Journal of Pharmaceutical Research International

24(3): 1-11, 2018; Article no.JPRI.45124
ISSN: 2456-9119
(Past name: British Journal of Pharmaceutical Research, Past ISSN: 2231-2919, NLM ID: 101631759)

The Synergistic Effect of Curcumin and Ziziphora Extract Due to Their Anti-inflammatory and Antioxidant Properties on Ovarian Tissue Follicles

Amir Raoofi[1], Abdollah Amini[2*] and Reza Masteri Farahani[2*]

[1]Student Research Committee, Department of Anatomy, Shahid Beheshti University of Medical Sciences, Tehran, Iran.
[2]Department of Biology and Anatomical Sciences, School of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran.

Authors' contributions

This work was carried out in collaboration between all authors. Authors AR and AA designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors AA and RMF managed the analyses of the study. Author RMF managed the literature searches. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JPRI/2018/45124
Editor(s):
(1) Dr. Jinyong Peng, Professor, College of Pharmacy, Dalian Medical University, Dalian, China.
(2) Dr. Jongwha Chang, University of Texas, College of Pharmacy, USA.
Reviewers:
(1) Sharafudeen Dahiru Abubakar, Katsina State College of Health Sciences and Technology, Nigeria.
(2) Rachel Rocha Pinheiro Machado, Faculdade de Ciências Médicas e da Saúde de Juiz de Fora, Brazil.
(3) Veeravan Lekskulchai, Srinakharinwirot University, Thailand.
Complete Peer review History: http://www.sciencedomain.org/review-history/27217

Original Research Article

Received 21 August 2018
Accepted 29 October 2018
Published 14 November 2018

ABSTRACT

Background: The aim of this study was to evaluate the synergistic effect of curcumin and Ziziphora extract due to their anti-inflammatory and antioxidant properties on ovarian tissue follicles in the rat model of the polycystic ovary syndrome (PCOS).
Methods: This experimental study was conducted on 60 female Wistar rats randomly divided into 6 groups of 10. The sham group (receiving curcumin and Ziziphora solvents), the control group (without injection), the PCOS group (injection of 2 mg of estradiol valerate to each rat) and the experimental groups that received intraperitoneal injection of curcumin (600 mg/kg), Ziziphora extract (300 mg/kg) and the combined curcumin and Ziziphora extract after syndrome induction. After 14 days of treatment, the animals were sacrificed by chloroform to collect blood and ovary specimens for histological and serological studies.

**Corresponding author: E-mail: d.amini2008@yahoo.com and mast_far@yahoo.com;*

Results: The PCOS caused a significant decrease in body weight and ovaries ($p<0.001$), follicular and granulosa sheath thickness, number of corpus luteum ($p <0.001$) compared to control group. However, there was no significant difference in tissue changes in experimental ovaries compared to the control. A decrease in LH, estradiol and testosterone, and an increase in FSH and progesterone were significant in the experimental groups compared to the PCOS group ($p<0.001$). Also, in the PCOS group, the LH, estradiol and testosterone levels were increased significantly, while the FSH and progesterone levels were decreased. In addition, the serum lipid profiles in the experimental group were significantly higher than the PCOS group ($p<0.001$).
Conclusion: Curcumin and Ziziphora extract due to their antioxidant and anti-inflammatory properties were effective in reducing cysts and modifying the hormonal levels in the polycystic ovary syndrome.

Keywords: Curcumin; ziziphora; polycystic ovary syndrome; rat.

1. INTRODUCTION

The polycystic ovary syndrome (PCOS) is a collection of signs and symptoms including of the menstrual cycle irregularities with hyperandrogenism, ovulation failure, obesity and various manifestations associated with metabolic disorders and hyperlipidemia [1]. This syndrome is manifested with early and rapid follicular growth, accumulation of at least 10 cysts around the necklace-shaped central stroma, enlargement of the ovarian volume, stromal volume, and increased thickness of theca layer [2]. The most probable cause of ovulation failure seems to be inadequate FSH secretion [3]. In fact, the PCOS is associated with abnormal secretion of gonadotropins of LH (luteinizing hormone) and FSH (follicle-stimulating hormone), increased ovarian steroid secretion and insulin resistance [4]. Insulin increases the level of androgens in three ways: 1. Affecting insulin receptors enhancing the androgenic response of theca cells to LH. 2. Reducing the production of sex hormone binding protein (SHBG) in the liver. 3. Decreasing the production of insulin-like growth factor (IGF)-binding proteins [5]. In the PCOS, the ovarian follicles progress only to the medial antral stage. After that, the maturity process stops. Apoptotic granulosa cells form atrophic, cystic and atritic follicles [6]. In patients with PCOS, the number of small antral follicles and the volume of theca cells are increased in ovaries [7,8].

The clarification of the complications and harmful effects of chemical drugs directed recently the attentions toward the natural and medicinal plants, and new attitudes have been started over the past decades to study herbs and to investigate their physiological and pharmacological effects. The medicinal plants are also an important source of new chemicals with potent therapeutic effects [9]. The herbal medications are used as alternative treatments with fewer complications and multiple properties and as effective therapies in some cases [10].

Turmeric consists of three main compounds, including demethoxycurcumin, bisdemethoxy curcumin and curcumin. Among these, Curcumin Longa, with the chemical name of diferuloyl methane, is an effective ingredient in turmeric rhizome, which is a yellow, fluorescent and hydrophobic molecule that can penetrate rapidly into the cell membrane [11]. This compound, is responsible for protecting the brain and organs and preventing the growth of cancer cells [12]. Anti-inflammatory and anti-cancer effects of curcumin are mainly due to the antioxidant effects and its effect on cellular enzymes, inhibition of signalling pathways at different levels, angiogenesis and cell adhesion [13]. The Ziziphora, *Ziziphora tenuior L.* belonging to the Lamiaceae family, is also one of the native plants of Iran. The most active and effective ingredient of this plant is a substance called Pulgone, whose analgesic and anti-inflammatory effects are well characterised.

This compound is used in traditional medicine in the treatment of fever, menstrual pain and stomach tonus. The anti-inflammatory effects of this plant were due to inhibition of the toxicity of acetic acid and lipid peroxidation, the metal chelation involved in oxidation and reduction, the weakening of the processes leading to the production of oxidative active compounds and, consequently, reduction of intracellular oxidative stress in inflammation-induced rat models [14]. The Ziziphora components have anti-tumour activity and can reduce the growth of some types of malignant tumours by 32.6% and cancerous glands by 47.5% [15].

Regarding the role of oxidative stress and inflammatory factors in the development and promotion of PCOS, and considering the prevalence of PCOS and its effects, we decided to investigate the synergistic effects of these two substances on the induction of ovulation in PCOS patients, opening promising window to future of fertility in these patients with a lower side effect and a higher success rate while reducing the treatment cost to give patients better and safer treatment.

2. MATERIALS AND METHODS

2.1 Ethical Approval

All procedures were conducted in accordance with the standards set forth in the guidelines for the care and use of experimental animals by the Committee for the Purpose of Control and Supervision of Experiments on Animals and the National Institutes of Health. The study protocol (care and handling of experimental animals) was approved by the Animal Ethics Committee of the Shahid Beheshti University of Medical Sciences.

2.2 Animals

This study was performed on female Wistar rats with the mean weight of 170 ± 20 gr. The rats were kept at 22°C and 12:12 h light-dark cycle wit free access to regular food and water. The sexual cycle of all rats was determined by providing vaginal smear for 14 days. The selected rats had four regular oestrous cycles. The animals were randomly divided into six groups. The control group was not under any treatment, in fact, contained healthy rats. The sham group received curcumin solvent (olive oil) and Ziziphora solvent (normal saline). The PCOS group had the rats with polycystic ovary without any drug interactions. The three experimental groups were: A) Curcumin group, the rats with polycystic ovary received 600 mg/kg of curcumin, B) Ziziphora extract group, the rats with polycystic ovary received 300 mg/kg of Ziziphora extract, and C- Combined curcumin and Ziziphora extract, the rats with polycystic ovary received simultaneously curcumin and Ziziphora extract.

2.3 Induction of PCOS

In order to induce syndrome, the rats received 2 mg/kg of estradiol valerate (EV) (Aburaihan Pharmaceutical Company) as a single subcutaneous injection. In order to investigate the successful induction of syndrome, after 60 days of induction, the vaginal smears were performed and persistent vaginal cornification (PVC), which is one of the symptoms of follicular cysts in the ovary, was observed. This test was carried out by sampling vaginal discharge and observing the cells in the smear under an optical microscope [16].

2.4 Preparation of Ziziphora Extraction

The Ziziphora leaves were used to prepare the extraction. The leaves of this plant were prepared from the traditional herbal medicine centre and verified by the herbalist. The preparation of the extract was carried out using percolation method described in previous studies. In this method, Ziziphora leaves (200 g) were powdered and added to 400 cc of 70% ethanol and were left to macerate at room temperature for 4 h. Then, the soaked seeds were extracted by percolation method and the obtained extract was concentrated in a vacuum and was dried in the flat surface. The weight of the obtained extract was 6.5 g. The extract was dissolved in distilled water and was immediately administered interaperitoneally to rat, expressed as mg per kg of body weight for 14 days followed by Induction of PCOS [17].

2.5 Injection of Curcumin and Ziziphora Extract

The rats in the PCOS group received intraperitoneally the curcumin (600 mg/kg) dissolved in the olive oil solvent for 14 days with an insulin syringe and hydroalcoholic extract of Ziziphora (300 mg/kg) dissolved in a saline solvent for the same time. The weight of rats in each group was recorded at each stage of the study. After 14 days of treatment, the animals were sacrificed by inhalation of chloroform. The blood samples were collected from each of the 6 groups using a blood collection needle from the heart, and centrifuged at 3500 rpm for 5 minutes to obtain the serum samples that were analysed serologically by radioimmunoassay method, and hormonal results were recorded.

2.6 Measurement of Serum Biochemical Parameters and Lipid Metabolism

At the end of the experiment, the blood samples were separated by centrifuge at 2500 rpm for 15 minutes at 30°C to measure some biochemical factors including serum cholesterol, triglyceride, LDL, HDL and VLDL. These parameters were

measured by an enzymatic method with commercial kits (Nanjing, China). Furthermore, the level of VLDL was calculated by subtracting HDL and LDL from total cholesterol. The luteinizing hormone (LH), follicular stimulation Hormone (FSH), testosterone, progesterone and estradiol hormones were measured by ELISA Kit (Rat/mouse ELISA Kit, Cosmo Bio Co. Ltd. Japan).

2.7 Tissue Preparation

The ovaries of all three groups were dissected separately, the ovarian fats were removed under the loop and the ovarian tissue was taken for histological examination. In all groups, the ovaries of the rats were weighed with calibrated digital scale (A & D GF600), and the results were recorded to 3 decimal digits, and the ovarian tissues were fixed in 10% formalin.

2.8 Morphological Classification of Follicles

The follicles were divided into five distinct categories, including primordial follicles (a single layer of squamous granulosa cells surrounding the oocyte), primary (a single layer of cubic granulosa cells surrounding the ovum), preantral (multiple layers of granulosa cells), antral (containing antrum) and cysts.

2.9 Stereological Study

The orientator method was used to obtain isotropic uniform random (IUR) sections [18,19]. For this purpose, the cylindrical paraffin blocks containing ovaries were randomly placed on the φ- clock which each half of it was divided into 9 equal parts. By choosing a random number from 1 to 9, an appropriate cut was made along the selected number. The block was then placed on the θ-clock, each half of it was divided into 9 unequal sine-weighted parts, along its cut surface on the 0- 0 axis and then the random number was selected and the cut was made along the selected number. Consecutive 5 and 20 µm thick sections were prepared using a microtome and stained with Hematoxylin and Eosin (H&E) (Merck company, Germany) method.

2.10 The Number of Follicles

To estimate the number of follicles, the optical disector method was used. The average of 12 sections was selected from 20 µm thick sections using systematic random sampling. The sections were studied using the Olympus microscope (BX41TE model) with 100x magnification and the microcator (ND 221 B, Heidenhain, Germany) connected to a computer. The nuclei of follicular cells were sampled by an unbiased counting frame superimposed on the monitor. Identification of the stage of follicles was carried out based on the Mayer et al classification [20]. Any nucleus that lied in the frame and did not touch the left and bottom lines of the frames was selected. The number density (Nv) of different types of follicles was estimated as:

$$Nv = \frac{\sum_{i=1}^{n} Q}{\frac{a}{f} . \ h \ . \sum_{i=1}^{n} p}$$

in which $\sum_{i=1}^{n} Q$ is the total number of counted follicles, **h** is the tissue thickness considered for counting, a/f is the frame area in the true tissue scale and $\sum_{i=1}^{n} p$ is the total number of the points superimposed on the selected fields. The result of the equation is then multiplied by the total volume of the ovary to obtain the total number of follicles [20,21].

2.11 Follicular Layers Thickness (µm)

To estimate the mean thickness of Follicular layers (Theca and Granulosa), an average of 12 sections from 5 µm thick sections was randomly selected and studied with 100x magnification. To identify measurement sites, the specific line grid (3 parallel lines) was randomly superimposed on the sampled fields. The Follicular layers thickness was measured using the orthogonal intercept method, in brief by measuring the length of a line extended perpendicularly from the inner membrane to the outer surface of Follicular layers at each intercept of the line of the grid with Follicular layers, and was considered as orthogonal intercept (oi). An average of 110 measurements was made to calculate the harmonic mean thickness using the following formula [22]:

Harmonic mean thickness= 8π /3 × Harmonic mean of orthogonal intercepts

Where harmonic mean = number of measurements / sum of the reciprocal of orthogonal intercepts lengths= number of measurements/ (1/oi1 + 1/oi2 +….)

2.12 Statistical Analysis

The statistical analysis of data was performed by SPSS 16 software using one-way ANOVA test at significance level of $p<0.05$. Charts were plotted using the EXCEL software.

3. RESULTS

3.1 Body and Ovarian Weight

After statistical analysis, due to the lack of significant difference between sham group and control group, the data related to sham group were excluded. The body and ovarian weight gain in the PCOS group was significantly different from the control group due to increased abdominal fat ($P<0.01$). The animals treated with curcumin and Ziziphora extract showed a significant weight loss ($P<0.01$) compared to the PCOS group (Fig. 1).

3.2 Hormonal Findings

Blood serum was collected from the heart blood samples. The results showed that the induction of this syndrome increased the levels of LH, testosterone and estradiol, and decreased FSH and progesterone, which were statistically significant compared to the control group. After treatment of the rats with curcumin and Ziziphora extract, significant changes were observed in levels of hormones (increased FSH and progesterone, decreased LH, testosterone and estradiol). These hormonal results suggest that these two substances have synergistically a positive effect on modulation of the hormones. In the PCOS group, the LH, estradiol and testosterone levels were increased significantly, while the FSH and progesterone levels were decreased ($P<0.001$). Compared with the PCOS group, there was a significant change in the level of progesterone in the group treated with curcumin and Ziziphora extract due to the presence of corpus luteum in this group. Also, the levels of LH, testosterone and estradiol in the treatments group were decreased compared to the PCOS group ($P <0.001$) (Table 1).

3.3 Lipid Metabolism Findings

The serum triglyceride, cholesterol, LDL, HDL and VLDL levels were significantly increased in the PCOS group compared to the control group ($P<0.001$). In the group treated with curcumin and Ziziphora extract, the values were increased in these parameters significantly up to a normal level ($P<0.001$) (Table 2).

3.4 Histomorphometric Findings

The results of histological studies and hematoxylin-eosin staining in each of the three groups are shown in Figs. 3 - 5. The PCOS group showed large cystic follicles with a narrow granulosa layer at three cellular layers and a few number of small follicles. No corpus luteum was seen in this group. In the control group, unlike the PCOS group, the ovaries lacked cysts and were full of corpus luteum, indicating normal ovulation. Also, the amount of small follicles has been far higher. After 14 days of treatment with curcumin and Ziziphora extract, the number of cysts showed a significant reduction, and the emergence of corpus luteum revealed the onset of ovulation in rats. A significant increase in small follicles was seen in the treatment group similar to the control group (Fig. 3).

According to Fig. 4, there was a significant decrease ($p<0.001$) in the number of corpus luteum, antral follicle, preantral follicle and primary follicle between the PCOS group and control group. However, it increased the number of cystic follicles compared to control group($p<0.001$). In the follicular count, the PCOS group and the group treated with curcumin and Ziziphora extract had a significant increase ($p <0.01$) in the number of these follicles count while the number of cystic follicles significantly decreased. It was notable that use of curcumin and Ziziphora synergic extract, the observed changes will be more significant (Fig. 4).

The measurements of the follicular sheath(Theca) and granulosa layer thickness in different groups showed significantly an increase in the follicular sheath in the PCOS group compared to control group and a decrease in the granulosa layer thickness compared to the control group($P<0.001$). There was a significant decrease in the follicular sheath in the treated group compared to the PCOS group, also there was a significant increase in the thickness of the granulosa group compared to the PCOS group ($P<0.01$) (Fig. 5).

4. DISCUSSION

In this study, the PCOS phenotype was induced by injecting estradiol valerate and the synergistic effect of curcumin and Ziziphora extract, which have anti-inflammatory compounds, was evaluated on the changes in follicular number and ovarian morphology.

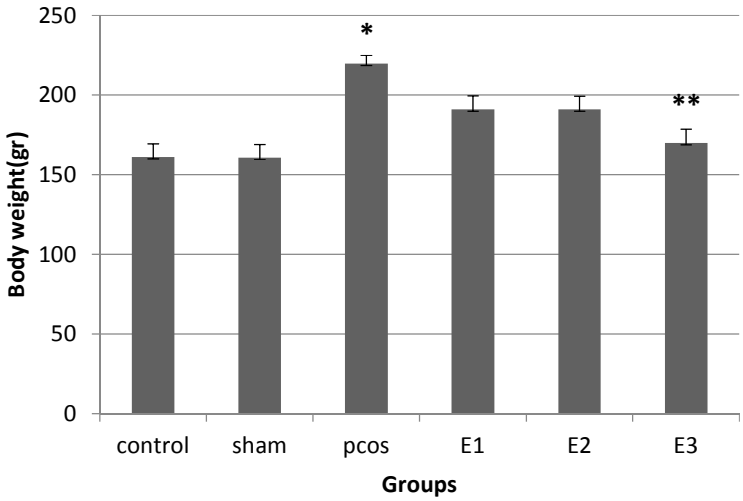

Fig. 1. Comparison of the mean levels of body weight of the rats in the control, sham, PCOS and experimental groups. Measuring the body weight showed in the PCOS control group significantly increased compared to the control group. However, the body weight in the experimental groups significantly decreased compared to the PCOS group

*The groups (Horizontal axis) are E_1: curcumin, E_2: Ziziphora tenuior L., E_3: curcumin+ Ziziphora tenuior L. (*P < 0.01 in comparison with the control group and **P < 0.01 in comparison with the pcos group)*

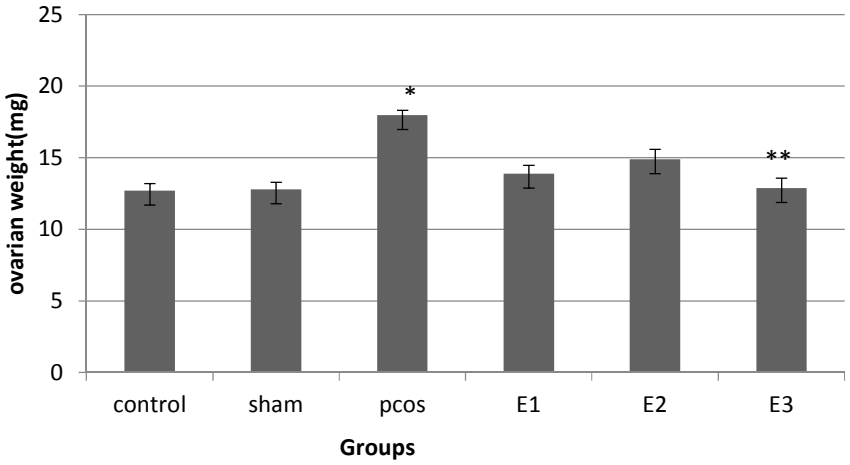

Fig. 2. Comparison of the mean levels of ovarian weight of the rats in the control, sham, PCOS and experimental groups. Measuring the ovarian weight showed in the PCOS control group significantly increased compared to the control group. However, the ovarian weight in the experimental groups significantly decreased compared to the PCOS group

*The groups (Horizontal axis) are E_1: curcumin, E_2: Ziziphora tenuior L., E_3: curcumin+ Ziziphora tenuior L. (*P < 0.01 in comparison with the control group and **P < 0.01 in comparison with the PCOS group)*

The peripheral endocrine system playing a major role in oocyte maturation before ovulation during the course of follicular development is abnormal in the PCOS and is associated with increased follicular vascularity and abnormal performance of granulosa cells [23]. Increasing the ovarian weight in this disease can generally be attributed to body weight gain, increased follicle size, decreased atretic follicles, and overall increased folliculogenesis [24]. Loucks et al. reported the dependence of LH on body fat and reduced production of this hormone to changes in body fat [25]. In the patients with PCOS, the LH/FSH ratio is higher than that of the control group, and the insulin resistance is higher in these patients than in the control group.

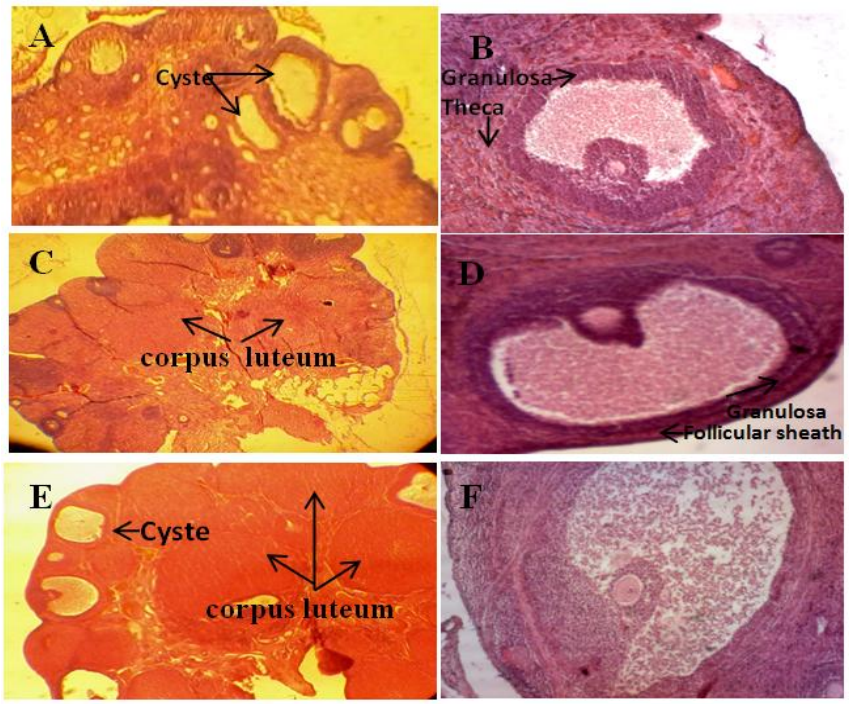

Fig. 3. Histological analysis of ovarian induced oestradiol valerate (PCOS) compared to healthy ovaries treated with curcumin and Ziziphora extract: ovarian stained with hematoxylin and eosin. Polycystic ovary (A, B) has a large number of large cystic follicles with a thin granulosa and thick follicular sheath. Fig. (C, D) shows a healthy ovary that is full of corpus luteum. The treated ovary with curcumin and Ziziphora extract (E, F) have a large follicle with a thin follicular sheath, which is a symptom of normal ovulation

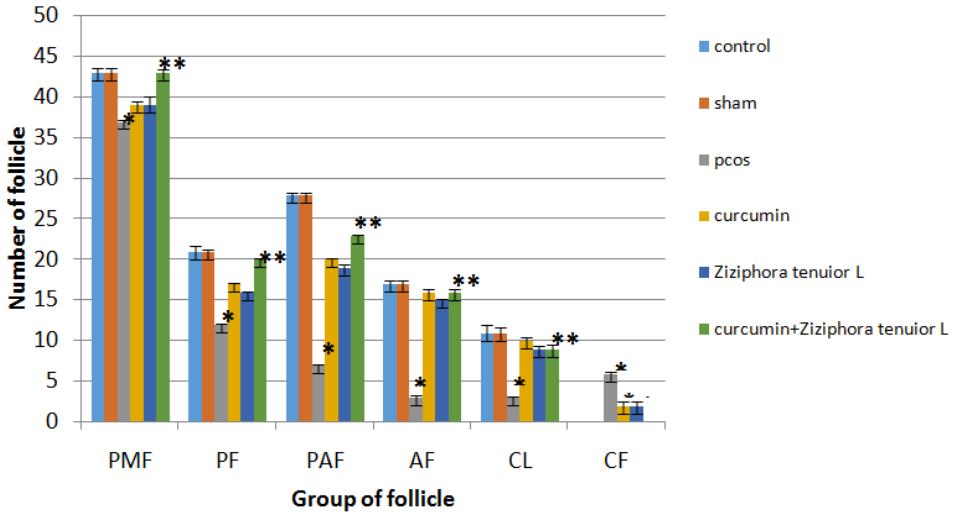

Fig. 4. Comparison of the number of different follicular groups in control and PCOS and the Curcumin and Ziziphora extract -treated ovaries. Note the increase in the numbers of cysts and a decrease in the number of corpus luteum of PCOS ovaries, respectively. A significant increase in all follicular clusters is detected in Curcumin and Ziziphora extract extract -treated PCOS ovaries. Moreover, a significant decrease in the number of ovarian cysts is also detected

*PMF, Primordial Follicle; PF, Primary Follicle; PAF, Preantral Follicle, AF, Antral Follicle; CF, Cystic Follicle; CL, Corpus Luteum.*** P<0.05, **P<0.01; *P<0.001*

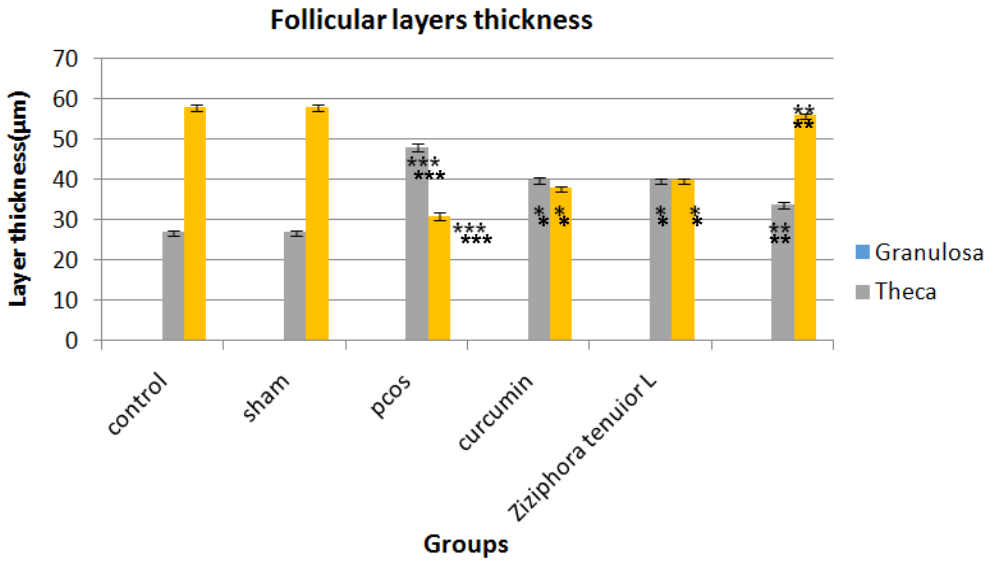

Fig. 5. Diameter of the granular and theca layers thickness in control, PCOS control and experimental groups. Measuring the thickness of follicular layers showed the thickness of the theca layer in the PCOS control group significantly increased compared to the control group. However, the thickness of the theca layer in the experimental groups significantly decreased compared to the PCOS control group. Relative to PCOS control group, 14-day sequential treatment with The Curcumin and Ziziphora extract resulted in a significant decrease in theca and a significant increase in granulosa layers thickness

**** P<0.001; **P<0.01; *P<0.05*

Table 1. Comparison of the mean levels of LH, testosterone, FSH, Progesterone and estradiol of the rats in the control, PCOS and experimental groups

Groups	LH(ng/ml)	T (ng/ml)	FSH (ng/ml)	P4(ng/ml)	E2(ng/ml)
Control	3 ± 0.29	0.59 ± 0.02	1987.94 ± 172.7	78.54 ± 1.09	0/027 ± 0.003
Sham	3 ± 0.18	0.59 ± 0.09	1889.94 ± 172.7	76.24 ± 1.02	0/027 ± 0.003
Pcos	5. 84 ± 0.13 *	1.02 ± 0.04*	622 ± 197.31 *	32.05 ± 1.03*	0.052 ± 0.001*
ZTE	2.71 ± 0.07**	0.6 ± 0.02**	1754.6 ± 170**	60 ± 3.09**	0.042 ± 0.002**
Curcumin	2.91 ± 0.07 **	0.7 ± 0.02 **	1591/21±44.97**	53/95±0/2**	0.041 ± 0.002**
ZTE+ Curcumin	2.71 ± 0.07*	0.47 ± 0.01 *	1854.6 ± 170*	67.54 ± 0.42*	0.035 ± 0.002*

*P<0.001; **P<0.01.*

LH: Luteinizing Hormone, FSH: Follicle Stimulating Hormone, T: Testosterone, P: Progesterone, E: 17β-estradiol, ZTE: Ziziphora tenuior L extract

Table 2. Comparison of the mean levels of TG, Chol, LDL, HDL and VLDL of the rats in the control, PCOS and experimental groups

Groups	TG(mg/dl)	Chol(mg/dl)	LDL(mg/dl)	HDL(mg/dl)	VLDL(mg/dl)
Control	64 ± 9.42	95.70 ± 16.2	32.55±19.67	52.56±12.08	13.90±2.05
Sham	63 ± 9.30	95.70 ± 16.2	31.25±13.67	52.56±12.08	13.90±2.05
Pcos	134.06±20.14*	124.58 ± 8.67*	67±10.55*	32 ± 4.66*	23.8±4.88*
ZTE	110±11.02**	98.4 ± 9.55**	29.54±2.16 **	49.95±4.05**	18.8±4.88**
Curcumin	105 ± 8.02 **	88.4 ± 3.55**	27.54±4.16**	45.15±3.21**	18.8±4.88**
ZTE+ Curcumin	90 ± 10.85*	88.4 ± 7.55*	27.54±4.16*	45.95±4.25*	14.5±3.65*

**P<0.001; **P<0.01.*

VLDL: Very Low-Density Lipoprotein, HDL: HIGH-Density Lipoprotein, LDL-C: Low-Density Lipoprotein-Cholestrol, TG: Triglyceride, Chol: Cholesterol

Increasing the production of androgenic precursors in follicular sheath cells leads to an increase in the production of androstenedione, which is then converted to testosterone by 17β-hydroxysteroid dehydrogenase, or to estrone by aromatase [26]. Considering the production of testosterone by follicular sheath cells and increasing the thickness of this layer due to

hypertrophy or cell proliferation in polycystic ovaries, the study of serum levels of this hormone is considered as one of the main variables. Given that the granulosa cells receive testosterone in the form of estrone from follicular sheath cells and convert to estradiol, and more estradiol is produced by the growth of follicles [27], Schulster et al. [28] stated that women with PCOS show higher concentrations of estradiol compared to healthy women. Desjardins et al. reported that induction of PCOS in rats by Estradiol valerate leads to the formation of many cysts in the ovary and the origin of these cysts is from the atretic antral follicles, which has features such as degenerated granulosa cell layers and follicular sheath thicker than the control group [29]. The changes in ovarian tissue in the PCOS group in the present study were consistent with the results of Desjardins et al. and indicated the successful induction of this syndrome and defect in the development of follicular groups in adult rats as compared to the control group.

Spaczynski et al. [30] stated that insulin and insulin-like factors are of autocrine and paracrine regulators of these cells in humans and rats, and have the ability to stimulate the proliferation and steroidogenesis of these cells, thereby increasing the synthesis of androgens in the theca and granulosa cells.

Moreover, due to a decrease in the corpus luteum formation and an increase in the number of unruptured antral follicles forming cysts, the serum level of progesterone in the PCOS group was decreased significantly, in agreement with Krattenmacher's research on progesterone changes in the PCOS group [31]. In addition to proving the estradiol valerate-induced syndrome, this study showed that intraperitoneally injected curcumin and oral administration of Ziziphora extract modulated the levels of hormones, improved ovarian layers, and reduced cysts. Curcumin and Ziziphora extract have anti-oxidative stress, anti-cancer and anti-inflammatory effects. The two substances also play a role in processes such as cell proliferation, differentiation and migration.

The wide spectrum of curcumin function is due to its interaction with intracellular signaling pathways [32]. The curcumin can inhibit the expression of inducible nitric oxide synthase (iNOS) gene and prevent the early stages of carcinogenesis. Additionally, the curcumin at certain concentrations protects the body from cellular damage caused by radioactive radiation. When radiotherapy or chemotherapy, the curcumin can be used as an antioxidant under the supervision of an oncologist [33]. It prevents angiogenesis and the formation of new blood vessels in tumor cells and stops their growth by inhibiting vascular endothelial growth factor (VEGF), its specific receptor (VEGF receptor) or angiopoietin [34]. Matthew Miller et al in 2008 stated that the curcumin inhibits the proliferation of pituitary tumour cells and induction of apoptosis and reduces the production and release of the hormone. Therefore, they suggested that treatment with curcumin could be used as a new therapeutic approach [35].

Yeging Shan et al. [36] showed that the curcumin can be used as a potential antioxidant against oxidative stress and its effects. In this study, the antioxidant effects of the curcumin were used to improve the symptoms of the syndrome and the results confirmed that their suggestion on the use of antioxidants in treating the patients with PCOS.

Koretz et al. indicated that the Ziziphora extract, which contains phenolic compounds and flavonoids, shows anti-inflammatory activity through controlling the levels of inflammatory cytokines or inflammatory mediators including IL-1, IL-6 and IL-10, TNF-α, NF- KB, NO, iNOS and COX-2. Therefore, the extract of this plant has pharmacological effects and therapeutic benefits [37]. Debnath and Konyalioglu showed that Ziziphora compounds have the ability to inhibit colitis in mice. Biochemical measurements clearly demonstrated that administration of total extract of Ziziphora reduces the activity of Myeloperoxidase (MPO) and Thyrotropin receptor-stimulating antibodies (TRABS) concentration, as both of these are important oxidative stress indicators and colitis markers. Interestingly, the effects of Ziziphora, especially at the 300 mg/kg/BW concentration, were very similar to that of prednisolone, which protected the animal well against colitis. The prednisolone also inhibits the phospholipase A2 enzyme, thereby reducing the amount of prostaglandins and leukotrienes. Therefore, the first idea that may come up to mind is that the Ziziphora has inhibitory effects on the synthesis or release of these inflammatory mediators [37,38].

5. CONCLUSION

The Curcumin and Ziziphora extract due to their antioxidant and anti-inflammatory properties caused an increase in the antioxidant capacity of the tissues and a decrease in the PCOS

symptoms in the rat model, as well as can be considered as a new method of promoting the fertility rate with increasing number of corpus luteum in the ovary.

CONSENT

It is not applicable.

ETHICAL APPROVAL

All procedures were conducted in accordance with the standards set forth in the guidelines for the care and use of experimental animals by the Committee for the Purpose of Control and Supervision of Experiments on Animals and the National Institutes of Health. The study protocol (care and handling of experimental animals) was approved by the Animal Ethics Committee of the Shahid Beheshti University of Medical Sciences.

ACKNOWLEDGEMENT

This study was funded by the School of Medicine Shahid Beheshti University of Medical Sciences; grant number 8515. No conflict of interest existed.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Fowler B. Disorders of homocysteine Fowler B. J Inherit Metab Dis. 2001;20(2): 270-85.
2. Kelly C, Lyall, H, Petrie J, Gould G, Connell J, Sattar N. Low grade chronic inflammation in women with polycystic ovarian syndrome. J Clin Endo Metab. 2001;86(6):2453–5.
3. David S, Guzick DS. Polycystic ovary syndrome. J Obstet Gynecol. 2004;103: 181-93.
4. Marx TL, Mehta AE. Polycystic ovary syndrome: Pathogenesis and treatment over the short and long term. Cleveland Clinic J Med. 2003;70:31-45.
5. Balen AH, Tan SL, Jacobs HS. Hypersecration of Luteinising hormone: A significant cause of infertility and miscarriage. Br J Obs Gyn. 1993;10:82.
6. Benson S, Janssen O, Hahn S, Tan S, Dietz T, Mann K, et al. Obesity, depression, and chronic low-grade inflammation in women with polycystic ovary syndrome. Brain Behavior and Immunity. 2008;22:177–84.
7. Hye M, Hwa L, Myung S, Dong J, Ho S, Min J, et al. JNK pathway is involved in the inhibition of inflammatory target gene expression and NF-kappa activation by melting. Journal of Inflammation. 2008;5:7.
8. Welt CK, Taylor AE, Martine KA, Hall JE. Serum Inhibin B in polycystic ovary syndrome: Regulation by insulin and leuteinizing hormone. J Clin Endocrinol Metab. 2002;875:559-65.
9. Goel A, Kunnumakkara AB, Aggarwal BB. Curcumin as Curecumin: From kitchen to clinic. Biochem Pharmacol. 2008;75(4): 787-809.
10. Salem ML, Hossain MS. Protective effect of black seed oil from *Nigella sativa* against murine cytomegalovirus infection. Int. J. Immunopharmacol. 2000;22(9):729-40.
11. Lin JK. Molecular targets of curcumin. Adv Exp Med Biol 2007;595:227-43.
12. Sharma RA, Gescher AJ, Steward WP. Curcumin: The story so far. Eur J Cancer. 2005;41(13):1955-68.
13. Thangapazham RL, Sharma A, Maheshwari RK. Multiple molecular targets in cancer chemoprevention by curcumin. AAPS J. 2007;8(3):443-449.
14. Sezik E, Tumen G, Baser KH, Ziziphoratenuior L. A new source of polygon. Flavour and Fragrance Journal. 1991;6(1):101-104.
15. Franks S. Polycystic ovary syndrome. N Engl J Med. 1995;333(13):853-61.
16. Chachoyan AA, Oganesyan GB. Antitumor activity of some spices of the family Lamiaceae. Rastitelnye Resursy. 1996; 32(4):59-64.
17. Raoofi A, Khazaei M, Ghanbari A. Protective effect of hydroalcoholic extract of *Tribulus terrestris* on Cisplatin induced renal tissue damage in male mice. International Journal of Preventive Medicine. 2015;6:11.
18. Howard C, Reed M. Unbiased stereology: Three-dimentional measurement in microscopy. United Kingdom: Bios Scientific Publishers; 1998.
19. Mouton PR. Principles and practices of unbiased stereology: An introduction for bioscientists. Baltimore and London: The Johns Hopkins University Press; 2002.
20. Myers M, Britt KL, Wreford NG, Ebling FJ, Kerr JB. Methods for quantifying follicular numbers within the mouse ovary. Reproduction. 2004;127:569-580.

21. Wang Y, Newton H, Spaliviero JA, Allan CM, Marshan B, Handelsman DJ, et al. Gonadotropin control of inhibin secretion and the relationship to follicle type and number in the hpg mouse. Biol Reprod. 2005;73:610-618.
22. Ferrando RE, Nyengaard JR, Hays SR, Fahy JV, Woodruff PG. Applying stereology to measure thickness of the basement membrane zone in bronchial biopsy specimens. J Allergy Clin Immunol. 2003;112:1243-1245.
23. Baravalle CSR, Mira G, Pezzone N, Ortega H. Microscopic characterization of follicular structure in letrosole-induced poly cystic ovarian syndrome in the rat. Archives of Medical Research. 2006;37: 830-839.
24. Jefferson W, Newbold R, Padilla-Banks E, et al. Neonatal genistein treatmont alters ovavian differentiation in the mouse: Inhibition of oocyte nest breakdown and increused oocyte survival. Biol Repord. 2006;74:161-8.
25. Loucks AB, Thuma JR. Luteinizing hormone pullstility is disrupted at a threshold of energy availability in regularly menstruating women. J Clin Endocr Metabol. 2003;88(12):97-311.
26. Rajesh Agarwal, Charu Agarwal, Haruyo Ichikawa, Rana P. Singh, Bharat B. Aggarwal. Anticancer potential of silymarin: From bench to bed side. Anticancer Research. 2006;26:4457-4498.
27. Fernández M, Bourguignon N, Lux-Lantos V, Libertun C. Neonatal exposure to bisphenol a and reproductive and endocrine alterations resembling the polycystic ovarian syndrome in adult rats. Environmental Health Perspectives. 2010;118(9):1217.
28. Schulster A, Farookhi R, Brawer JR. Polycystic ovarian condition in estradiol valerate-treated rats: Spontaneous changes in characteristic endocrine features. Biol Reprod. 1984;31:587-93.
29. Desjardins GC, Beaudent A, Brawer JR. Alternation in opioid parameters in the hypothalamus of rat with estradiol-induced polycystic ovariyan disease. Endocrinology. 1990;127(6):2669-76.
30. Spaczynski RZ, Arici A, Duleba AJ. Tumor necrosis factor-a stimulates proliferation of rat ovarian theca- interstitial cells. Biol Reprod. 2000;61(4):993–8.
31. Krattenmacher R. Drospirenone: Pharmacology and pharmacokinetics of a unique progestogen. Contraception. 2000;62(1):29–38.
32. Karimi Jashni H, Kargar Jahromi H, Bagheri Z. The effect of palm pollen extract on polycystic ovary syndrome (POS) in rats. Int J Med Res Health Sci. 2016;5(5): 317-21.
33. Walters KA, Allan CM, Handelsman DJ. Rodent models for human polycystic ovary syndrome. Biology of Reproduction. 2012;86(5):149.
34. Thangapazham RL, Sharma A, Maheshwari RK. Multiple molecular targets in cancer chemoprevention by curcumin. AAPS J. 2006;8(3):443-449.
35. Matthew M, Shenglin Ch, Jeffrey W, Sanjay K. Curcumin (Diferuloylmethane) inhibits cell proliferation, induces apoptosis, and decreases hormone levels and secretion in pituitary tumor cells. Endocrinology. 2008;149(8):4158–4167.
36. Shang YJ, Jin XJ, Shang XL, Tang JJ, Liu GY, Dai F, et al. Antioxidant capacity of curcumin-directed analogues: Structure–activity relationship and influence of microenvironment. Food Chem. 2010; 119(4):1435-42.
37. Trishna Debnath, Da Hye Kim, Beong Ou Lim. Natural products as a source of anti-inflammatory agents associated with inflammatory bowel disease. Research Institute of Inflammatory Disease. 2013;380-701.
38. Konyalioglu S, Ozturk B, Elgın Meral G. Comparison of chemical compositions and antioxidant activities of the essential oils of two Ziziphora. Taxa from Anatolia. Pharm Biol. 2006;44(2):121-6.

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sciencedomain.org/review-history/27217