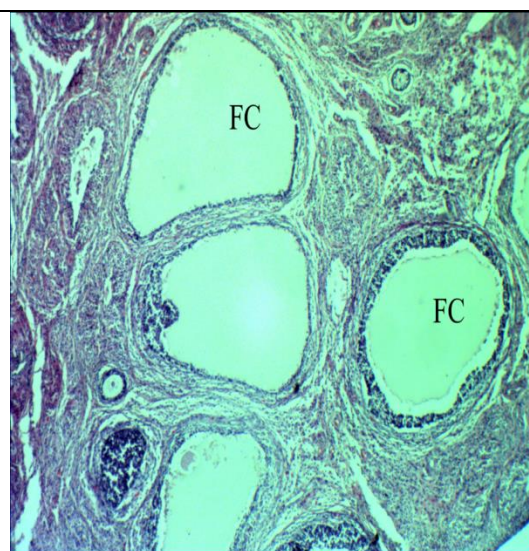


Figure (5) Photomicrograph letrozole induced PCOS ovary rat after 21 day, show multi follicular cyst (FC) and thickness in theca cell. Stain (H&E) 4X



Figure (6-A) histopathological section shows the ovarian follicle (yellow arrow) retains to normal after 21 days of treatment. Some ovarian cysts appear with partially response to kisspeptin treatment with 20 nmol/kg B.W. while some necrotizing tissues can be seen in the center of follicle (granulosa cells) (black arrow). The section stains with H&E stain.



Figure (6-B) histopathological section shows the ovarian follicle (black arrows) retains to normal after (21 days of treatment) days with kisspeptin. Some ovarian cysts appear with completely response to kisspeptin treatment with 40nmol/kg B.W while some fibrous connective tissues can be seen in the centre of follicle (granulosa cells) (yellow arrow). The section stains with H&E stain.

Table (1): Effect of letrozole induced polycystic ovary syndrome on LH and FSH in serum female rats. (Mean±SE)

Parameters Groups	FSH (mIU/ml)	LH (mIU/ml)
C	0.2273±0.01 B	0.2354±0.029 A
C+	0.1705±0.029 A	1.786±0.070 B
Sig. (2-tailed)	0.002	0.000

Table (2): Effect of letrozole induced polycystic ovary syndrome on estrogen, progesterone and testosterone in serum female rats. (Mean±SE) (n=5)

Parameters Groups	Estrogen (pg/ml)	Progesterone (ng/ml)	Testosterone (ng/ml)
C	25.30±0.59 B	18.166±0.90 A	0.0277±0.003 A
C+	13.27±0.62 A	21.23±1.43 A	0.741±0.070 B
Sig. (2-tailed)	0.000	0.108	0.000

-Mean±SE -C= control group. -C+= polycystic group. -Sig. (2-tailed) =the significance of the t-test (The significance of the t-test is less than 0.05 so that we reject the null hypothesis and accept the alternative hypothesis by having significant difference at the level of 0.05). - Capital letters denote differences between groups, P<0.05.

Table (3): Effect of administration kisspeptin on serum FSH and LH in induce polycystic ovary female rat. (Mean±SE) (n=5)

Parameters Groups	FSH (mIU /ml)	LH(mIU/ml)
C	0.244±0.011 B	0.215±0.020 A
C+	0.170±0.008 A	1.269±0.059 B
T1	0.229±0.022 B	0.143±0.022 A
T2	0.266±0.007 B	0.338±0.153 A
LSD	0.041865	0.2513

Table (4): Effect of administration kisspeptin on serum estrogen, Progesterone and Testosterone in induce polycystic ovary female rat.

Parameter Groups	Estrogen(pg/ml)	Progesterone(ng/ml)	Testosterone(ng/ml)
C	25.383±0.56 D	21.52±2.53 A	0.043±0.005 A
C+	13.73±0.57 A	21.72±1.18 A	0.529±0.081 B
T1	16.99±0.11 B	21.79±1.12 A	0.142±0.006 A
T2	22.46±0.24 C	22.46±1.80 A	0.089±0.009 A
LSD	1.2675937	5.0777257	0.1230239

-Mean±SE - C= control group. -C+= polycystic group. -T1= Animals s/c injected with kisspeptide (20 nmol/kg B.W.). -T2= Animals s/c injected with kisspeptide (40 nmol/kg B.W.). - Capital letters denote differences between groups, P<0.05.