Medical Principles and Practice

DOI: 10.1159/000494300
Received: 3/7/2018 5:41:23 AM
Accepted: 10/7/2018
Published(online): 10/7/2018

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ISSN: 1011-7571 (Print), eISSN: 1423-0151 (Online)
https://www.karger.com/MPP
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Investigation of Therapeutic Effects of Erdosteine on Polycystic Ovary Syndrome in a Rat Model

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Short title: Therapeutic Effects of Erdosteine in PCOS

Key Words: Polycystic ovary syndrome • Metformin • Erdosteine • Letrozole

Significance of the Study

Important treatment options that influence hyperandrogenism and insulin sensitivity are available for PCOS, while long-term treatment options for PCOS are still controversial. This study investigated the therapeutic and protective effects of erdosteine and treatment with metformin in an experimental rat model of PCOS. The results suggest that the therapeutic and protective effect of erdosteine could be due to suppression of biosynthesis of ovarian androgens. Thus, erdosteine may have therapeutic potential in the treatment of PCOS.

Abstract

Objective: Polycystic ovary syndrome (PCOS) is a serious endocrine disorder. In the present study, we investigated the therapeutic effects of erdosteine in letrozole induced-PCOS in rats.

Methods: Thirty two wistar albino female rats were grouped as control group (C), PCOS group (PCOS), PCOS-metformin group (PCOS+MET), PCOS-erdosteine group (PCOS+Erd). Polycystic ovary syndrome was induced by administering letrozole; such rats presented with sex hormone disorder, abnormal estrous cycles determined by daily vaginal smear, large cystic follicles, and increasing fasting insulin levels. After induction of PCOS, Metformin (500 mg/kg/day) and Erdostein (100 mg/kg/day) were given orally to the treatment groups for 30 days. Serum concentrations of glucose, total cholesterol, low- and high-density lipoprotein, triglyceride, as well as the total oxidant and antioxidant status, oxidative stress index, circulating estrone (E1), estradiol (E2), testosterone, androstenedione were evaluated. The ovaries were graded histologically. ***Results:*** Weights of ovarian tissues ($p < 0.05$) and the number of atretic follicles ($p < 0.001$) and cystic follicles ($p < 0.01$) decreased in PCOS+Erd group; the corpus luteum number was significantly higher in PCOS+Erd group ($p < 0.01$) as compared with PCOS group. Lipid parameters (Total-C, LDL-C and TG), E1 (Estrone), E1/E2 ratio, testosterone and androstenedione significantly decreased, while HDL-C, E2 (Estradiol) significantly increased in PCOS+Erd group as compared with PCOS group. Moreover glucose, insulin and HOMA-IR were reduced with treatment of Erdosteine ($p > 0.05$, $p < 0.001$, $p < 0.001$ respectively). ***Conclusion:*** It is suggested that Erdosteine may be used in treatment of PCOS as an alternative to metformin. It appears that our findings might be supported by clinical and molecular studies.

Introduction

Polycystic ovary syndrome (PCOS) is one of the most common endocrine disorders in women of reproductive age [1]. As far as different diagnostic criterias are concerned, the prevalence of PCOS ranges from 6 - 15% [2]. Though the etiology and pathogenesis of PCOS has not been explained fully, it is characterized by anovulation, hyperandrogenism findings and abnormal ovarian morphology [3, 4]. Furthermore, PCOS is associated with obesity, metabolic syndrome, hyperinsulinemia, type 2 diabetes, dyslipidemia and cardiovascular syndrome [5, 6]. Thus, PCOS is an endocrine disorder that presents long-term health risks [7, 8].

Important treatment options that influence hyperandrogenism and insulin sensitivity are available for PCOS while long-term treatment options for PCOS are still controversial [9]. The aim of treatment of PCOS is to decrease hyperandrogenism and improve insulin sensitivity [9]. Insulin-sensitizers, oral contraceptive pills (OCPs) and anti-androgenic agents are used commonly for the treatment of PCOS [10, 11]. Treatment with metformin, an insulin-sensitizer, increases insulin sensitivity and improves ovulatory function in PCOS [12]. In addition, treatment with metformin improves hyperinsulinemia, menstrual irregularity and reduces androgen levels in women with PCOS [13]. Metformin not only reduces inflammation, it also has an effect on steroidogenesis in ovarian theca cells and granulosa cells [14].

Several agents are used to investigate treatment of PCOS in distinctive experimental and clinical models. Erdosteine [*N*-(carboxymethylthioacetyl)-homocysteine thiolactone] is a mucoactive agent with antioxidant activity that involves a thiol groups [15]. Erdosteine has been reported to protect against hepatic, renal, retinal and cardiotoxicity [16]. In addition it is used in clinical practice as a mucolytic drug. Erdosteine is absorbed from the intestine, and after entering the circulation it is transformed into three metabolites in hepatic circulation. As

erdosteine includes thiol groups, it inhibits free radicals present in the circulation. It brings about strong antioxidant effects by increasing the activities of antioxidant enzymes [17].

Although several drugs have been used for treatment of PCOS, the effectiveness of metformin and erdosteine in PCOS has not been compared. Experimental animal models may help shed light on the pathophysiology of this syndrome. To the best of our knowledge, this is the first study to investigate the therapeutic and protective effect of erdosteine and treatment with metformin in an experimental rat model of PCOS. The main objective of this study was to investigate the potential protective effects of erdosteine in PCOS.

Materials and Methods

Animals

The experimental protocol used in this study was confirmed by Institutional Animal Care and Ethics Committee of Mustafa Kemal University and performed in accordance with the National Institute of Health Guide for Care and Use of Laboratory Animals (Publication No. 86-23, revised 1996).

Female adult Wistar albino rats (10 weeks old; weigh 275-325 g) were obtained from the Experimental Animal Laboratory of Mustafa Kemal University, Hatay. Rats were caged individually in temperature-controlled steel cages (20-22°C, 55%-65% humidity) with a 12-hour light/dark cycle and were provided with standard rat feed without phytoestrogen free and water ad libitum. Rats were allowed to acclimatize for 5 days before the experiments. All animals were subjected to daily collection of vaginal secretions for seven consecutive days in order to evaluate ovarian function; the estrous cycles of rats were evaluated by using vaginal smear method as described by Biswal et al. [18]. Subsequently, only rats with regular estrous cycles were included in the study. Thirty-two rats were randomly classified as four groups with 8 animals in each group. Groups were control group (C), PCOS group, PCOS-metformin treatment group (PCOS+MET), PCOS-erdosteine treatment group (PCOS+Erd).

Reagents

Erdosteine (Erdosteine suspension) was obtained from Sandoz Pharmaceuticals (Istanbul-Turkey), and metformin was obtained from Bilim Pharmaceuticals (Istanbul-Turkey) and letrozole was obtained from Femara-Novartis Pharmaceuticals (Istanbul, Turkey). Ketamine hydrochloride (Ketalar 80 mg/ml) was purchased from Pfizer (Turkey) and xylasine hydrochloride (Rompun 12 mg/ml) was purchased from Bayer (Turkey).

Experimental Animal Model of PCOS

Animal model of PCOS was induced in 24 rats by oral gavage with letrozole once daily at a concentration of 1 mg/kg dissolved in DMSO for 21 consecutive days [19, 20]. Subsequently, the PCOS model evaluation with an anovulatory estrous cycle was verified by analysis of vaginal smear, for which the first one-third of the vaginal wall was taken daily to evaluate the stage of the 4-day ovarian cycle during the examination of study period. Following the induction of PCOS, 8 rats with PCOS were included in PCOS group and did not receive any treatment. Rats in the PCOS-metformin group (PCOS+MET, n=8) were treated orally with 500 mg/kg/day of metformin, while rats in the PCOS-erdosteine treatment group (PCOS+Erd, n=8) were treated orally with 100 mg/kg/day of erdosteine for 30 days [15]. Animals in the control group received 1 mL/kg/day of saline solution orally for 30 days.

Collection of Tissue and Blood Samples

All animals were weighed at the beginning and end of study. The rats were anesthetized with a single intraperitoneal injection of ketamine (80 mg/kg) and xylasine (12 mg/kg). The blood samples were obtained by cardiac puncture and transferred to EDTA-containing tubes. Plasma was separated by cold-centrifugation (4 °C) at 3000 rpm for 10 min. Ovarian tissues were excised for histological analysis and fixed in 10% formaldehyde (PBS 10 mM, pH 7.4). Plasma samples were stored in a freezer at-80 ^{0}C for biochemical analysis.

Histopathological Analyses

Ovaries and uterus of rats were removed and weighed prior to histological examination. After cutting through at the longest length-wise dimension, ovarian tissues were fixed in 10% neutral formalin for 24 h. Then all samples were cleaned, dehydrated and embedded in paraffin. 4-mm thick sections were prepared using a microtome (Leica RM 2145 RTS; Nussloch-Germany) and stained with hematoxylin and eosin. A light microscope (Olympus Clinical Microscope BX53, Tokyo-Japan) was used to evaluate all samples according to the methods of Ergenoglu et al. [21] and Atis et al.[22]. All histological slides were examined in a blinded fashion by an experienced pathologist. Follicles containing an oocyte with a nucleus were counted and described as healthy. Follicles were grouped according to the following definitions:

- Preantral follicle: A follicle with an intact, enlarged oocyte with a visible nucleus and a single layer of cuboidal granulosa cells.
- Antral follicle: A follicle with two or more layers of cuboidal granulosa cells, whether the cavity was apparent or not.
- Atretic follicle: A follicle containing a degenerating ovum or pyknotic granulosa cells
- Cystic follicle: A follicle that is a large fluid-filled structure with an attenuated granulosa cell layer and a thickened theca internal cell layer.

For each group, the number of corpus luteum, preantral, antral, atretic and cystic follicles of ovaries were evaluated and counted. The thickness of the tunica albuginea, hyperplasia of the theca internal cells, reduce in corpus luteum, and sub-capsular follicular cysts in the control group and PCOS groups were analyzed (Fig. 1).

Biochemical Analyses

Serum total cholesterol (Total-C), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), and triglycerides (TG) were measured using an automated clinical chemistry analyzer (Abbott diagnostic, Architect C 8000, Abbott Park, IL).

Serum insulin levels were measured using a chemiluminescence method (Siemens, Immulite 2000 XPI, Malvern, PA). Insulin resistance was measured with a homeostasis model assessment for insulin resistance (HOMA-IR) calculated as previously [23]. Serum levels of estrone (E1), estradiol (E2), testosterone (TT), and androstenedione (AS) were determined using a competitive enzyme-linked immunosorbent assay [24] using commercially available ELISA kits (Awareness Technology Inc., Chromate ELISA Reader, Palm City, FL).

Serum samples for the measurement of total antioxidant status (TAS) and total oxidant status (TOS) were stored at -80°C until needed. The TOS and TAS of the rat serum samples were measured automatically with an Architect C8000–Abbott auto-analyzer device (Abbott, Wiesbaden, Germany) at the biochemistry laboratory of MKU Hospital. The oxidative stress index (OSI) was calculated by the formula TOS (μmol H2O2 equivalent/L)/TAS (μmol Troloxequivalent/L).

Measurement of TOS in Serum

Oxidants present in a sample oxidize the ferrous ion-o-dianisidine complex to ferric ion. The oxidation reaction is enhanced by abundant glycerol molecules in the reaction medium. The ferric ion forms a colored complex with xylenol orange in an acidic medium. The color intensity, which can be measured spectrophotometrically, is related to the total amount of oxidant molecules in the sample. The assay was calibrated with hydrogen peroxide. The results are expressed in terms of micromolar hydrogen peroxide equivalent per liter (μmol H2O2 Eq/L).

Measurement of TAS in serum

This was done by the new automated measurement technique described by Erel [25]. This technique measures the antioxidant effect of the sample against the potent free radical reactions initiated by hydroxyl radicals. The assay had sensitivity of <3%. The findings are expressed as mmol Trolox Eq/l [25].

Statistical Analyses

The normality of the distribution of variables was analyzed by the Kolmogorov–Smirnov normality test. Normally distributed data was analyzed by One-way analysis of variance and also nonparametric variables between different groups were compared using Kruskal–Wallis test. The data were analyzed by one-way ANOVA followed by Tukey's post hoc test for multiple comparisons. Also, Wilcoxon test was used for between Initial and last rat weights. All values were reported as mean ± SEM. Statistical significance was considered to be $p < 0.05$. The analyses were performed using Graph Pad Prism software (La Jolla, CA).

Results

Histopathologic Analyses

The average value of uterine and ovarian weights and histopathologic parameters shown in Table 1 and Figure 1. Histopathological analysis of PCOS-only ovaries appeared to be substantially increasing in subcapsular cyst formation, capsular thickness and theca cell hyperplasia in addition to a reduced corpus luteum. There was no difference between groups in terms of average of preantral and antral follicle counts, despite the fact that preantral follicles was statistically lower in the PCOS-Erdosteine group as compared to PCOS-Metformin group ($p < 0.05$), and antral follicles seem to be higher in PCOS-Erd group when compared to PCOS group (Table 1). Statistically significant differences between groups were seen in terms of average counts of atretic and cystic follicles (Table 1). The cystic follicle count of the PCOS group was significantly higher when compared with control, PCOS+MET and PCOS+Erd groups ($p < 0.01$; $p < 0.01$; $p < 0.01$ respectively). The atretic follicle counts were significantly lower in control, PCOS+MET and PCOS+Erd groups when compared with the PCOS group ($p < 0.05$; $p < 0.01$; $p < 0.001$ respectively). There was no significant difference between PCOS+MET and PCOS+Erd group with respect to numbers of cystic and atretic follicles ($p > 0.05$). Corpus luteum numbers were lower in the PCOS group as

compared with control, PCOS+MET and PCOS+Erd groups ($p < 0.05$; $p < 0.001$; $p < 0.01$ respectively). There was no significant difference between the PCOS+MET and PCOS+Erd groups with respect to corpus luteum number ($p > 0.05$).

Biochemical Analyses

Biochemical parameters and weights of rats are presented in Table 2. The final weights of rats in the PCOS, PCOS+MET, PCOS+Erd groups were statistically higher than their initial weights ($p < 0.001$, $p < 0.05$, and $p < 0.05$ respectively). Fasting glucose levels were higher in the PCOS group than in the control and metformin groups ($p < 0.001$; $p < 0.05$ respectively). There was no significant difference in the PCOS+Erd group as compared to the control and PCOS groups. HOMA-IR was substantially higher in the PCOS group as compared with control, PCOS+MET and PCOS+Erd groups ($p < 0.001$, $p < 0.01$, $p < 0.001$, respectively). However, there were no substantial differences in HOMA-IR levels between PCOS+MET and PCOS+Erd groups. Total cholesterol levels were higher in the PCOS group as compared with control and PCOS+MET groups ($p < 0.01$, $p < 0.05$ respectively). Triglyceride levels were also substantially higher in the PCOS group as compared to the control, PCOS+MET and PCOS+Erd groups ($p < 0.05$, $p < 0.05$, $p < 0.05$ respectively). Low-density lipoprotein cholesterol levels were statistically lower in control, PCOS+MET and PCOS+Erd groups as compared to the PCOS group ($p < 0.01$, $p < 0.05$, $p < 0.01$, respectively). On the other hand high-density lipoprotein cholesterol levels were lower in the PCOS group as compared to the PCOS+MET and PCOS+Erd groups ($p < 0.05$, $p < 0.05$ respectively). Levels of TAS were not substantially difference between groups. TOS was statistically lower in control, PCOS+MET and PCOS+Erd groups as compared to the PCOS group ($p < 0.0001$, $p < 0.05$, $p < 0.01$ respectively). Furthermore,OSI was statistically lower in control, PCOS+MET and PCOS+Erd groups as compared to the PCOS group ($p < 0.0001$, $p < 0.01$, $p < 0.0001$

respectively). There was a significant difference between PCOS+MET and PCOS+Erd group with respect to OSI ($p < 0.05$).

Hormone Parameters

A statistically significant reduction was seen in levels of estrone in the control, PCOS+MET and PCOS+Erd groups as compared to the PCOS group ($p < 0.001$, $p < 0.05$, $p < 0.001$ respectively); estradiol levels were lower in the PCOS group as compared to the control, PCOS+MET and PCOS+Erd groups ($p < 0.01$, $p < 0.001$, $p < 0.001$ respectively). The E1/E2 ratio was significantly higher in the PCOS group as compared to the control, PCOS+MET and PCOS+Erd groups ($p < 0.001$, $p < 0.001$, $p < 0.001$ respectively). Likewise, levels of testosterone and androstenedione were substantially higher in the PCOS group as compared to the control, PCOS+MET and PCOS+Erd groups (Table 3).

Discussion

This study shows that erdostein ameliorates lipid profile, insulin resistance, hormone profiles and follicle counts in PCOS. Women with PCOS are in need of long-term treatment because PCOS is a life-long endocrinopathy. Even though several drugs have been used to treat PCOS, the effectiveness of erdosteine in PCOS has not been investigated. Previous experimental studies have shown that erdosteine could be used at a wide range of dose without any side effects; however, metformin induces many side effects after long term use.

Metformin and oral contraceptive pills (OCP) have been used to treat the symptoms experienced by women with PCOS [13]. When metformin is administered as an insulin-sensitizer, substantial reductions in serum insulin levels and insulin resistance are observed and it also improves ovulatory functions. Although Metformin and oral contraceptive pills (OCP) have been beneficial in treating PCOS symptoms, their efficacy and safety are still not entirely elucidated [13, 26]. The observed effects of drugs used for PCOS are usually not significant enough for treatment. Furthermore, previous studies have indicated that metformin

produces many side effects after prolonged usage [27]. These observations made us interested in exploring new therapeutic options for the therapy of PCOS.

Erdosteine is associated with a low incidence of adverse events, most of which are gastrointestinal and generally mild. The LD_{50} (median lethal dose) is very high, 3,500-5,000 mg/kg [28]. PCOS is a low grade chronic inflammatory oxidative state [29]. In this study, we compared the effects of erdosteine and metformin. There was a significant reduction in the numbers of atretic and cystic follicles in both metformin and erdosteine groups. Furthermore, there was a substantial reduction in the number of preantral follicles in the erdosteine group as compared to the metformin group, probably due to its antioxidant effects. Our histological analysis indicates that erdosteine may be benefit follicular development in the rat model of PCOS.

There was a substantial difference in ovarian and uterine weights between groups; while uterine weights were low, while ovarian weights were high in the PCOS group. Similar findings regarding ovarian and uterine weights were reported in letrozole-induced PCOS models [19]. Uterine weights in the metformin and erdosteine groups are higher than in the PCOS group, probably due to an increase in estrogen levels.

In addition to morphological improvements in the ovaries, we observed that levels of total testosterone in the metformin and erdosteine groups were significantly lower than in the PCOS group. There have been different findings on E1, E2 and androstenedione concentrations in letrozole-induced PCOS rat models [30]. In our study, it appears that erdosteine substantially affected levels of E1, E2 and androstenedione. These findings may be considered as a reflection of histopathologic amelioration brought about by erdosteine. However, the histological data does not reveal the mechanism by which erdosteine decreases the appearance of PCOS in ovaries. Erdosteine may affect androgen levels. On the other hand it seems that the precise effect of metformin on androgen levels compared to erdosteine

treatment group was not studied. This may be due to the possible effect of metformin on reduction of androgens by regulating insulin resistance [31].

PCOS has been shown to be associated with several forms of dyslipidemia with low levels of HDL-C, high levels of triglycerides, total cholesterol, and LDL-C [24]. In our study, levels of HDL-C were significantly higher in metformin-treated and erdosteine-treated animals than in untreated rats with PCOS. Furthermore, levels of triglycerides, total cholesterol and LDL-C were significantly lower in metformin-treated and erdosteine-treated rats than in the PCOS group.

The etiology of insulin resistance in PCOS is still not clear. Insulin resistance was described as HOMA-IR [32]. In our study we observed that HOMA-IR is significantly lower in the PCOS+MET and PCOS+Erd groups compared to the PCOS group. Glucose also is significantly lower in the PCOS+MET group as compared to the untreated PCOS group. In previous studies it was indicated that hypovitaminosis D may contribute to worsen insulin-resistance that is a feature of women with PCOS [33]. Therefore, vitamin D replacement therapy is given to PCOS patients. In terms of the same effect of vitamin D, erdosteine may have a beneficial effect on insulin resistance in women with PCOS [34].

We suggest that these clinical results are associated with the antibacterial, antioxidant and mucolytic effects of erdosteine [35, 36]. The positive effect of erdosteine in PCOS is likely to be associates with the thiol groups in its structure. Erdosteine has been used to ameliorative ischemia reperfusion injury in several organs [15]. Erdosteine may also protect against ovarian ischemia/reperfusion with the release of oxidant free radicals and prevention of pro-inflammatory processes [15]. Also erdosteine, due to its antioxidant effects, may reduce the adverse effects caused by increased levels of reactive oxygen species (ROS). We chose to test erdosteine because of its antioxidant effects, because it has been used successfully in clinical

and experimental studies and because it is cheap and is easily available. Taken together, our analysis support a protective role of erdosteine in a rat model of letrozole-induced PCOS.

Conclusions

This study shows that erdosteine treatment increases the numbers of antral follicle and corpus luteum, and reduces the numbers of atretic and cystic follicles. In addition, it decreases levels of total testosterone, E2 and androstenedione. This study also shows that the therapeutic effect of erdosteine could be due to suppression of biosynthesis of ovarian androgens. Thus, erdosteine may have therapeutic potential in the treatment of PCOS.

Conflict of interest: None declared.

Author contributions

AK and RD designed and conducted the study; RD provided the rats for the experiments. HD, OT and GA performed the application of treatment agents. OT, HD, RD and GA performed together surgical technique. RD and AK wrote the manuscript. CT provided supervision. OT, ZAT evaluated histological analysis. All of authors participated in interpretation of the studies and analysis also approved the final version of the manuscript for submission.

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Table 1. Comparison of the histopathologic scores in groups.

	Control	PCOS	PCOS+MET	PCOS+ERD
Uterine weight (mg)	1.0±0.1	0.5±0.0 [a,**]	3.7±0.4 [b,**]	4.4±0.6 [c,***]
Ovary weight (mg)	1.00±0.1	1.81±0.2 [a,*]	1.08±0.1 [b,*]	1.11±0.2 [c,*]
Preantral Fc	3.62±0.7	2.62±0.6	3.22±0.7	1.55±0.3 [d,*]
Antral Fc	5.87±0.7	5.37±0.3	5.55±0.7	6.88±0.6
Atretic Fc	4.75±0.2	6.25±0.6 [a,*]	2.11±0.3 [b,**]	1.88±0.2 [c,***]
Cystic Fc	0.5±0.3	3.1±0.7 [a,**]	0.55±0.3 [b,**]	0.66±0.2 [c,**]
Corpus Luteum	13.00±1.4	8.87±1.1 [a,*]	14.56±1.4 [b,**]	15.22±1.6 [c,**]

PCOS, Polycystic ovary syndrome; MET, Metformin; ERD, Erdosteine; Fc: Follicle Count.
Mean±SEM. *: $p<0.05$; **: $p<0.01$; ***: $p< 0.001$;
[a] PCOS vs. Control; [b] PCOS+MET vs. PCOS;
[c] PCOS+ERD vs. PCOS; [d] PCOS+ERD vs. PCOS+MET

Table 2. Comparison of the biochemical parameters in groups.

	Control	PCOS	PCOS+MET	PCOS+ERD
Initial rat weight (g)	274.1±6.4	282.7±6.0	280.5±9.9	275.8±10.7
Last rat weight (g)	291.5±6.6	323.5±4.2 †***	311.5±10.8 †*	314.0±11.2 †*
Glucose (mg/dl)	232.7±7.8	353.0±15.9 a***	306.8±16.7 b*	342.4±8.4
Insulin (µIU/ml)	0.9±0.00	3.2±0.00 a***	2.5±0.01 b**	2.3±0.01 c***
HOMA-IR	0.52±0.03	2.77±0.12 a***	1.76±0.01 b**	1.66±0.28 c***
Total-C	49.25±1.8	60.65±1.20 a**	55.01±1.5 b*	57.06±3.3
TG	48.03±5.2	63.85±3.2 a*	55.18±2.3 b*	53.77±2.9 c*
LDL-C	4.37±0.3	8.37±0.82 a**	5.11±0.4 b*	4.75±0.3 c**
HDL-C	13.84±1.1	12.79±1.1	17.55±0.7 b*	17.56±1.1 c*
TAS (mmol/l)	1.106±0.22	0.713±0.03	0.856±0.06	0.950±0.06
TOS (µmol/l)	24.70±5.0	104.80±12.4a***	65.87±12.6 b*	45.69±4.4 c**
OSI (TOS/TAS)	22.41±8.0	146.90±15.7 a***	78.38±13.5 b**	48.18±9.8 c***, d*

HOMA-IR: Homeostasis model assessment of insulin resistance; TC: Total cholesterol; TG: Triglyceride; LDL-C: Low-density lipoprotein cholesterol; HDL-C: High-density lipoprotein cholesterol. TAS: Total antioxidant status; TOS: Total oxidant status; OSI: Oxidative stress index
Mean±SEM. *: p<0.05; **: p<0.01; ***: p< 0.001;
†: Initial vs. Last; [a] PCOS vs. Control; [b] PCOS+MET vs. PCOS;
[c] PCOS+ERD vs. PCOS; [d] PCOS+ERD vs. PCOS+MET

Table 3. Comparison of the hormone parameters in groups.

	Control	PCOS	PCOS+MET	PCOS+ERD
Estrone (E1) (pg/ml)	113.9±3.2	277.6±7.7 ^{a***}	209.4±25.4 ^{b*}	147.7±23.9 ^{c***}
Estradiol (E2) (pg/ml)	109.0±3.9	76.3±10.0 ^{a**}	155.5±14.5 ^{b***}	125.8±4.7 ^{c***}
E1/E2	1.05±0.03	4.01±0.44 ^{a***}	1.34±0.08 ^{b***}	1.17±0.17 ^{c***}
Testosterone (ng/ml)	3.55±0.59	9.01±0.93 ^{a***}	4.63±0.69 ^{b**}	4.12±0.85 ^{c***}
Androstenedione ng/ml)	0.43±0.09	1.11±0.14 ^{a***}	0.71±0.09 ^{b*}	0.62±0.07 ^{c**}

Mean±SEM. *: p<0.05; **: p<0.01; ***: p< 0.001;
[a] PCOS vs. Control; [b] PCOS+MET vs. PCOS;
[c] PCOS+ERD vs. PCOS.

Figure Legend

Fig. 1: A (Control): Section of ovary from a control rat showing follicles at various stages (prf: preantral follicle, antf: antral follicle, cl: corpus luteum: cl, atretic follicle: af), Hematoxilen&Eosin X40). B (PCOS): Section of ovary from a PCOS rat showing cystic, atretic and antral follicles and decreased corpus luteum (cf: cystic follicle), Hematoxylin Eosin X40). C (Metformin) – D (Erdostein): Decrease in numbers of atretic and cystic follicles with increase in antral follicles and corpus luteum in the sections of ovaries of erdosteine and metformin groups, Hematoxylin Eosin X40.

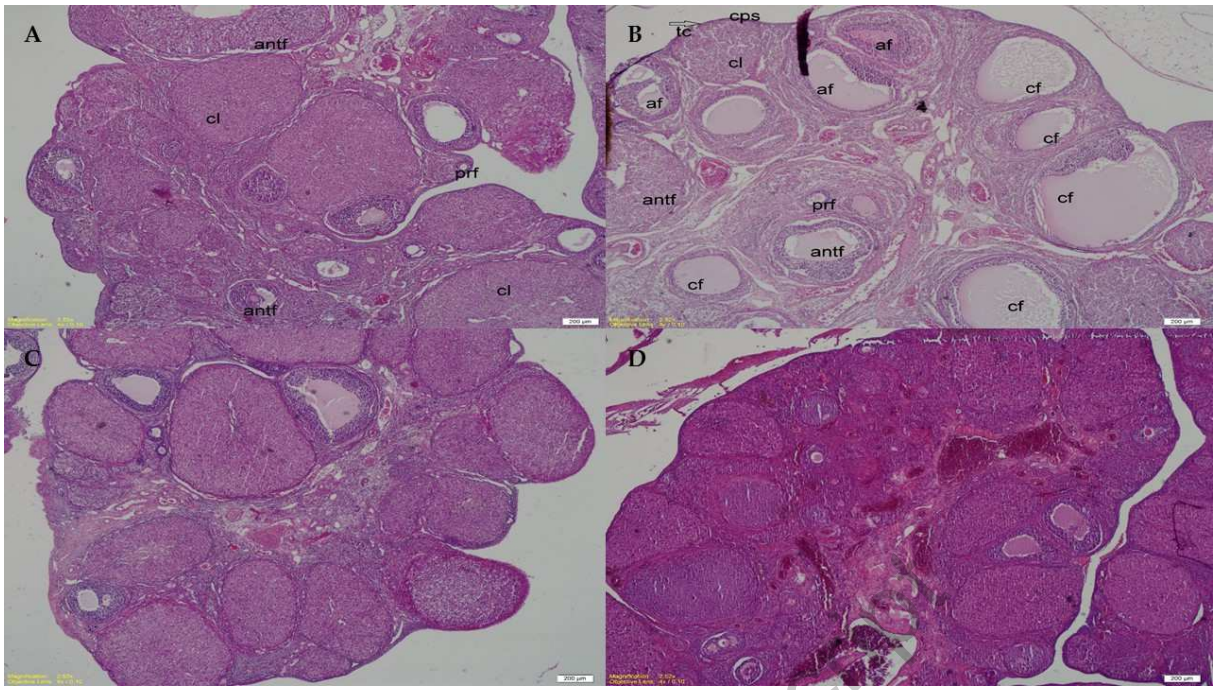

Fig 1.