



Histochemical study for Effect of *Asparagus officinalis L.* roots extract on ovarian histology in female rat with Polycystic Ovary Syndrome

Farah Jawad Al-masoudi ^{1*}, Ashwaq Kathum Obeid ², Alaa Hussein Al-Safy ³

¹ Biomedical engineering Department / College of engineering / Kerbala University / Karbala / Iraq.

^{2,3} Biology Department / College of Education for pure sciences / Kerbala University / Karbala / Iraq.

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ABSTRACT

Polycystic ovarian syndrome (PCOS) is characterized by several symptoms, such as hirsutism hyperandrogenism, amenorrhea, anovulation, sterility, as well as metabolic and endocrine disorders. This study was conducted to evaluate the efficacy of an extract from the roots of *Asparagus officinalis L.* in treating female rats with induced polycystic ovarian syndrome. In this study, the impact of 400mg/kg of *asparagus officinalis L.* roots extract on the polycystic ovarian syndrome was examined over a period of 28 days in order to scrutinize the effects of *asparagus officinalis L.* roots extract on letrozole-induced polycystic ovary syndrome.

Thirty female albino rats were chosen. They were divided into five groups. Group 1 Control, Group 2 *asparagus officinalis* Extract 400 mg/ kg for 28 days, Group 3 is the PCOS inducer. Rats were given letrozole (1 mg/kg) orally for 28 days in a 0.5% Carboxy-Methylcellulose (CMC) solution. Group 4 is a treatment group in which the animals were dosed with letrozole for 28 days. After that, they were dosed with *asparagus* root extract for another 28 days, and Group 5 is the preventive group, which received *asparagus* root extract for 28 days. Then, it was dosed with letrozole for the same period. The Trichrome stain showed results low in stroma and collagen fibers of the structure ovaries in all groups, including control except the PCOS group that indicates a high presence of collagen. In addition to the number of primary, antral, and graphene follicles increased considerably. The treatment and preventative groups showed fewer cystic follicles than the PCOS group. The findings reveal decrease in collagen fibers by *asparagus* root extract. Therefore, it speeds up oogenesis and early cell divisions.

1. INTRODUCTION

Chronic anovulation, hyperinsulinemia [1], and elevated androgen levels are all factors in PCOS, a group of reproductive problems that primarily affect women. [2] This illness affects the metabolic and endocrine systems, [3] but its underlying etiology is still unknown. [4,5] It is the predominant cause of female anovulatory infertility. [6], an increase in ovarian volume, follicle collecting, and a lack of corpus luteum. [7,8]. Several therapeutic medicines, including metformin [9,10,11], glucocorticoids, aromatase inhibitors, and clomiphene citrate, have been recommended for the treatment of the PCOS. [12,13,14]. However, a number of negative effects, such as nausea, vaginal bleeding, and abdominal pain, are associated with these medications. [15,16]. Therefore,

people prefer herbal remedies to the pharmaceuticals mentioned above for treatment of the PCOS. [17,18,19]. In reality, *asparagus officinalis L.* (AR) is a food crop that has been farmed for its therapeutic properties by a number of ancient cultures. As a result, the crop has a long and interesting history. It is also a valuable plant because of its medicinal and nutraceutical properties. [20]. *asparagus officinalis L.* has been used to treat cardiac issues, palpitations, dyspnea, and toothaches. Saponins, one of its main constituents, are essential for the anti-tumor action and lowering the risk of diseases including obesity, diarrhea, and constipation. [21]. *asparagus officinalis L.* roots have diuretic and potent cardiac sedative diuretic and powerful cardiac sedative effects are present in *A. officinalis* roots. [22,23]. This study was conducted to evaluate the efficacy of an extract from the roots of *Asparagus officinalis L.* in treating female rats with induced polycystic ovarian syndrome.

*Corresponding Author Institutional Email:
farah.j@uokerbala.edu.iq (Farah Jawad Al-masoudi)

2. MATERIALS AND METHODS

2.1. Experimental Animals

In this experiment, thirty female adult albino rats were employed weighing 180-230 g. They were bought at the pharmacy college Karbala University and they were kept in breeding cages with meticulous care. The rats were divided equally into five groups, with six rats (n=7/group), to guarantee statistical significance and fairness. Group 1 Normal Control, Group 2 Animals were received *asparagus officinalis* roots extract 400 mg/kg up to 28 days, Group 3 animals with induced the PCOS by letrozole up to 28 days, Group 4 is a treatment group in which the animals were dosed with letrozole up to 28 days, Afterwards, they were dosed with asparagus root extract up to another 28 days, and Group 5 is the preventive group, which received asparagus roots extract for 28 days. Then, it was dosed with letrozole for the same period.

2.2. Induction the pcos and prepare asparagus officinalis l. extract

To induce PCOS, rats were given letrozole (1 mg/kg bw) orally up to 28 days in a 0.5% Carboxy-Methylcellulose (CMC) solution. [24]. However, the roots were procured from Iraqi markets and fully dried in a laboratory before being pulverized into powder. The resultant powder was mixed with 70% ethyl alcohol for 24 hours at room temperature to produce a solid extract, after filtering, the mixture was dried for 48 hours. The 400 mg/kg concentration of the solid extract was then diluted in 1 cc of distilled water. The solutions were kept in a refrigerator until use [25].

2.3. Histological Study

After 24 hours from the last day of the experiment, rats were given a 3-5 minute chloroform-soaked cotton sedation. Ovaries were dissected, then fixed by 10% formalin for 48 hours, and processed as tissue before being employed in study.

2.4. Statistical Analysis

For the presentation of the results, standard error of the mean (SEM) is employed. ANOVA is a comparison of statistical variances between groups. P 0.05 served as the threshold for statistical significance.

3. RESULTS and DISCUSSION

The Masson's trichrome stain showed results low in stroma and collagen fibers of the structure ovaries in all groups, including the control one **Figure 1,2,4,5** except the PCOS group that indicates a presence of fibrosis. **Figure 3**. Due to the absence of corpus luteum and antral follicles, the mean number of primary follicles in the PCOS group fell in comparison to control and other groups, whereas the mean number of

cystic follicles in the PCOS group increased considerably in comparison to other groups. While corpus luteum and antral follicle counts significantly increased in the treatment and prevention groups, the average number of cystic follicles sharply fell in both. Additionally, there was a rise in the quantity of ovarian follicles in the group consuming Asparagus roots extract **TABLE 1**. Letrozole, an aromatase inhibitor, was administered to female Wistar rats in the study to induce polycystic ovarian syndrome. [26]. The collagen fiber density in the control group and the other groups that received 400 mg of Asparagus root extract decreased, according to the results of histological sections stained in trichrome. In contrast, with including the asparagus group without cyst formation, the therapeutic group and the preventive group, compared with the cyst formation group, which was characterized by fibrosis around the cystic follicles and the stroma. The strong staining in the ovarian cortex is also attributed to an increase in the theca externa layer because it is composed of collagen fibers, which led to its deposition in the ovarian cortex [27].

When ovarian follicles grow and develop, the connective tissue's constituents, particularly the forms of collagen around them, go through significant modifications [28]. PCOS is characterized by abnormal collagen synthesis and increased ovarian stroma volume and density [29]. In this case, the ovary is characterized by an increase in androgen production in the ovary with a thickness of the tunica albuginea and the appearance of fibrous ligaments in the ovarian cortex consisting of many cystic vesicles [30]. Additionally, the extract from asparagus roots contained a variety of amino acids, vitamins, minerals, and estrogenic compounds that promoted follicle growth and maturation and decreased fibrosis. [31] Such substances as quercetin, tryptophan, and arginine, [32] which function as intermediary factors to activate the activity of hormones, also regulate the action of hormones. Granulosa cells also help ovarian follicles differentiate and expand by secreting estrogen, which controls its activity and thins the integumentary layer [20,33].

These results agree with those from the preceding studies [34,35,36]. The results of histopathological measurements in the group of animals treated with letrozole in which polycystic ovaries were induced showed a significant decrease (P<0.05) in the numbers of ovarian follicles and corpus luteum and a significant increase (P<0.05) in the number of cystic follicles. It is an aromatase inhibitor, which negatively affects hormone levels, thus leading to interruption of ovulation and infertility of the ovary [37]. The table also offered a significant increase (P<0.05) in the total number of ovarian follicles and the corpus luteum and a significant decrease (P<0.05) in the number of cystic follicles for

the groups treated with asparagus ovarian, The reason for this increase is due to the properties of the asparagus root extract from the effective and biologically active compounds, as quercetin promotes the growth of ovarian follicles [38], and arginine and aspartic acid participate in the formation and maturation of eggs due to the stimulatory ability in the response of the pituitary gland that releases gonadal hormones [39,40]. Conclutoin asparagus showed helpful effects in letrozole induced pcos in female wistar rats. Its effect was comparable to that of clomiphene citrate, most widely used treatment for ovulation induction in pcos condition. These results suggest that asparagus roots may be a potential natural supplement for promoting the health of female reproductive systems. It is a relevant article and participate in genuine remedies for problems in this sector generally.

TABLE 1. The influence of *asparagus officinalis L.* on ovarian follicle numbers in adult female rats.

| Parameters | control | Asperges | PCOS | Treatment | preventive |
|---------------|----------------------------|----------------------------|----------------------------|----------------------------|----------------------------|
| Primordial F. | 5.3 ±0.21 ^b | 6.5 ±0.22 ^a | 1.84 ±0.26 ^e | 4.6 ±0.20 ^c | 3.67 ±0.21 ^d |
| Primary F. | 4.33 ±0.21 ^b | 5.5 ±0.22 ^a | 1.33 ±0.21 ^e | 3.66 ±0.21 ^c | 2.67 ±0.21 ^d |
| Secondary F. | 3.3 ±0.21 ^b | 4.6 ±0.21 ^a | 0.67 ±0.21 ^e | 2.8 ±0.17 ^{bc} | 1.67 ±0.21 ^d |
| Graphain F. | 2.5 ±0.22 ^b | 3.5 ±0.22 ^a | 0.50 ±0.22 ^e | 1.83 ±0.17 ^c | 1.17 ±0.17 ^d |
| Corpus L. | 4.5 ±0.22 ^b | 5.66 ±0.21 ^a | 0.33 ±0.21 ^e | 3.83 ±0.17 ^c | 2.83 ±0.17 ^d |
| Cysts F. | 0 ±0.00 ^d | 0 ±0.00 ^d | 5.83 ±0.31 ^a | 1.66 ±0.33 ^c | 3.50 ±0.34 ^b |

The values are displayed as mean ±SD, values in the same column with different letters, statistically significant (P < 0.05)

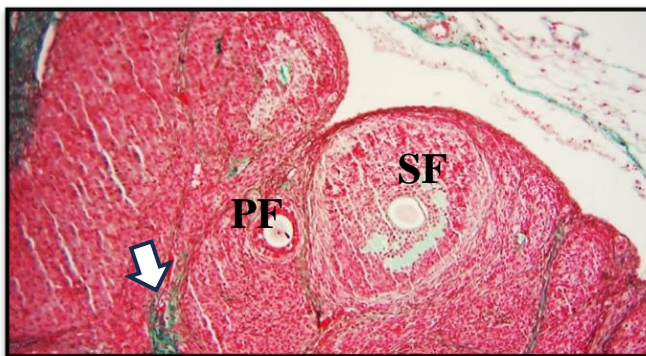


Figure 1. Histological section in ovaries in control group Shows less

of stroma (arrow) secondary follicle (SF), Primary follicle (PF) (masson trichrome stain X10).

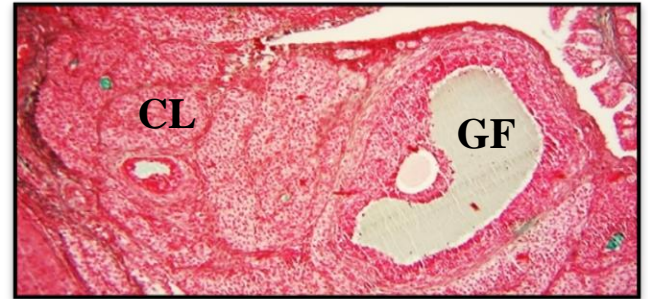


Figure 2. Histological section in ovaries in asparagus group Shows graafian follicle (GF), corpus luteum (CL) (masson trichrome stain X10).

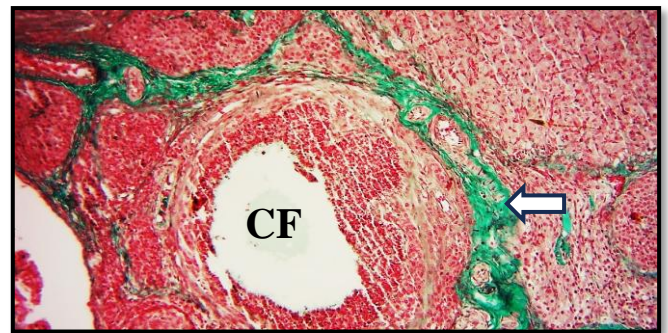


Figure 3. Histological section in ovaries in PCOS group Shows aggregation of stroma plenty (collagen fibers) (arrow), Cysts follicle (CF) (masson trichrome stain X10).



Figure 4. Histological section in ovaries in treated group with asparagus Shows less of stroma (collagen fibers) (arrow), graafian follicle (GF), Primary follicle (PF) (masson trichrome stain X10).

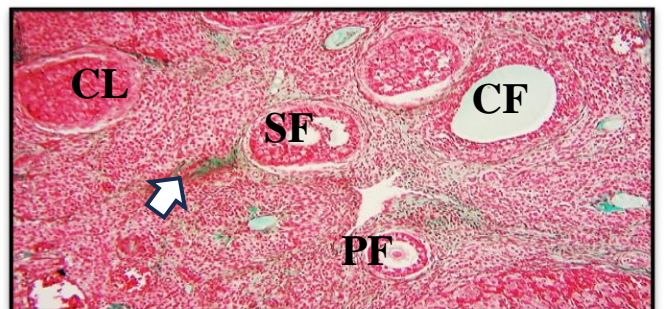


Figure 5. Histological section in ovaries in preventive group Shows less of stroma (collagen fibers) (arrow), secondary follicle (SF), Primary follicle (PF), corpus luteum (CL) , Cysts follicle (CF) (masson trichrome stain X10).

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Arabic Abstract

متلازمة المبيض متعدد الكيسات (PCOS) لها عدة أعراض مثل كثرة الشعر، فرط الأندروجين، انقطاع الطمث، انعدام الإباضة والعقم بالإضافة إلى الاضطرابات الأيضية واضطرابات الغدد الصم. تهدف الدراسة الحالية تقييم فعالية مستخلص من جذور نبات الهليون في علاج إناث الفئران المستحث بها متلازمة تكيس المبايض. في هذه البحث، تم دراسة تأثير 400 ملغم / كغم من مستخلص جذور نبات الهليون على متلازمة تكيس المبايض لمدة 28 يوماً من أجل فحص تأثيرات مستخلص جذور نبات الهليون على متلازمة تكيس المبايض المستحثة بالليترزول. قسمت ثلاثين أنثى من فئران الالبيو إلى خمس مجموعات، المجموعة الأولى 1 هي مجموعة التحكم، المجموعة الثانية 2 جرعت بمستخلص جذور الهليون 400 مجم/كجم لمدة 28 يوم، المجموعة الثالثة 3 هي المجموعة المستحث فيها متلازمة تكيس المبايض ولتحفيز متلازمة تكيس المبايض، تم إعطاء الفئران ليترزول (1 مجم/كجم) عن طريق الفم لمدة 28 يوماً في محلول 0.5% كربوكسي ميثيل سلولوز (CMC)، المجموعة الرابعة 4 هي مجموعة علاجية تم تجريع الحيوانات بالليترزول لمدة 28 يوم بعدها جرعت لمستخلص جذور الهليون لمدة 28 يوم أخرى اما المجموعة الخامسة 5 هي المجموعة الوقائية مستخلص جذور الهليون لمدة 28 وبعدها تم التجريع بالليترزول لنفس المدة. أظهرت نتائج ملون التميز الثلاثية الألوان قلة في ألياف السدى والكولاجين في التركيب النسجي للمبايض في جميع المجموعات، بما في ذلك المجموعة الضابطة باستثناء مجموعة متلازمة تكيس المبايض التي تشير إلى وجود نسبة عالية من الكولاجين، بالإضافة إلى زيادة عدد الجريبات الأولية، الثانوية (الغارية) والحوصلة جراف بشكل كبير. أظهرت المجموعة العلاجية والوقائية عدداً أقل من الجريبات المتكيسة مقارنة بمجموعة متلازمة تكيس المبايض كما تظهر النتائج انخفاضاً في ألياف الكولاجين في المجموعة التي جرعت بمستخلص جذور الهليون حيث انه يعمل على تسريع عملية تكوين البويضات والانقسامات المبكرة للخلايا.