Effects of *Pimpinella anisum* oil Extract on Some Biochemical Parameters in Mice experimentally induced for human Polycystic Ovary Syndrome

تأثير المستخلص الزيتي لنبات الينسون على بعض المعايير الكيماحياتية والمرضية في الفئران المستحدث فيها متلازمة تكيس المبايض

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Abstract

In this study, the effect of *Pimpinella anisum* oil (Anise oil) extract is evaluated on some biochemical parameters in PCOS mice as a model for human PCOS. Estrous cyclicity of 24 adult cycling mice was monitored by vaginal smears. After about 4 days, 18 mice received an intramuscular (I.M.) injection of a single dose of estradiol valerate (EV), one mg in 0.1 ml of corn oil to induce (PCOS), corn oil was injected to the six mice in the control group. After 60 days from injection, mice with (PCOS) were orally administered multiple doses (200, 400) mg/kg Body weight of anise oil extract for 15 days. The histological (ovary) and hormonal (FSH, LH, estradiol and progesterone) results showed that anise oil can decrease signs of PCOS in the ovarian tissue and altered concentrations of luteinizing hormone.

المستخلص

تم في هذه الدراسة تقييم تأثير المستخلص الزيتي لنبات الينسون على بعض المعايير الكيمياحياتية في الفئران المستحدث فيها تكيس المبايض . تم عمل المسحات المهبلية لـ 24 انثى ناضجة بشكل دوري كل اربعة ايام . 18 انثى حقنت بالاسترادايول عن طريق العضل1ملغم في 0.1 مللتر من زيت الذرة لاستحداث متلازمة تكيس المبايض ، في حين حقنت 6 اناث بزيت الذرة كمجموعة سيطرة . بعد 60 يوم من الحقن ، جرعت الفئران المستحدث فيها متلازمة تكيس المبايض بجرعتين من المستخلص الزيتي لنبات الينسو ن (200،400) ملغم/كغم من وزن الجسم عن طريق الفم لمدة 15 يوم . اظهرت نتائج الدراسة النسجية للمبيض والهرمونية (الهرمون المحفز لنمو الجريبات ، الهرمون اللوتيني، الاسترادايول والبروجستيرون) بان المستخلص الزيتي لنبات الينسون يقلل من علامات متلاز مة تكيس المبايض في نسيج المبيض ويساعد عل ى افراز الهرمون اللوتيني في الفئران .

Introduction

Polycystic ovary syndrome, the most common female endocrine disorder, is a heterogeneous endocrine and metabolic disorder, affecting 6 - 10% of women of reproductive age [1]. Features of PCOS may manifest at any age, ranging from childhood (premature puberty), teenage years (hirsutism, menstrual abnormalities), early adulthood and middle life (infertility, glucose intolerance) to later life (diabetes mellitus and cardiovascular diseases) [2]. Several of these features increase the risk of cardiovascular diseases (CVD) in women and the prevalence of hypertension in women with PCOS is about 40% in comparison with a prevalence of about 25.8 in the general population [3]. The PCOS is also associated with a higher risk of myocardial infarction (relative risk) and with a compromised cardiovascular profile independent from obesity in young women [4]. Hyperandrogenism and insulin resistance or deficiency is linked to (PCOS), as early as 1921, when Achard and Thiers published

their classic description of a bearded woman with diabetes [5]. The PCOS is then called the Stein-Leventhal syndrome, which is first described in 1935. Originally, diagnosis required pathognomonic ovarian findings 9 and the clinical triad of hirsutism, amenorrhea and obesity [6].

Experimental induction of a PCOS in rodents is first introduced by [7] which made possible by the use of a single (I.M.) injection of EV in 8-week-old mice. The mice ceased ovulation and developed characteristics of human PCOS, including large cystic follicles in the ovaries and altered concentrations of luteinizing hormone [7].

As an aromatic plant, anise (*Pimpinella anisum* L.) is an annual herb indigenous to Iran, India, Turkey and many other warm regions in the world. Anise oil has anothole (85%) as active ingredient and also it has eugenol, methylchavicol, anisaldehyde and estragole. As a medicinal plant, anise has been used as a stimulating effect of digestion and antiparasitic antibacterial, antifungal and antipyretic [8]. Additionally, the plant and especially its fruit essential oil has been used for treatment of some disease including seizures and epilepsy [8]. Furthermore, it has been shown to have anticonvulsant effects and has been used for the treatment of constipation and possesses muscle relaxant effect [9]. Other constituents include coumarins, lipids and flavonoids (flavonol, flavotie, glycosides, rutin, isoorientin, isovitexin) which are usually conjugated with sugars and are present in all vascular plants. The benzopyranonring system is a molecular scaffold which is found in flavonoid natural products and has weak aromatase inhibitory activity [10].

The aim of this project was to study the effects of anise oil extract on some biochemical and clinical parameters in PCOS mice as a model for human.

Materials & Methods

Extraction of *Pimpinella anisum*

Pimpinella anisum leaves were taken from local market and identified by prof. Dr. Ali AL-Mosawy, biology department, college of science, Baghdad University, after grinding the dried leaves; the plant material was extracted by clavenger operator and the dose (200, 400) mg/kg B.W determined according to [11].

Animals and Care: Twenty-four 8-week-old mice, weighting (23-27) gm were divided into two groups and housed every six mice in a cage under standard conditions (21 ± 2°C, 12- hour light/ 12-hour dark cycles) for at least one week before and throughout the study, with free access to standard chow and tap water ad libitum. The study and all the procedures were carried out in accordance with the guidelines for the care and use the laboratory animals of AL-Nahrain University, Biotechnology Research Center.

Hormonal Treatment and Study Procedures: After one week of acclimatization, mice were divided into two groups of control and PCOS mice. Six mice in the control group received 0.1 ml corn oil and eighteen mice assigned to the (PCOS) group received an I.M. injection of 1 mg Estradiol Valerate (EV), in 0.1 ml of corn oil, to induce PCOS as described by [7]. All the EV-treated mice were evaluated 60 days after the injection, PCOS mice were subdivided into three Groups (8 mice in each group): one group did not receive anise oil extract and other two groups daily received different doses (200, 400) mg/kg B.W of anise oil extract orally for 15 days .

Experiment Design

Vaginal Smears: Estrous cyclicity was monitored by vaginal smears obtained, and assessed by light microscopy for the relative proportion of leukocytes, epithelial and cornified cells found in daily vaginal lavages, which characteristically change during different stages of the estrous cycle. The mice estrous cycle (estrus, diestrus, metestrus, and proestrus) usually lasts about 4 days, in both control and PCOS mice.

Measuring Circulating Gonadotropins and Gonadal

Steroids: Blood samples were collected by heart puncture and serum was isolated by centrifugation (2000 RBMI/10 minutes). Serum LH, FSH, progesteron and estradiol levels were determined by ELISA method. The kits used for these hormones supplied by (Diagnostic Automation, INC.).

Ovarian Morphology: Ovaries of the controls and EV-treated mice were removed after treatment period, cleaned from adherent fat and connective tissue, and fixed in 10% formaldehyde buffer for at least 24 hours.

Histopathological studies

Ovaries were carefully isolated, weighed, washed in buffered saline and fixed in 10% formalin. Ovaries sections (5-6)µm were routinely processed by standard histological techniques, then Ovaries were stained with hematoxyline - eosin stain as described by Guyer [12], and examined by light microscope (Nikon Eclipse E400) to assess histopathological changes among, control and experimental animals.

Statistical Analysis: Differences between the two groups were analyzed by Student's t-test. Comparisons between the control and EV-treated mice were made by ANOVA, followed by the Student-Newman-Keuls post-hoc test. A p-value less than 0.05 was considered statistically significant [13].

Results and Discussion

Mice with PCOS which had been treated by (200, 400) mg/kg of *Pimpinella anisum.* Oil extracts showed recovery demonstrated by macroscopic and microscopic morphological examination of the ovaries. In the ovarian tissue, the cysts had mainly appeared Figure (1) and the cysts were disappeared by anise oil administration Figure (2).

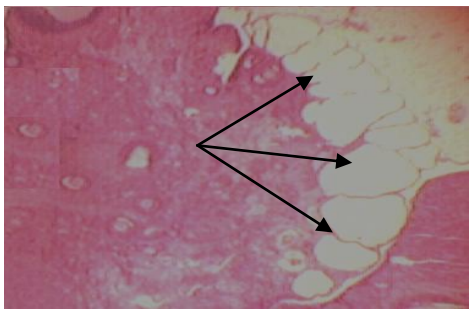


Figure (1): The ovary. In the ovarian tissue, the cysts were mainly appeared by a single intramuscular dose of estradiol valerate, 0.1 mg in 0.1 ml of corn oil (H&E 40X)

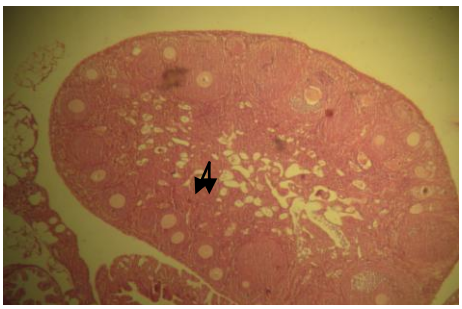


Figure (2): The ovary. In the ovarian tissue, the cysts were mainly disappeared after treated with anise oil extraction at concentration 400mg.(H &E 10X)

A type of polycystic ovary resembling some aspects of human (PCOS) can be induced in the mice with a single injection of long-acting estradiol valerate. Among several theories behind the development of PCOS, the involvement of the sympathetic

nervous system draws much attention, and herbal medicine is known to relieve the abnormal symptoms of PCOS.

For an experimental induction of PCOS, a long-acting EV has been used [12]. The morphological changes include atretic antral follicles, follicular cysts with a well-developed theca cell layer, a diminished granulosa cell compartment and luteinized cysts [14].

For example changes in serum levels of gonadotropin-releasing hormones (GnRH) and appearance of cysts, was induced by injecting a long-acting estradiol valerate. Classical neuroendocrinological studies indicate that in female mice, the neuronal component responsible for the induction of the LH surge is located in the preoptic area (POA) [15].

The results indicated that the administration of 200 and 400 mg/kg B.W of Anise oil extract caused a significant boost in ovarian weight Table (1).

Table (1): ovarian weight and serum hormonal levels of in contrast to the control group at the end of the experimental period

	Ovarian weight (mg/100mg B.W)	LH (mIU/mL)	FSH (mIU/mL)	Estradiol pgr/ml	Progesterone ngr/ml
	A	A	A	A	A
Control	**0.12+0.002**	**0.63+0.14**	**0.36+0.12**	**39.42+2.40**	**28.54+3.67**
	A	B	B	B	B
PCOS	**0.10+0.0013**	**1.65+0.34**	**1.09+0.26**	**122.61+6.39**	**14.56+1.84**
PCOS + Oil extract Anise (*pimpinella anisum*) 200mg/kg B.W	B **0.16+0.0021**	A **0.60+0.12**	B **0.92+0.10**	AC **44.64+4.92**	AC **32.60+2.53**
PCOS + Oil extract anise (*pimpinella anisum*) 400mg/kg B.W	B **0.18+0.003**	A **0.65+0.09**	B **0.88+0.15**	C **51.05+3.82**	C **36.43+4.71**

Differentes letters are significant at (P<0.05) to compression columns

Previous studies have shown that the compounds present in anise extracts enhance oxygen diffusion in tissues [16]. [17] have shown that ovarian weight increases by rising in the numbers of small and medium follicles (secondary follicles), these follicles can be a considerable source of ovarian weight [17]. Thus, different compounds of anise oil extracts can increase ovarian weight by enhancing oxygen diffusion to granulosa cells and decreasing atresia in small and medium follicles. In this study, the improved feed utilization with (200, 400) mg anise oil could be due to the positive effects of anise oil on the digestive system. Similarly reported that essential oils were positively affected digestibility of nutrient. Studies showed that, essential oils increased digestion of protein, cellulose and fat [18], improved apparent whole-tract and ileal digestibility of the nutrients and increased effects of pancreatic lipase and amylase [19].

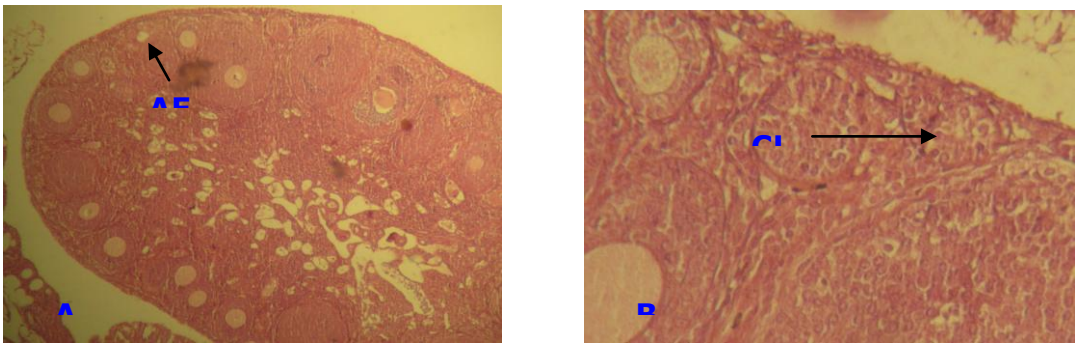
The mechanism of the effects of aniseed on ovary appeared to be related to its high content of antioxidant polyphenolic compounds such as lignans and flavonoids, which would influence oxidative stress [20].

Table (2): Changes in means between primary, secondary, graffian follicles and corpus luteum in all groups

	Primary follicle	Secondary follicle	Graffian follicle	Corpus luteum
	A	AC	A	AC
Control	5.21+0.36	6.48+0.29	0.87+0.09	3.7+0.23
	B	B	B	B
PCOS	2.42+0.26	4.01+0.30	0.54+0.06	1.20+0.09
PCOS + Oil extract Anise	A	A	C	A
(*pimpinella anisum*) 200mg/kg B.W	4.85+0.31	6.26+0.38	0.69+0.07	3.4+0.05
PCOS + Oil extract Anise	A	C	D	C
(*pimpinella anisum*) 400mg/kg B.W	5.94+0.47	7.43+0.61	0.78+0.058	4.2+0.17

Differences letters are significant at (P<0.05) to compression columns

The ovarian functions are influenced by FSH and LH secretion from anterior pituitary, as well as, progesterone and estrogen produced by the sex organs [21]. The observed higher levels of serum PH in the experimental groups may have resulted from the *anisum* oil extract which may have fastened the oestrous cycle Table(l). This may be the reason for the presence of atretic follicles and corpora lutea in the histology of the experimental groups compared with the control Table (2) Figure (3) (A and B).



Figure(3):(A, B) Photomicrograph of groups section treated with anise leaf extract shows primordial, atretic, AF, and matured Graafian follicles, GF, as well as corpus luteum, CL. (H&E 10 k 40 X)

were significantly (p<0.05) higher in the two treatment groups treated with 200mg/kg and 400mg/kg of *anisum* oil extracts Table (1). No difference was found in the FSH serum level of the treatment groups compared with the control. This may suggest that (200,400) mg/kg of anise oil extract stimulated ovulation, which may have resulted in subsequent stimulation of progesterone synthesis, and a resulting inhibition of the LH surge. This study is in line with [22] who reported reduction in sera LH levels after treatment with extract of anise oil, while [23] reported that anise oil did not possess any estrogenic, anti-estrogenic or progestational activity, while appearing not to have interfered with the actions of progesterone.

FSH indirectly stimulates gametogenesis in both sexes and directly stimulates estrogen synthesis and follicular development. It also maintains the structure of the gonads in conjunction with LH [24]. On the other hand, LH is critical to luteinization of the ovarian follicles and post-ovulatory follicular function [21]. Ovulation occurs as a result of the "LH surge" which takes place from the onset of oestrous [24] and

decreases after ovulation due to the increase in the serum level of PH. During post-ovulation, the ruptured follicle is stimulated by LH to become near structure, corpus luteum, which secrets both estrogen and progesterone [21]. When this occurs, there is a negative feedback inhibition of FSH and LH [24].

The most characteristic constituents of *Pimpinella anisum* species are volatile oil, sesquiterpene lactones and phenolics including flavonoids. The effects of flavonoids on the central nervous system have been considered only in the past 10 years. One of the predominant flavonoids in anise oil, was found to competitively inhibit the binding of flunitrazepam, a benzodiazepine derivative, and was fitted into a pharmacophore model for ligands binding to the receptor benzodiazepine site. This reflected the affinities of the compounds in the [(3) H-flumazenil binding assay [25]. Therefore, the effects of anise oil extract in mice which were shown in the present study can be attributed to the direct activation of the central benzodiazepine site.

In conclusion, treatment with (200,400)mg/kg of oil extract of the leaf of anise does decrease signs of model PCOS mice by effect on the histo-morphologies of the ovary and changes in the serum levels of FSH, LH and progesterone of female model PCOS mice.

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