

Immunohistochemical Changes of Polycystic Ovaries in rats Treated with *Asparagus Officinalis* L. Roots Extract

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Abstract

Background: Polycystic ovary syndrome (PCOS) which a series reproductive, metabolic and psychological condition that is characterized by abnormal gonadotropin secretion or hyperandrogenism and associated with insulin resistance and most commonly found in the reproductive age female. **Objectives:** This research paper were conducted to examine the effects of *Asparagus officinalis* L. roots extract on letrozole induced PCOS where the effect of 400mg/kg *A. officinalis* L. root extract on the PCOS was studied for 28 days in female rats. **Materials and Methods:** Thirty-five female Albino rats were randomly divided into five groups, including control negative group, positive control, PCOS, treatment and preventive group. During 28 days, the positive control group got an extract of the *A. officinalis* roots. Letrozole 1 mg/kg dissolve in 0.5% carboxymethyl cellulose solution was used to cause PCOS for 28 days. Following the induction of PCOS, the treatment group received extract *A. officinalis* roots for 28 days. The preventative group received an extract of asparagus roots during PCOS induction. **Results:** Immunohistochemical results are shown Ovary slices from of the therapeutic and preventative groups next to the asparagus group were immunostained for Ki 67, which showed high positive statistically ($P < 0.05$) immunological activity in the granulosa nuclei and less in the theca cells. When compared to the PCOS group, both the granulosa and theca layers had very weak positive immunostaining ($P < 0.05$). According to the morphological findings, granulosa cells from the Control group and asparagus group proliferated much more than granulosa cells from the PCOS group statistically significant ($P < 0.05$). **Conclusion:** The findings show that theca and granulosa cells are quickly stimulated to proliferate by asparagus root extract. Moreover, it therefore speeds up oogenesis and early cell divisions.

Keywords: *Asparagus officinalis* L., immunohistochemical, Ki67, PCO

INTRODUCTION

The most prevalent reproductive condition, polycystic ovarian syndrome (PCOS), has a destructive impact on a number of metabolic systems.^[1] Stein-Leventhal syndrome was first identified in 1935 by American gynecologists Irving F. Stein Sr. and Michael L. Leventhal.^[2] (Polycystic ovarian syndrome, a complicated metabolic, psychological, and reproductive problem that is characterized by abnormal gonadotropin secretion or hyperandrogenism and associated with insulin resistance and Obesity, It causes of up to 30%of infertility,^[3,4] In the case of PCOS the appearance of ovaries in the form cysts pearls are from eggs or follicles size of approximately 2–10mm.^[5] There are different clinical significances like infertility, irregular periods, hirsutism, alopecia.^[6,7]

Several treatments were used to address the symptoms of polycystic ovarian syndrome and to trigger ovulation.^[8,9] Yet, some treatments have had significant serious side effects^[10] Hence, emphasis is increasingly being placed on medications derived from natural sources that exhibit fewer or no negative effects, Lifestyle adjustment is vastly considered to be the cornerstone of PCOS treatment.^[11-13]

From ancient times, *Asparagus officinalis* L., a perennial grass with many bioactivities, has been utilized

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primarily as food and medicine.^[14] Because of its health advantages on the market, academia has become increasingly interested in it.^[15] Due to its medicinal and nutraceutical qualities, *A. officinalis* (L.) is regarded as a high value plant. Its constituents and properties have revealed a rich variety, including saponins, flavonoids and (kaempferol, quercetin, rutin), phenolics, fructans, saponins, vitamins, and minerals. These substances also play a role in anti-tumor activity and the reduction of the risk of disorders and diseases like obesity, constipation, disease rheumatism, diarrhea, as well as like, diabetes, cardiovascular, and osteoporosis.^[16,17] Asparagus roots have medical use as well since they are utilized as tonics, erogenous, and extracts from the roots have been linked to the regulation of key reproductive hormones and oogenesisides^[18] increased folic acid levels are necessary for the creation of new red blood cells.^[19] Tyrosine, asparagine, and arginine are three other key chemical constituents. It is also to blame for the kidneys' increased excess rate of urine output.^[20]

MATERIALS AND METHODS

Animals

Female Wistar rats weighing 160–200g were used in the investigation. The animals were obtained from the pharmaceutical collage of Karbala University of Iraq and allowed to get acclimatized for 1 week. They were in a temperature-controlled environment (21–22 °C, with 12-hour cycles for light and dark), Water and a regular diet were given to the animals, Every animal procedures were carried out in accordance with the guidelines of Supervisory Committee of housing department/ education collage of pure sciences/ Animal Council/ Karbala university.

Experiment design

A total of 35 female Wistar rats were randomly divided into five groups, each with six rats, Group I, which acted as the control; Group II the positive control group got 400mg an extract of the *A. officinalis* roots for 28 days; Group III Letrozole 1 mg/kg dissolve in 0.5% carboxymethyl cellulose solution was used to cause PCOS for 28 days; group IV Following the induction of PCOS, the treatment group received 400mg extract *A. officinalis* roots for 28 days. However, Group V The preventative group received an extract of asparagus roots during PCOS induction.

Letrozole induced polycystic ovarian syndrome

One dose of 1 mg/kg letrozole was used to produce PCOS for 28consecutive days and dissolved 0.5% Carboxy-Methylcellulose (CMC).^[21]

Prepare asparagus extract

Roots were Purchased from Iraqi markets and they were completely Powder was created in the lab after being

dried. To create a homogeneous solution, the resulting powder was combined with 70% ethyl alcohol, and the mixture was agitated in a rotodoxy device for 24 hours at room temperature, to get a solid extract free of alcohol, the solution was filtered and dried at room temperature for 48 hours, the solid extract was then diluted in 1 ml of distilled water using 400mg. Prior to usage, the solutions were kept in a refrigerator.^[18]

Statistical analysis

The data are presented as mean standard deviation SD, and one-way ANOVA and revised Least Significant Differences (LSD) tests are used to analyze them, statistically significant were considered as $P < 0.05$.

Ethical approval

All animal operations were carried out in compliance with the guidelines of Supervisory Committee of housing department/ education collage of pure sciences/ Animal Council/ Karbala university, A local ethics committee examined and approved the study protocol in accordance with document number 1818/14/3 on June 7, 2023.

RESULTS

Immunohistochemical results are shown Sections of the control group's ovaries (Group1) and asparagus group (Group 2) significant immunostaining for Ki 67 in the granulosa cell nuclei and less in the theca cell nuclei ($P < 0.05$) [Figures 1 and 2]. while PCOS group (Group 3) showed statistically significant ($P < 0.05$) Very weak positive immunostaining of the two layers of theca and granulosa cells [Figure 3]. According to the statistical results ($P < 0.05$) treatment with asparagus group (Group4) exhibited granulosa and theca layer immunostaining that was positive [Figure 4], whereas preventative group (Group 5) showed few positive immune reactions of granulosa and theca layer ($P < 0.05$) [Figure 5].

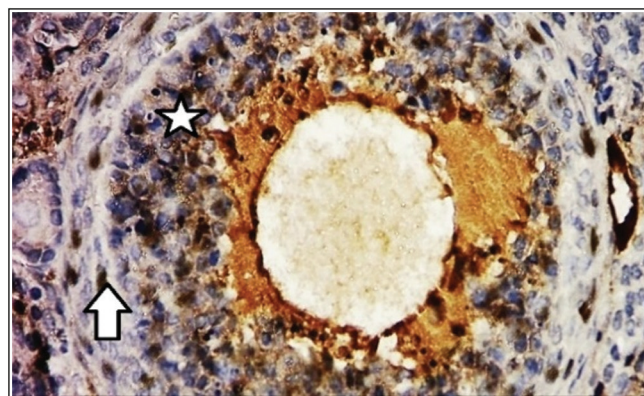


Figure 1: Histological section of an ovarian section of the control group, displaying granulosa layer (star) and theca layer (arrow) in a secondary follicle with high positive Ki 67 antibody. (Ki 67 immunostaining X400)

DISCUSSION

Based on the results *A. officinalis* roots increases cellular proliferation of granulosa and theca cells, as well as preservation of ovarian vesicular development [Figures 2, 4, and 5] and [Table 1]. This study agreed with^[22,23] It is believed that the presence of phytoestrogen components in the extract from asparagus roots contributes to the rise in ovarian hormones, Diosgenin, Protodioscin, and Sarsaponin are steroid saponins that are the estrogenic components taken out of asparagus roots,^[24,25] these substances boost production and serve as a precursor to the progesterone hormone.^[18,26] the Phytoestrogens effects of an assortment of structurally various asparagus roots derived compounds are interposed through the estrogen receptors ER β and ER α , which function as inducible factors for cell growth, proliferation, and differentiation.^[27] The amino acid molecules arginine and aspartic acid are abundant in roots.^[17] Nitric oxide, one of the key

components, is produced when arginine, a component of asparagus root, is transformed in key proceedings of reproduction inclusive gamete activation, early cell divisions (ovarian cells), fertilization, and implantation^[28] while Aspartic acid promotes the release of hormones that release gonadotropins that involved in reproduction and development of the oocyte in function, promotes growth and differentiation of the theca cells and granulosa cells, where the Granulosa cells are significant cells in the ovary that undergo changes physiologically and morphologically during the processes of follicular proliferation, ovulation, differentiation, lutenization and atresia,^[29,30] besides elements including calcium, magnesium, zinc, and phosphorus are abundant in asparagus roots^[5,6] that control follicle growth and steroidogenesis, and different levels of vitamins A, B1, B2, C and E, and folic acid additionally

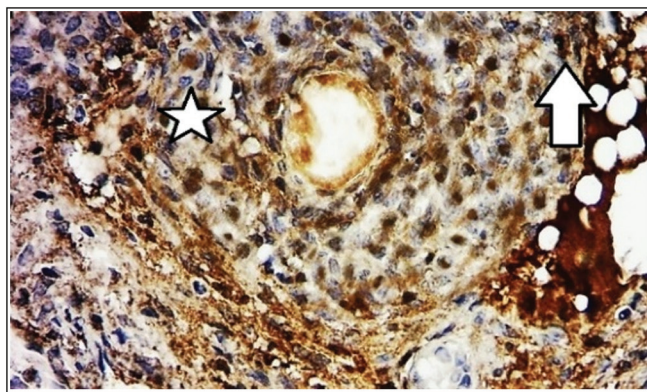


Figure 2: Histological section of an ovarian section of the asparagus group, which displayed a lot of theca layer and granulosa layer Ki 67 immunoreactivity (arrow) (Ki 67 immunostaining X400)

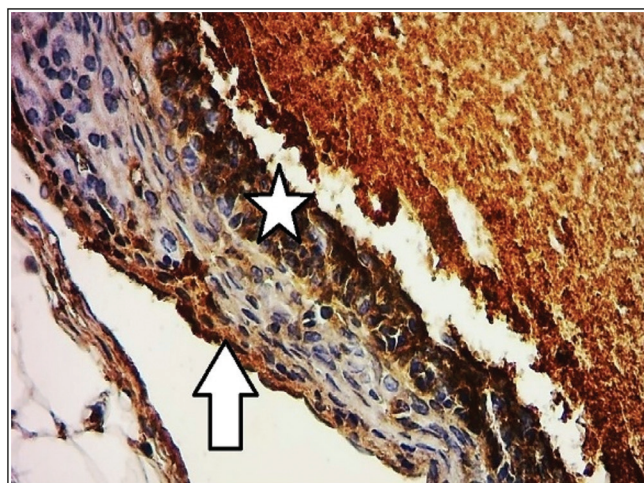


Figure 4: Histological section of an ovarian section of treatment group, showing positive Ki67 immunostaining in both two layers the granulosa cells (star) and theca cells (arrow). (Ki 67 immunostaining X400)

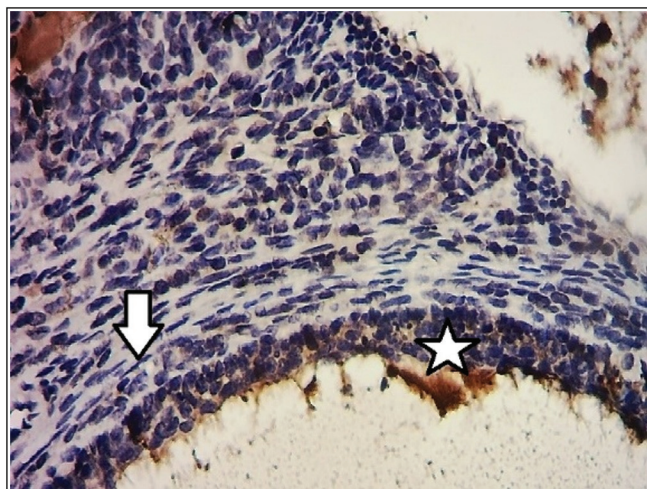


Figure 3: Histological section of an ovarian section of PCOS group, the granulosa layer (star) exhibits positive Ki67 immunostaining, but the theca layer exhibits negative response (arrow). (Ki67 immunostaining X400)

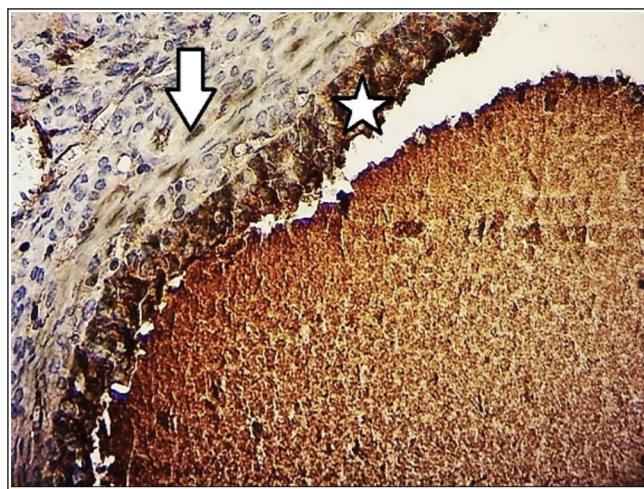


Figure 5: Histological section of an ovarian section of protective group, with positive Ki67 immunostaining of the granulosa layer (star), but minimal positive reactivity of theca layer (arrow). (Ki67 immunostaining X400)

Table 1: The effect *Asparagus officinalis* L on main value to ovarian layers in adult female rats for 28 day

Parameters (μm)	Control (G1)	Asperges (G2)	PCOS (G3)	Treatment (G4)	Preventive (G5)
Granulosa layer	61.74 \pm 1.24 ^b	68.74 \pm 1.67 ^a	27.89 \pm 1.42 ^c	58.24 \pm 1.44 ^{bc}	47.83 \pm 1.68 ^d
Theca layer	1.31 \pm 0.1 ^c	1.32 \pm 0.06 ^c	1.82 \pm 0.07 ^a	1.40 \pm 0.09 ^b	1.67 \pm 0.08 ^{ab}

The values are displayed as mean \pm SD

Values in the same column with different letters, statistically significant ($P < 0.05$)

impact ovarian function and fertility.^[31,32] while in results [Figure 3] all the process of folliculogenesis disturbed in PCOS, inclusion that could be an substantial ovarian abnormality in PCOS.^[33]

CONCLUSION

Asparagus had positive effects in female Wistar rats with PCOS induced by letrozole. Its results were comparable to those of the medication used most frequently by PCOS patients to induce ovulation.

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Conflicts of interest

There are no conflicts of interest.

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