

Promising histological and functional effects of asparagus officinalis L. roots extract on letrozole induced polycystic ovary syndrome in female rat

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Abstract

Polycystic Ovarian Syndrome A variety of symptoms, including metabolic and endocrine abnormalities, hirsutism hyperandrogenism, amenorrhea, anovulation, and sterility, describe. The goal of the current study is to assess how well an extract from the roots of *Asparagus officinalis* L. can treat female rats with induced polycystic ovarian syndrome when different hormonal levels are present. Female rats were given daily doses of letrozole 1 mg/kg for 28 days in order to produce PCOS. *Asparagus officinalis* 400mg/kg was administered to the rats after PCOS was induced, and the hormonal parameters were examined after 28 days, Roots of *Asparagus officinalis* L. were used as a conventional pharmaceutical beside that its treatment and preventive role, In the therapy group compared to the PCOS group, there was a discernible rise in primary, antral, and graphene follicles, Compared to the PCOS group, the treatment and preventative groups had less cystic follicles, Moreover, compared to the control group and other groups, PCOS shows a large rise in testosterone and LH levels and a considerable decline in estradiol and FSH levels, When compared to control and other groups, the conventional medicine made from asparagus roots significantly increased levels of estradiol and FSH while significantly decreased levels of testosterone and LH were seen, Moreover, a rise in the number of ovarian follicles is seen. Conclusion: asparagus officinalis L. roots extract improved the endocrine hormones and the histomorphometric features of the ovary in the Treatment group.

Keywords: *PCOS, Sex hormones, Asparagus Officinalis L.*

INTRODUCTION

PCOS is a variety of reproductive issues that affects women and is associated with chronic anovulation, hyperinsulinemia, and high levels of androgen (1) but its underlying etiology is still obscure, this syndrome has an impact on the metabolic and endocrine systems, and it is the main contributor to female anovulatory infertility(2), Some of the obvious symptoms of ovaries with PCOS include an increase in

ovarian volume, follicle gathering, and lack of corpus luteum(3). For the treatment of PCOS, a variety of therapeutic drugs, including clomiphene citrate, glucocorticoids, aromatase inhibitors, and metformin, have been suggested(4). However, these drugs have a variety of side effects, including nausea, vaginal bleeding, and abdominal pain. As a result, the uses of herbal medicines nowadays are more widespread(5), particularly in the treatment of polycystic ovarian

syndrome(6,7).In truth, asparagus officinalis L. (AR) is a medicinal food crop that has been grown by numerous ancient civilizations(8) As a result, the crop has a rich and varied history, beside that due of its medicinal and nutraceutical qualities, a high value plant(9). A. officinalis has been used to treat toothaches, dyspnea, palpitations, and heart problems. Its constituent saponins have a crucial role in the anti-tumor action and reduction of the risk of illnesses like obesity, diarrhea, and constipation(10). A. officinalis roots have diuretic and potent cardiac sedative properties(11). Galactogogic characteristics can be seen in powdered roots(12). They help cure metabolic disorders, the irregular menstrual cycle, and the symptoms of PCOS, as well as general senility. Moreover, it enhances female hormones and fertility(13).

MATERIALS AND METHODS

Experimental animals

In the Karbala University of Iraq's pharmacy college, 30 mature female Wistar rats weighing 180–230 g were purchased. They were then separated into five groups of six rats each. Group (1) Normal Control, Group (2) asparagus officinalis Extract (400 mg/ kg), Group (3) PCOS induce, Group (4) PCOS+ asparagus officinalis (treatment) , and Group (5) PCOS with asparagus officinalis (preventive). Rats were administered Letrozole (1 mg/kg bw) in a 0.5% Carboxy-Methylcellulose (CMC) solution orally for 28 days to produce PCOS. After Letrozole induction, the rats were separated into three groups: PCOS untreated, PCOS plus asparagus officinalis Extract (400 mg/ kg), and PCOS plus Extract.

Induction of PCOS and prepare AR extract

Rats were given letrozole (1 mg/kg) dissolved in 0.5% carboxymethylcellulose (CMC) for 28 days to induce PCOS (14). Whereas roots for the treatment group were bought from Iraqi marketplaces and completely dried in the lab

before being ground into powder. To create a solid extract, the resulting powder was combined with 70% ethyl alcohol for 24 hours at room temperature. The mixture was then filtered and dried for 48 hours. The solid extract was then diluted in 1 ml of distilled water at a concentration of 400 mg/kg. Prior to usage, the solutions were kept in a refrigerator (15).

Blood sampling and histological study

Once 24 hours had passed since the experiment's final day, rats were sedated for 3-5 minutes using cotton that had been soaked in chloroform before entire blood was drawn via a cardiac puncture to measure hormones like estrogen, testosterone, LH and FSH. Blood was maintained in an Eppendorf tube without EDTA for no more than 4 hours, and the serum was separated for further biochemical analysis by centrifuging 3000 times for 5 minutes. Ovaries were removed via dissection, fixed with formalin, and tissue processing before being used in a histomorphometric investigation. For histological study, serial slices of ovaries were cut into 5 m thick pieces and stained with hematoxylin and eosin using the routine dye.

STATISTICAL ANALYSIS

Standard error of the mean (SEM) is used to present all results. Comparing statistical variances between groups (ANOVA). The cutoff for statistical significance was P 0.05.

RESULT

In the PCOS group (Fig 3), the mean number of cystic follicles increased dramatically in comparison to other groups, while the mean number of primary follicles decreased in comparison to control and other groups due to absence of antral follicles and corpus luteum. In contrast to the PCOS group, the average number of cystic follicles dramatically decreased in the treatment and preventative groups (Fig4,5) while the number of corpus

luteum and antral follicles significantly rose. in the group using asparagus extract there was also a rise in the number of ovarian follicles (Fig 2). When PCOS was induced, testosterone and LH levels significantly increased, although estrogen and FSH levels were decreased when compared to the control group and other groups, next to the treatment group When compared to PCOS rats, oral administration of asparagus resulted in a

significant increase in estradiol and FSH levels and a significant drop in testosterone and LH levels, While the extract asparagus roots significantly increased estradiol and FSH levels while significantly lowering testosterone and LH levels when compared to control and other groups(Tabel1). there was also a rise in the number of ovarian follicles (Fig 2).

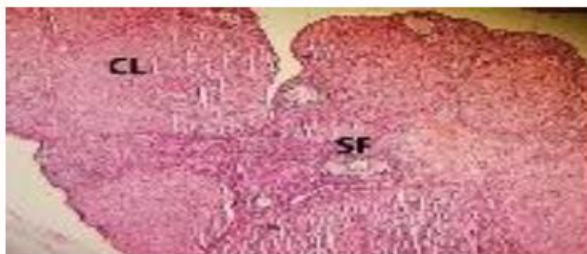


Figure (1) Ovarian tissue from the control group. Show the existence of corpus luteum (CL) and secondary follicles (SF), and normal histological structure.(H and E X100)

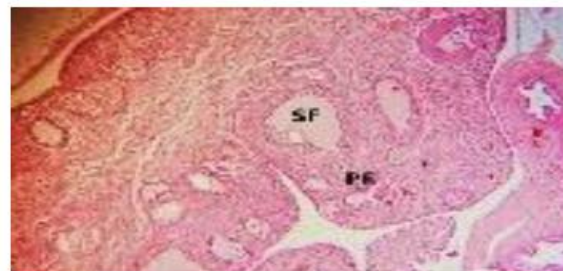


Figure (2) Asparagus group's ovary histological section displaying follicles in various stages of development secondary follicles (SF) and primordial follicles (PF) (H and E X100)



Figure (3) Ovarian histology in the PCOS group. displaying several deteriorating follicles along (arrows)

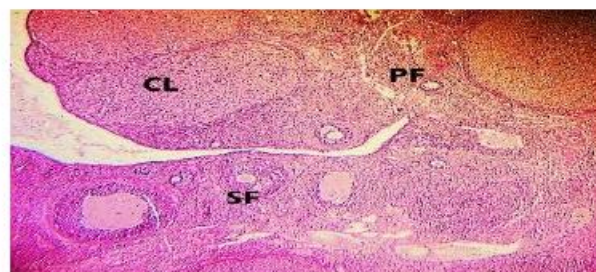


Figure (4) Histological portion of ovary in treated group with asparagus Show The corpus luteum (CL) and secondary follicles (SF). (H and E X100)

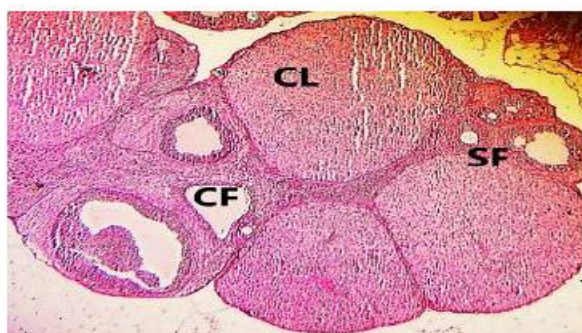


Figure (5) Ovarian histology in the preventative group shown the corpus luteum (CL), secondary follicles (SF), cystic follicles (CF). (H and E X100)

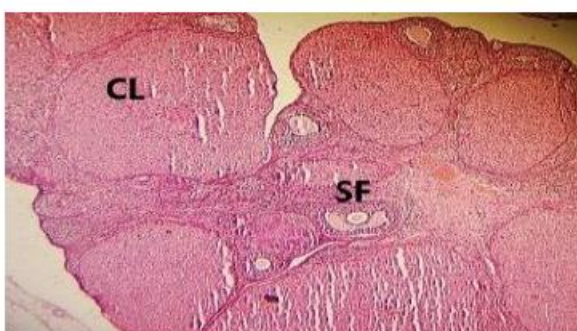


Figure (1) Ovarian tissue from the control group. Show the existence of corpus luteum (CL) and secondary follicles (SF), and normal histological structure.(H and E X100).

Table (1) the effect Asparagus Officinalis L on main value of the number of ovarian follicles in adult female rats

<i>Parameters</i>	<i>control</i>	<i>Asperges</i>	<i>PCOS</i>	<i>Treatment</i>	<i>preventive</i>
<i>Primordial F.</i>	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
<i>Primary F.</i>	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
<i>Secondary F.</i>	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
<i>Graphain F.</i>	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
<i>Corpus L</i>	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
<i>Cysts F.</i>	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)

Table (2) the effect Asparagus Officinalis L on main value to serum hormones level in adult female rats.

<i>hormones</i>	<i>control</i>	<i>Asperges</i>	<i>PCOS</i>	<i>Treatment</i>	<i>preventive</i>
<i>Estrogen(pg/ml)</i>	29.32±0.54 ^b	32.82±0.73 ^a	18.18±0.67 ^e	27.41±0.76 ^{bc}	24.33±0.77 ^d
<i>Testosterone(ng/ml)</i>	0.82±0.03 ^{cd}	0.69±0.01 ^e	2.73±0.04 ^a	1.22±0.02 ^c	1.65±0.07 ^b
<i>LH (ng/ml)</i>	1.56±0.07 ^d	1.21±0.03 ^e	3.17±0.07 ^a	1.83±0.09 ^c	2.18±0.09 ^b
<i>FSH (ng/ml)</i>	7.76±0.09 ^b	8.11±0.14 ^a	4.77±0.10 ^e	7.23±0.09 ^c	6.73±0.13 ^d

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

DISCUSSION

In the current work, female Wistar rats were given the aromatase inhibitor letrozole to cause polycystic ovary syndrome ,As shown, testosterone levels significantly increased when compared to control animals, showing the hyperandrogenism status of PCOS are a sign of anovulation (16).however, Gonadotropin-releasing hormone (GnRH) levels rise in pcos induce as a result of hypothalamic-pituitary-gonadal axis

malfunction in PCOS, and inappropriate FSH and LH secretion impairs follicle growth and may result in cysts and loss of ovarian function(17).the results showed that asparagus was effective and able to normalize blood hormone levels to those of the control group. These findings are consistent with those of the earlier research(18,19,20) The cause may be related to the aspartic acid and arginine effect of AR, which stimulates pituitary gonadotropin secretion, and the phytoestrogen action of the root, which can reduce the

quantity of free testosterone(21,22). Asparagus effectively returns its level to normal Due to the abundance of phenolics, steroidal glycosides, terpenes, and polycyclic alkaloids in AR roots(23). Also, asparagus revealed the presence of the phenolic component caffeic acid in the roots of a plant that is mechanistically implicated in the growth and modification of estrogen receptors(24).It appears that the phytoestrogen components found in asparagus extract are similarly efficient at raising ovarian hormone levels. Phytoestrogens are organic substances generated from plants that perform comparable roles to estrogen, Sarsaponin, protodioscin, and diosgenin are three steroid saponins that are most likely to contain estrogenic properties when recovered from asparagus roots(25).These substances also function as progesterone precursors, increasing the hormone's release(26),While the granulosa and theca cells of the ovaries are among the cells in the body that synthesize steroid hormones because they have the necessary enzymes for hormone production(27,28).Calcium, magnesium, phosphorus, and zinc are among the minerals abundant in asparagus roots(29)Minerals in the follicular fluid control steroidogenesis and follicle development ,Minerals influence ovarian function and fertility in addition to serving as cofactors in several enzymatic activation mechanisms for oocyte growth and maturation(15,30).

CONCLUSION

asparagus officinalis L. roots extract improved the endocrine hormones and the histomorphometric features of the ovary in the Treatment group compared to the PCOS group. Beside this study showed that 400 mg/ml of this extract, maintains follicular and promotes the development.

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