Original Article

The Histological and Hormonal Effects of Irisin on Polycystic Ovary Syndrome Induced by Letrozole in Adult Female Rats

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ABSTRACT

Introduction: Polycystic ovary syndrome (PCOS) is a complex hormonal status that impacts around 10% of women globally. PCOS is associated with multiple health conditions and there is currently no cure available.

Aim of the Work: This study aims to evaluate the effects of irisin on female rats with letrozole-induced PCOS.

Materials and Methods: Twenty-one female rats have been divided into three groups. The negative control group (n=7, CMC), received carboxymethyl celluose (CMC) at a dose of 0.5 mg per 100 ml distilled water by oral gavage, and PCOS group (n=7, PCOS) received letrozole that dissolved of (CMC) at a dose of 1 mg per kg\body weight by oral gavage. In addition, a treatment group (n=7: Letrozole +Irisin) females rats received letrozole orally for 21 days. In addition to Irisin injection intra peritoneal (IP). The generalization of PCOS ended for 21 days, and the Irisin treatment continued for 4 weeks. All rats were selected in the estrous phase depended on vaginal smears. The effects of irisin on PCOS were evaluated by vaginal smear investigation, histological analysis, and measuring the levels of sex hormone (Luteinizing hormone (LH), follicle-stimulating hormone (FSH) and Testosterone), along with insulin levels.

Results: According to the study, irisin showed improvements in the estrus cycle. Histological investigation revealed that letrozole elicited the development of polycystic ovary syndrome in the PCOS group, resulting in multiple cysts in the cortical structures of the ovaries. Irisin treatment led to an increase in serum FSH levels and a significant decrease in testosterone levels when compared to the PCOS group.

Conclusion: It concluded by these findings that irisin can enhance ovarian histological structure and regulate sex hormones involved in PCOS pathogenesis to enhance the estrous cycle, which can be beneficial in PCOS therapy.

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Key Words: Irisin, letrozole, polycystic ovary syndrome.

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INTRODUCTION

Polycystic ovary syndrome is one of the most essential complex diseases defined by ovarian abnormalities and hormone disorder linked with fertility, such as luteinizing hormone (LH), androgen and FSH[1]. The Polycystic ovary syndrome has been found in 1935 by Stain and Leventhal[2]. Were considered the first researchers to scientifically characterize a collection of symptom of PCOS therefore, in the beginning the disease was named after them "Stein-Leventhal syndrome". There are various metabolic disorders that were linked with PCOS including several genes especially those work to regulate insulin action, androgen biosynthesis, gonadal function, and cardiovascular disease. Change in the environmental conditions was also found as the main cause that track the polycystic ovary syndrome especially that is associated with complex diet including high fat and carbohydrate[3,4,5,6,7,8]. Today more than one study verify a change in the life style especially weight loss thoughtful the most important consideration in the treatment of PCOS[9]. Researchers have done a hard work in order to find a natural treatment that has the ability to cure or at least stop the progress of the PCOS[10,11]. Irisin may be taken as vital duty in the future in order to treat PCOS[12]. Irisin is a myokine which was first described by Bostrom *et al*.[13]. A little amount of irisin also, is synthesizing and secreting from liver and adipose tissue[14]. Irisin increases thermogenesis and energy uptake by increasing (browning) is a process that convert white into brown fatty tissues[15]. Irisin concentration could be increased by exercises and cold exposure[13]. Also, it was expressed in several tissues such as ovaries, testes, epididymis and leydig cells[16]. Irisin increases thermogenesis and energy uptake especially in skeletal muscles and adipose tissue[17]. However, according to the previous reason, irisin may be used to for enhanced PCOS[18].

MATERIALS AND METHODS

Drug and chemical materials

Carboxymethyl celluose (CMC) (SDFCL, Mumbai, India), powder 0.5 mg/kg was dissolved in 95% distal water. Letrozole powder (Solarbio, China) dose of 1 mg/kg body

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weight was dissolved in carboxymethyl celluose (CMC) solution[19]. Irisin (HEK 293, Med Chem Express, NJ, USA.

Determination of estrous cycle (EC)

To ensure the phases of the estrous cycle (EC) and ensure its steadiness, daily vaginal smear checks were done on each rat, following the scheme outlined by Matsuzaki *et al*.[20] In brief, vaginal discharges were obtained using an eye dropper with a drop of normal saline inserted into the vaginal cavity. Smears were prepared by placing one drop in the center of a clean glass slide, followed by adding a specific amount of Giemsa stain (JOURILABS, Ethiopia). Then, a cover slip was gently applied, and the slide was immediately tested under a light microscope with (x400) objectives. All females rats were ensured have regular estrus cycle (4-5 stages) by microscopic examination of the vaginal smears. In brief the high number of the nucleated epithelial cells and few the cornified epithelial cells indicated the (proestrus stage), the cornified squamous epithelial cells observed may reflected to the (estrus stage), the presence of both few cornified epithelial cells and mainly leukocytes meaning the (metestrus stage), and a prevalence mainly of leukocytes referred to the (diestrus stage) following the criteria figured out by some authors[10,20].

Experimental animals

This study was performed at the Animal House of Veterinary Medicine at Basra University, correspond to ethical guidelines for the use and care of laboratory animals (Approval code: 6\37\2024). All procedures were approved by the local Ethics Committee. The rats were housed in polypropylene cages within a well-ventilated room, with free access to water and a standard diet. A 12:12-hour light-dark cycle was maintained. Twenty-one adult female rats weighing 150-200 g were utilized in the study. The experimental animals were divided according to regular estrus cycle into two groups: A total of twenty one females rats (n=21) were divided into three groups according to the following:

1. Negative control group (CMC): It consisted of 7 Female rats which received carboxymethyl celluose (CMC) (the vehicle) (0.5 ml aqueous solution of carboxymethyl celluose) orally by (gavage) one does per day for 21 days.
2. Induction of PCOS group: A Seven (n=7, PCOS) group of female rats have regular (EC) used. They received letrozole at a concentration of 1 mg/kg dissolved of carboxymethyl celluose solution, orally by (gavage) once daily for 21 days. Female rats of negative control group (n=7, CMC) and seven females rats (n=7, PCOS) group were anesthetized and scarified after 21 days, blood samples were collected by cardiac puncture for serum hormones tests like testosterone, FSH, LH, and insulin. Ovaries from each group were separated and kept in 10% formalin for anatomical and histological study.
3. (Letrozole + Irisin) group: A Seven of females rats (n=7, Letrozole + Irisin) as therapeutic group, were induced PCOS by receiving Letrozole 1 mg\kg BW, dissolved of carboxymethyl celluose solution, orally once daily by oral gavage for 21 days then animals received intra-peritoneal injection (IP) of 100 ng\kg Irisin (HEK293, Fc, Med Chemical Express) on (deionized water; 1 ml\kg) for four weeks. At the end experiment the female rats were anesthetized and scarified, the blood samples were collected by cardiac puncture for serum hormones tests such as Testosterone, FSH and Insulin. Ovaries were isolated for morphological stud and fixed on formalin 10% for histological study.

*The estrus cycle (EC) was remaining examination along the experiment.

Statistical analysis

Statistical analysis were presented as means ± SEM. using SPSS package version 12 (Inc., Chicago, IL., USA). All data were analyzed by computer. ANOVA was used to evaluate significance of differences between the groups at $P \leq 0.05$ levels.

RESULTS

Determination of estrus cycle (EC)

The female rats in (CMC) negative control group have regular estrus cycle (EC) whereas, the female rats in (PCOS) have irregular (EC). The collection of vaginal testing which mainly observed the cornified epithelial cells (estrus stage) (Figure 1). The female rats of (Letrozole + Irisin) treated group showed regular estrus cycle (EC). Four stages of (EC) could be observed including the proestrus which have nucleated cells in addition to cornified epithelial cells. estrus stage with cornified epithelial cells only, metestrus has few of nucleates cells and mainly Leukocyte cells. Finally, diestrus stage has mainly leukocyte cells (Figure 2).

Ovarian morphology and histology

The ovaries of females rats of (CMC) negative control group were normal with multiple follicles like bubbles noted (Figure 3A). Ovaries of females rats that received letrozole (PCOS) group had growing follicular cysts in the surface of ovary. Moreover, one ovarian sample was filled with fluid (Figure 3B) in contrast with (CMC) group experimental animals treated with (Letrozole + Irisin) has enhanced ovaries without showing the cysts (Figure 3C) comparison to (PCOS) group.

Ovarian tissues of (CMC) group showed that there is normal and healthy tissue in all different stages, with the existence of multiple and large corpus luteum, the normal structure of ovarian parenchyma and indicative a normal Graafian follicle (Figure 4).

Ovaries of the polycystic group (PCOS) had multiple follicle cysts found in various sizes, enclosed by dense fibers connective tissue, the corpus luteua were very

few or disappeared completely. Moreover, there were congestion and vacuolated appearance (Figure 5) in contrast to the ovaries of the control group (CMC) group. (Irisin + Letrozole) group showed that normal ovarian structure, mainly cysts disappeared in all the ovarian stages that appeared healthy with clear and multiple corpus luteua indicating that ovulatory function of the ovary was restored. Ovaries tissue was observed with collecting of adipose tissue and reduced of vacuolated (Figure 6).

Hormone serum level

The study showed that, significant ($P \leq 0.05$) decrease of Testosterone and Insulin hormones levels on the (Irisin + Letrozole) treated group compared with (PCOS) group. The results also showed that, a significant ($P \leq 0.05$) increase of FSH compared with the (PCOS). The LH hormone levels significant increased on the (PCOS) group compared with other groups (Table 1)

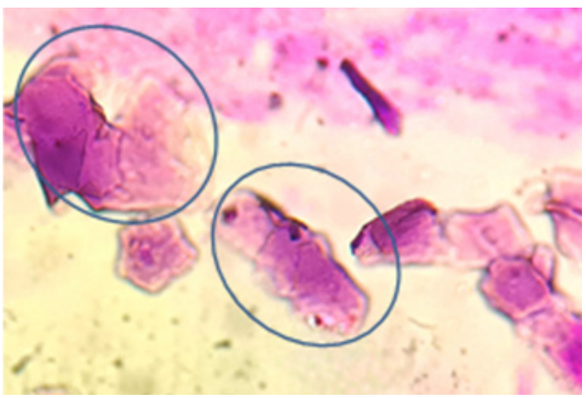

Fig. 1: A photomicrograph of vaginal smear from female rats in (PCOS) group of the estrous stage, mainly observed the collection of cornified epithelial cells were arranged in clumps (circles). with Giemsa stain (x400) under light microscope.

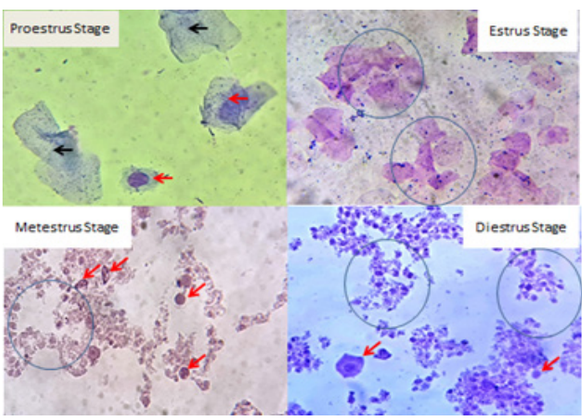

Fig. 2: Virginal smears were collected from females rats in (Letrozole + Irisin) group, stained with Giemsa stain (x400) showed regular estrus cycle. Four stages of (EC) could be observed including the Proestrus which have nucleated cells (red arrows), cornified epithelial cells (black arrows),. estrus stage with cornified epithelial cells only (circles). Metestrus has few of nucleates cells and mainly leukocyte cells (circles) and diestrus stage has mainly leukocyte cells (circles).

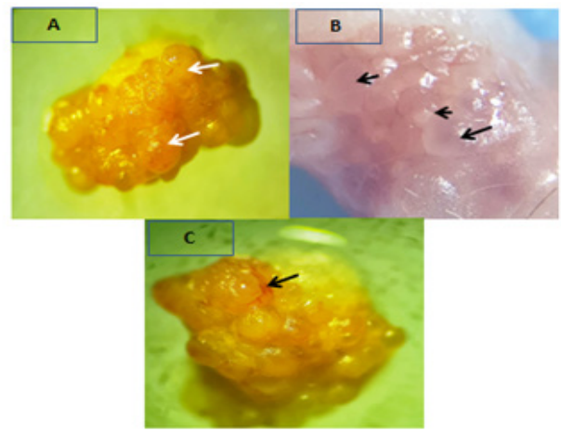

Fig. 3: Morphological gross sections of ovaries showed that: (A) Normal morphological structure of (CMC) group with mature follicles (white arrows). (B) Gross section of (PCOS) group shows multiple cysts (black arrows) of (PCOS) and (C): Gross section of (Letrozole + Irisin) treated group showed that disappeared of cysts and observed mature follicle (black arrow). Under descending microscope.

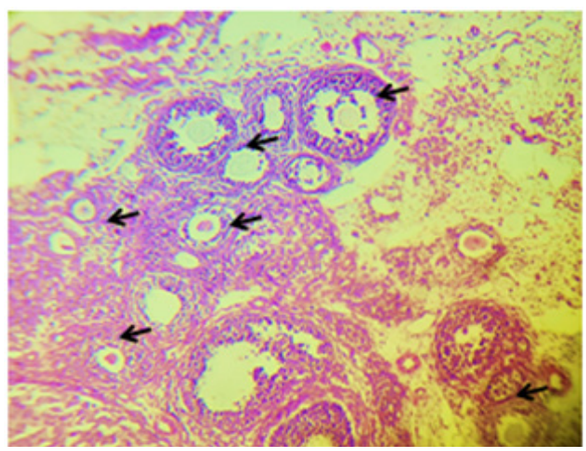

Fig. 4: Section Of ovaries in CMC group showed that normal ovarian structure with multiple and different ovarian stages stained with (H&E) (x100)

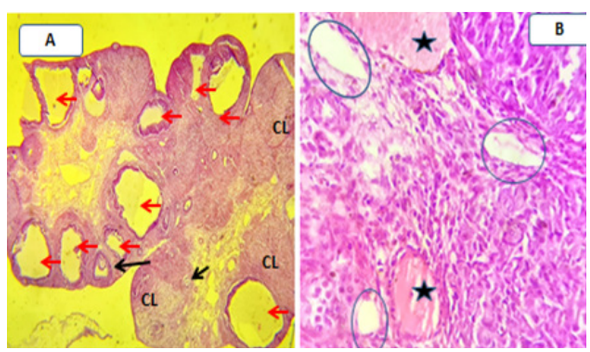

Fig. 5: Sections from (PCOS) group showed ovarian histological changes: (A): multiple of cystic follicles in ovarian cortical portion(red arrows) , few of corpus Luteum (CL) and a little of ovarian development stages (black arrows) (x40: H&E). (B): Section of ovarian structure from (PCOS) group showed that congestion (stars) and vacuolated (circles) (x100: H&E).

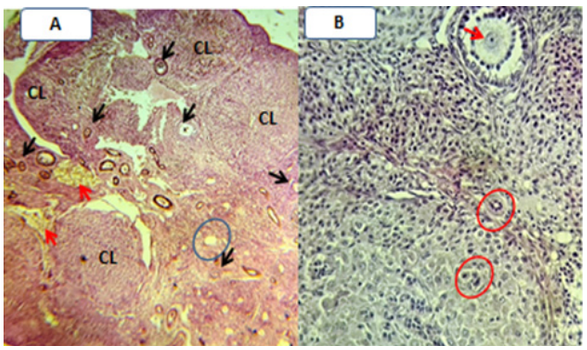

Fig. 6: Sections of ovarian histological structure of (Letrozole + Irisin) treated group showed that: (A): Enhanced of ovarian histological structure, cysts were disappeared, increased of development ovarian stages (Black arrows), corpus luteum presents (CL). Also, observed clusters of adipose tissues (red arrows) and reduced of vacuolated (blue circle), (H&E: x100). (B): Showed that developed of ovarian stages were noted the secondary follicle (red arrow) and primary follicles (red circles), (H&E: x400).

Table 1: The serum levels of all parameters

Groups	Insulin	FSH	LH	Testosterone
CMC	8.75 ± 0.81^{B}	0.81 ± 0.107^{A}	0.77 ± 0.32^{B}	0.40 ± 0.12^{B}
PCOS	11.76 ± 1.01^{A}	0.47 ± 0.076^{B}	1.38 ± 0.31^{A}	2.24 ± 0.36^{A}
Letrozole+ Irisin	9.77 ± 0.42^{B}	0.81 ± 0.079^{A}	1.06 ± 0.10^{AB}	0.80 ± 0.28^{B}

The data is expressed as the mean ± standard error. A High Significant differences between groups, B Less significant difference between groups, AB no significant differences between A and B. (CMC) carboxymethyl celluose, (PCOS) polycystic ovary syndrome induced by letrozole

DISCUSSION

In the current study, an Estrus Cycle (EC) was regular in females rats of negative control group (CMC) compared with polycystic ovary syndrome group (PCOS). While studies showed that the Polycystic ovary syndrome (PCOS) is a medical condition initially, irregularities in the estrus cycle were found through a study utilizing letrozole to cause PCOS in rats[21]. The study showed that the estrus stage is appears mainly in females rats with PCOS, this result agreed with study explained that the females rats when induced the PCOS have estrus stage via irregular epithelial cells[10].

The vaginal smear of (Letrozole + Irisin) female rats group observed has regular (EC), this result accepted with new report was noticing that the Irisin enhanced the reproductive system in females mice that have PCOS[22]. Normal ovarian morphology showed that in (CMC) group compared with (PCOS) group.

The morphological of ovarian gross on (PCOS) group appearance with multiple follicles filled with fluid. This result confirmed by with Nanees *et al*.[23], they showed that multiple features of ovarian morphology occurred with polycystic ovary syndrome such as increased of weight and cysts observed have fluid. Further, the ovarian morphology of (Letrozole + Irisin) group appeared mostly normal. This result was confirmed by study showed that, Irisin could enhance secretion each of (FSH and LH) hormones. These hormones play important role of management the ovulation[22]. Furthermore histological examinations which identified the ovaries on the PCOS group have multiple cysts, sometime more than five cysts with variable sizes. These cysts were enclosed by dense connective tissue fibers, with the corpus luteum vanishing. Furthermore, there was a congestion appearance when compared to the control group and the Irisin-treated group. Increased follicular cysts considered a histological marker of PCOS[24]. This result agreed with study mentioned that Researchers identified PCOS as a disorder involving many physiological alterations in female sex hormones and significant changes in the ovarian cortex[10].

The Irisin-treated group showed a healthier structure with multiple corpus luteua, to give feedback to the restoration of ovulatory function. This was explained by another study mentioned that Irisin illustrated positive effects by decreasing fibrosis and refining stromal ovarian follicles[21]. A research explained that Irisin had the potential to decrease inflammation by improving the production of anti-inflammatory agents such as cytokines and preventing vascular permeability[25].

In this study, we found that Testosterone and Insulin levels were high in the PCOS group compared to the control group (CMC), and these levels decreased in the Irisin-treated group. This results agreement with a study detected that Irisin has improved lipid metabolism, enhanced insulin hormone function, and regulated testosterone hormone levels[26]. Increasing the testosterone levels have also been mentioned in other projects[22,23]. In addition, Tekin, and his team revealed that Irisin increased the releasing of both LH and FSH hormones. These finding were in agreement with this study pointed that irisin has the ability to correct levels of LH, FSH, and testosterone hormones. In conclusion, this study assumed that irisin has the ability to recover ovarian histological structure, improve sexual reproductive hormones (LH, FSH, and testosterone), and enhances insulin hormone levels[27].

CONCLUSION

The study concludes that, irisin could improve the connective tissues of cortical ovarian with PCOS.

CONFLICT OF INTERESTS

There are no conflicts of interest.

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الملخص العربى

التأثير النسيجي والهرموني للايرسين على متلازمة تكيس المبايض المستحث بواسطة اليترزول في اناث الجرذان البالغة

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المقدمة: متلازمة تكيس المبايض هي حالة هرمونية معقدة تصيب حوالي ١٠٪ من النساء عالميا يرتبط بحالات صحية ولا يوجد حاليا علاج لمتلازمة تكيس المبايض.

ألهدف من الدراسة: تهدف هذه الدراسة الى فهم تأثيرات الايرسين على اناث الجرذان المصابة بمتلازمة تكيس المبايض بفعل اليتروزول

المواد وطرق العمل: واحد وعشرون من اناث الجرذان قسمت الى ثلاث مجاميع، مجموعة التحكم السالبة، (CMC: n=٧), جرعت كاربوكسيل مثيل سيليلوز بجرعة (٠,٥ لكل ٩٥٪ مل ماء مقطر) بواسطة انبوب التجريع الفموي، ومجموعة متلازمة تكيس المبايض (PCOS: n=٧)) والتي جرعت اليترزول ١ملغم \كغم من وزن الجسم المذاب في محلول (CMC) جرعت فمويا بواسطة انبوب التجريع الفموي. بالاضافة الى المجموعة الفرعية المعالجة (بالايرسين+ اليترزول :n=٧) ,والتي تشمل اناث الجرذان المجرعة ١ملغم \كغم ليترزول فمويا بالاضافة الى حقن الايرسين بالغشاء البريتوني. المعالجة باليترزول استمر (٢١) يوم واستمرت المعالجة بالايرسين الى (٤) اسابيع. تأثير الايرسين على متلازمة تكيس المبايض بالاعتماد على التحقق من المسحات المهبلية. التحاليل النسيجية، وقياس مستويات الهرمونات الجنسية (الهرمون المحرض للجسم الاصفر، الهرمون المحفز للحويصلات وهرمون التستوستيرون) بالاضافة الى مستويات الأنسولين.

النتائج: أكدت نتائج الدراسة ان المعالجة بالايرسين حسنت من دورة الشبق. النتائج النسيجية بينت ان اليتروزل يساعد على تطور متلازمة تكيس المبايض في مجموعة استحثاث متلازمة تكيس المبايض الاكياس الناتجة في التركيب القشري في المبايض. هذه التغيرات قلت بوضوح او اختفت في المبايض المعالجة بالايرسين. الايرسين المعالج رفع مستويات الهرمون المحفز للحويصلات المبيضية ومعنويا خفض التيستوستيرون مقارنة مع مجموعة متلازمة تكيس المبايض.

ألاستنتاجات: أقترحت هذه النتائج بإمكانيه الايرسين أن يحسن من دورة الشبق بواسطة تحسين التركيب النسيجي للمبايض وتنظيم الهرمونات الجنسية المتلازمة مع تكيس المبايض ومعالجة تكيس المبايض .