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Research Article

Curcumin is comparable to metformin for the treatment of PCOS in rats: a preclinical study

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Abstract

Background: Ploy cystic ovary syndrome (PCOS) is a common gynecological disorder affecting between 4–20% of females worldwide. Curcumin, the active ingredient in turmeric (*Curcuma longa* Zingiberaceae) is a yellow polyphenol with a wide range of pharmacological activities such as antioxidant, anti-inflammatory, anti-tumor, neuroprotective and cardioprotective properties.

Methods: Letrozole was used to induce PCOS in female Wistar rats. Curcumin, metformin, or a combination of both was given to assess their therapeutic effects. Body weight, estrogen, progesterone, testosterone, and glucose levels were measured after disease induction and at the conclusion of treatment. Additionally ovarian histomorphology was examined after sacrificing the animals and removal of the ovaries.

Results and conclusion: Weight was significantly reduced in the curcumin, metformin and curcumin and metformin combination groups. Testosterone was decreased, progesterone was increased, and normal ovarian morphology was restored in the three groups. Conclusions: curcumin is therapeutically effective for PCOS and is comparable to metformin.

Keywords

Turmeric, Testosterone, Progesterone, Letrozole

Introduction

Polycystic ovary syndrome PCOS is the most common endocrine/ metabolic disorder affecting women during their productive years (Azziz et al. 2004; Lizneva et al. 2016). Etiology of PCOS is not fully elucidated; genetic and environmental factors appear to be interconnected in the cause of this disorder (Escobar-Morreale 2018; Raperport and Homburg 2019).

PCOS is diagnosed by several criteria put forth via several scientific bodies. Hyperandrogenism, oligo- or anovulation, and polycystic ovary sonography are the three clinical characteristics of PCOS (Fauser et al. 2004; Azziz 2018). Consequences and comorbidities such as cardiovascular, diabetes, and mood instabilities risk accompany PCOS (Escobar-Morreale 2018). PCOS might even have negative consequences on the wellbeing of the offspring (Vanky et al. 2019). PCOS patients demonstrate low quality of life as a consequence of clinical conditions of the syndrome (Sidra et al. 2019).

The pro-inflammatory state of PCOS involving a chronic low-grade inflammation resulting in development of metabolic anomaly and ovarian dysfunction (Bannigida et al.

2020). Ovaries from PCOS patients showed an increase in inflammatory markers (Cirillo et al. 2019). A state of chronic inflammation is involved in the pathogenesis of PCOS and probably contribute to the clinical manifestations of the syndrome (Shaaban et al. 2019; Rocha et al. 2019). This chronic inflammation drives the outcomes of hyperandrogenism and insulin resistance (Shorakae et al. 2018).

No specific treatments for PCOS are available so far, only symptomatic remedies are used. Depending on the most urgent/ irritating symptom, treatment modalities range from hormonal (oral contraceptives) to insulin sensitizers (metformin) in addition to life style changes (Glintborg 2016; Lim et al. 2019). Several reports indicated the efficiency of traditional herbal supplements in the treatment of PCOS. Example traditional chinses medicine had been found to be advantageous in the treatment of PCOS (Fu et al. 2024). Metformin improves metabolic manifestations of PCOS as well as menstrual irregularities (Tiwari et al. 2019). Metformin was combined with either Glucagon like peptide 1(GLP-1) receptor agonist or Thiazolidinediones (TZDs) was found to be superior to metformin alone. Combined therapy appears to be better, particularly, in overweight women with PCOS in improving fasting glucose and decreasing Body Mass Index (BMI) (Xing et al. 2020).

Several experimental animal models of PCOS are employed to study the disorder (Divyashree et al. 2019). Letrozole, an aromatase inhibitor, produces hyperandrogenic state and mimics PCOS hormonal disruptions. Furthermore, letrozole also produces metabolic disturbances when given to female rats. Letrozole can be given to prepubertal as well as adult female rats to produce PCOS-like symptoms (Divyashree et al. 2019).

Curcumin is a major herbal constituent of turmeric (Nelson et al. 2017), it had been used in herbal medicine for ages, partially for its anti-inflammatory effects (Mahdizadeh et al. 2015). Curcumin, that golden concoction, is used for almost every ailment, acute and chronic (Amalraj et al. 2017; Kunnumakkara et al. 2017). From cancer to obesity, no health problem has not been presumed to profit from curcumin, mainly due to curcumin's antineoplastic, antioxidant, antimicrobial, and anti-inflammatory qualities (Kocaadam and Şanlier 2017; Li et al. 2018). Increasingly available evidence is accumulating regarding curcumin potential efficacy for pulmonary diseases (Lelli et al. 2017). Curcumin has also shown the potential to be effective for skin diseases such as psoriasis, atopic dermatitis, and wound care (Vollono et al. 2019). Additionally, curcumin is non-toxic and safe for humans' consumption (Soleimani et al. 2018). Curcumin anti-inflammatory properties make it potentially useful for many disorders and ailments whose pathophysiological basis implicates inflammation. Type II diabetes (T2D) a prevalent modern world disorder, is also potentially responsive to curcumin in pre- and clinical studies (Pivari et al. 2019). Endometriosis, a gynecological disorder marked by inflammation, appears to benefit from the anti-inflammatory effects of curcumin (Arablou and Kolahdouz-Mohammadi 2018).

This study was undertaken to examine the potential curative effects of curcumin on PCOS, and compared that to a popular remedy, metformin, on several hormonal parameters as well as blood sugar levels and ovarian morphology.

Methods

Animals

Eighty-one newborns female Wistar rat pups weighing between 5–10 grams were acquired from the animal facility at Jordan University of Science and Technology. Litters were standardized to 8–9 pups per cage, and were kept at 25±1 °C temperature, 12 hours light/dark cycle, fed a regular diet with food and water provided ad libitum. They were kept with their mothers until the time of weaning on postnatal day 26. After that, rats were housed 5–6 rats per cage until the time of the experiment. Rats were returned to their prospective cages in between treatments. At the conclusion of the experiments, rats were killed by decapitation, blood was collected for hormonal assays tests, and ovaries were excised for histopathological examination.

All experiment procedures were approved by Jordan University of Science and Technology animal care and use committee (ACUC) and funded by a grant from JUST-Deanship of Research (# 2017-40).

Drugs

Curcumin 95% pure was purchased from Santa Cruz Biotechnology Company®, USA. Metformin and letrozole were generous gifts from the Jordanian Pharmaceutical Manufacturing Company® and Alhikma Pharmaceutical Company® respectively, local drug manufacturers. Carboxymethyl cellulose (CMC) was purchased from Sigma Aldrich company®.

Study design

PCOS Induction

Six weeks old female rats were randomly divided into five groups; group one (n = 17), served as control group, received 2 ml/kg of 1% CMC solution via oral gavage. Groups two to five (n = 16 each); treatment groups, received 1 mg/kg of letrozole dissolved in 1% CMC solution (2 ml/kg) via oral gavage daily for 21 days (Kafali et al. 2004).

PCOS Treatment

After PCOS induction using letrozole, animals in groups two to five received treatment for a period of 20 days as follows; group 2 received metformin solution 500 mg/kg via oral gavage, group 3 received 100 mg/kg curcumin dissolved in olive oil as oral drops, group 4 received a combination of 100 mg/kg curcumin oral drops and 500 mg/kg metformin oral gavage, while group 5 served as control group, received 2 ml/kg olive oil oral drops.

The doses of metformin (Lemos et al. 2014), and curcumin (Nonose et al. 2014) were used based on previous studies.

Biochemical analysis

Body weight was measured every three days until the age of six weeks and daily thereafter.

A tail vein blood sample was withdrawn from each rat into a clot activating gel tube. The samples were left for 10 minutes to clot and then centrifuged for 10 minutes at a speed of 5000 rpm. Serum was poured into Eppendorf tubes and freezed at -60 °C for enzyme-linked immunosorbent assay (ELISA) hormonal analysis. Glucose was also measured after overnight fasting using a simple glucocheck device. Overnight fasting glucose was measured twice; once after PCOS induction and another after conclusion of treatment using a commercial glucocheck device (Mayyas et al. 2015).

Animals, at end of experiments, were anesthetized using 20 mg/kg of thiopental intraperitoneal injection 3 minutes before sacrificing. After scarification truncal blood was collected in clot activating gel tubes, kept for 10 min to clot and then centrifuged for 10 min at 5000 rpm speed. Serum was poured into Eppendorf tubes and freezed at -60 °C for hormonal assay. Serum samples were thawed at a cool room temperature for over one hour. Then the tests were done according to the manufacturer's instructions. Absorbance was read using Apoch ELISA reader at 450 nm wavelength and readings were analyzed using Elisa analysis software.

Estrogen, progesterone and testosterone levels were also measured twice, once after PCOS induction and another after conclusion of treatment using enzyme-linked immunosorbent assay (ELISA) kits from Demeditec company®, Germany. Tests were performed according to manufacturer's manual and specifications.

Ovarian histomorphology

The excised ovaries were immediately fixed in 10% formaldehyde followed by tissue processing: dehydration through an ascending grade of alcohol, clearance with xylene, infiltration and complete embedding in paraffin wax blocks. Then the blocks were serially sectioned at 3 µm thickness using microtome, tissue ribbons were carefully transferred to a warm water bath and allowed to float on the surface to be placed on glass slides, deparaffinized using xylene and rehydrated in downgraded ethanol series. Lastly, they were stained using hematoxylin and eosin stains.

Slides were examined by a veterinary specialist under Life Science Trinocular microscope and pictures of representative sections were taken via a 10 MP digital Euromex camera.

Statistical analysis

All statistical tests were carried out using GraphPad Prism software (version 8). Statistical comparisons of test results between the investigated groups were done by one-way ANOVA followed by Bonferroni posttest. Two-way ANOVA was performed to investigate differences among groups before and after treatment. All values were reported as the mean ± standard error of mean (SEM). $P < 0.05$ was considered statistically significant.

The data underpinning the analysis reported in this paper are deposited at "Dryad" at https://doi.org/10.5061/dryad.brv15dvgx.

List of abbreviations

BMI Body Mass Index;
PCOS Polycystic ovary syndrome;
GLP-1 Glucagon like peptide 1;
TZDs Thiazolidinedione.

Results

Letrozole induced PCOS rats, gained significant weight compared to controls

At age six weeks and just a day before letrozole injections (DI), all rats' weight were comparable with no significant difference between the groups. At age nine weeks and after three weeks of letrozole treatment (T) all 4 groups of rats had increased weight as compared to controls (vehicle only treatment) (Fig. 1; $F_{(4,228)} = 4.805$, $p = 0.001$). Interestingly, at age twelve weeks and following different treatments for PCOS (just before sacrifice Sac), only curcumin treated rats' weight was significantly different from control rats. Curcumin-treated rats had a significant increase in body weight compared to the control and curcumin + metformin groups.

Letrozole treatment resulted in an increase in testosterone and a decrease in progesterone

Three weeks of letrozole injections and at age nine weeks, rats had a significantly increased concentration of testosterone (Fig. 2A; $F_{(4,64)} = 4.238$, $p = 0.0042$), and a decreased concentration of progesterone (Fig. 2C; $F_{(4,68)} = 51.66$, $p < 0.0001$). Neither estradiol nor glucose levels were affected by letrozole treatment (Fig. 2B, D).

All treatment modalities normalized testosterone to control values

Metformin and curcumin, their combination as well as olive oil resulted in decreasing the concentration of testosterone to control group value (Fig. 2E; $F_{(4,75)} = 1.478$, $p = 0.2174$).

Two-way ANOVA testing treatment X time showed a significant difference over time ($F_{(4,137)} = 4.997$, $p = 0.009$), from end of disease induction to end of treatments. Following completion of treatment, testosterone concentrations were significantly decreased for all treatment groups (Fig. 3).

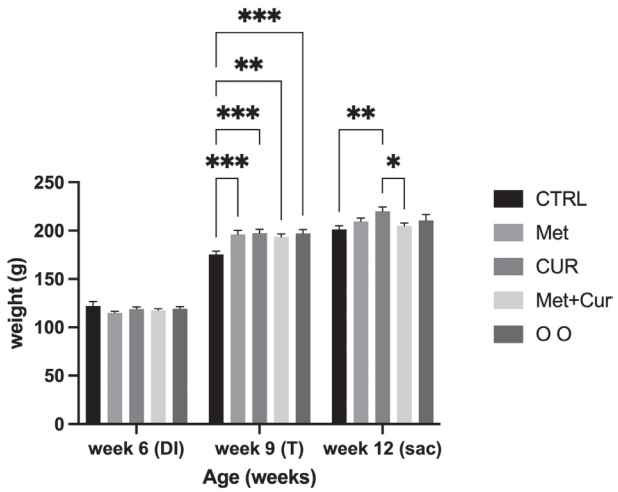

Figure 1. Changes in body weight before (DI, week 6) PCOS induction, after PCOS induction (T, week 9), and following treatments (sac, week 12) with metformin, curcumin, combination of met +cur and olive oil. Data expressed as mean ± SEM (n = 16–17). ** significant difference from control group.

All treatment modalities normalized progesterone to control values

Metformin, curcumin, their combination as well as olive oil resulted in increasing the concentration of Progesterone to control group value (Fig. 2G; $F_{(4,72)} = 2.478$, $p = 0.0.0516$).

Two-way ANOVA testing treatment X time showed significance difference over time ($F_{(4,66)} = 6.957$, $p < 0.0001$). Following completion of treatment progesterone concentrations were significantly increased for all treatment groups (Fig. 3).

Letrozole treatment did not affect the concentration of estrogen or glucose levels

At age nine weeks and following treatment with letrozole for three weeks, rats' glucose levels for all treatment groups were not significantly different from the control group (Fig. 2D; $F_{(4,73)} = 0.8934$, $p = 0.4724$). Similarly, estradiol concentration following letrozole treatment was also not different between treatment groups and control groups (Fig. 2B; $F_{(4,72)} = 0.9072$, $p = 0.4645$).

Histopathology

Hematoxylin & Eosin-stained ovarian sections from the control rats showed different stages of follicular development and normal ovarian morphology (Fig. 4).

Table 1. Histopathology of ovarian sections. +++: high, ++: moderate, +: low, -: not present.

Treatment group	Decrease number of corpora lutea	Decrease antral follicle	Subcapsular folliclualr cyst
Control group	-	-	-
Metformin 500 mg/kg	-	-	+
Curcumin 100 mg/kg	-	-	+
Metformin+ curcumin	-	-	+
Olive oil 2 ml/kg	+++	+++	+++

Sections from letrozole-treated rats showing multiple variably sized follicular cysts with disrupted normal follicular development, lack of corpora lutea and presence of follicular cysts (Figs 5, 6).

Sections of the three treated groups showed an increased number of corpora lutea and antral follicles and decreased number of subcapsular follicular cysts Table 1.

Discussion

Results of this study show that curcumin has the potential to ameliorate some of the signs of PCOS. The effects of curcumin were most pronounced on testosterone and progesterone levels. While estrogen and blood sugar values were not affected.

Letrozole an aromatase inhibitor widely used to induce PCOS-like disease in female rodents (Kafali et al. 2004; Du et al. 2014; Kauffman et al. 2015; Dăneasă et al. 2016; Ryu et al. 2019). Letrozole, as previous research showed, induced signs and symptoms in female rats when used for three weeks. Rats gained significant weight compared to non-treated rats. Weight gain is among the symptoms of PCOS in women (Azziz 2018; Patel 2018; Thackray 2019; Zhang et al. 2019). Weight gain, furthermore, is related to increased risk of metabolic disorders that accompany PCOS in many patients (Lim et al. 2019).

Other PCOS characteristics were changes in hormonal levels; testosterone levels went up, while progesterone levels went down. Women diagnosed with PCOS have higher testosterone levels (Glintborg 2016; Barrea et al. 2019). A good number of women with PCOS show raised levels of androgens, further this extra androgen correlates with the display of key features of PCOS (Cox et al. 2020). Progesterone levels at the end of the induction period were very low in comparison to non-induced rats. This is in accordance with typical characteristics of letrozole induced PCOS in rats (Kafali et al. 2004) and estradiol valerate induced PCOS (Mehraban et al. 2020). Human females suffering from PCOS also have low progesterone levels (Mobeen et al. 2016).

Estrogen, on the other hand, levels were not significantly changed in accordance with similar model of PCOS in mice (Kauffman et al. 2015). Even though other published reports saw a decrease in estradiol (Kafali et al. 2004; Ragy et al. 2019). This discrepancy could be due to many factors such as strain of rats, duration of disease induction among other variances in our experiment protocol.

Surprisingly, blood glucose values were also not changed, since one of the phenotypes of PCOS in humans is altered glucose tolerance and development of type 2 diabetes (Azziz 2018; Bannigida et al. 2020). This too was surprising especially considering that letrozole resulted in weight gain among rats treated as compared to control (non-treated) rats. We only measured fasting glucose at one point without measurements of A1C or even repeating the measurements. Constraints of time and finance are the reasons behind their limitations.

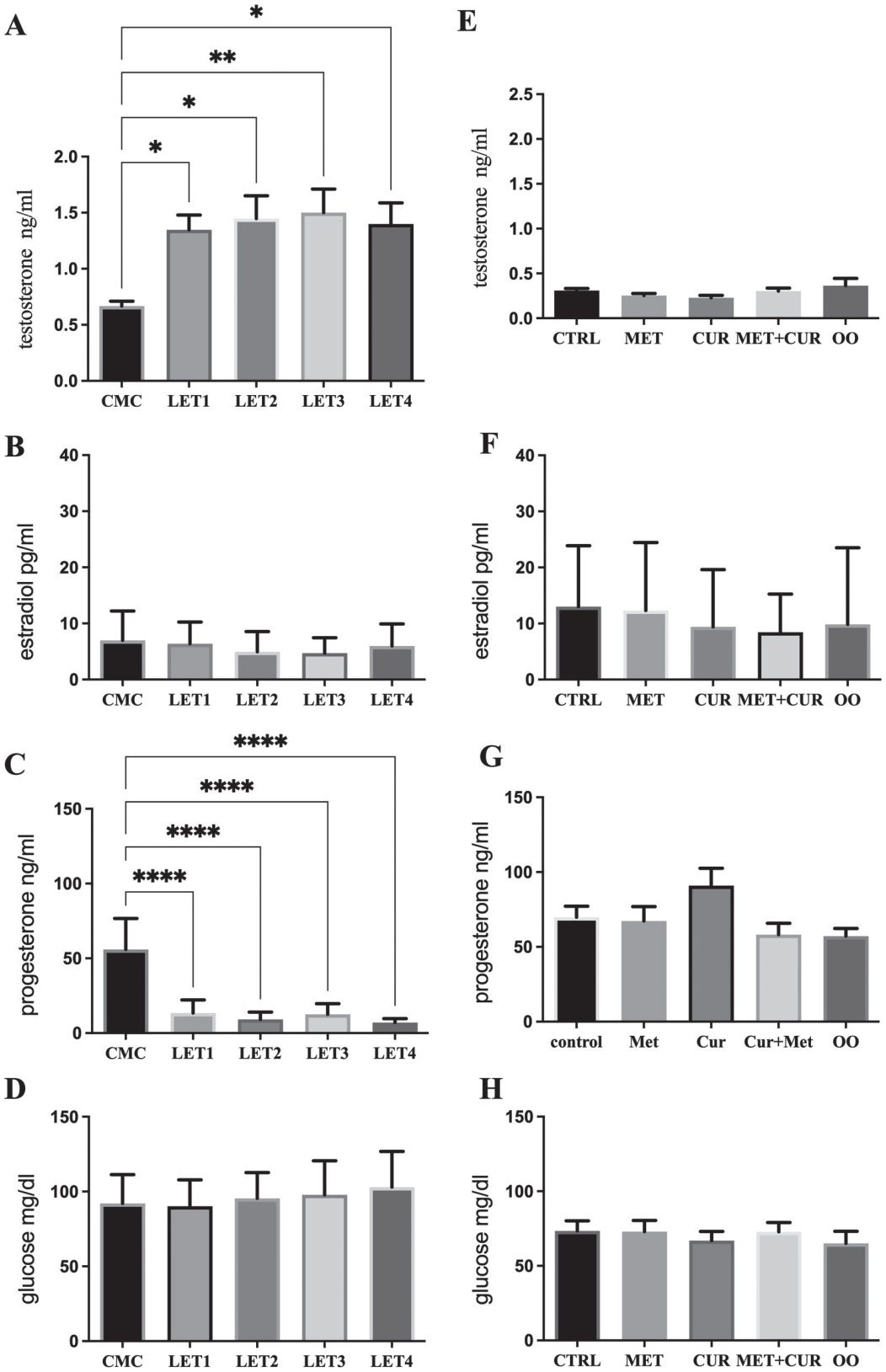

Figure 2. Hormonal and metabolic serum levels. Changes in serum levels of testosterone, estradiol, progesterone, and glucose before and after treatment with metformin, curcumin, metformin + curcumin, and olive oil compared to control rats. Data expressed as mean ± SEM (n = 16–17). *Significantly different form control. **A.** Testosterone levels in ng/ml following letrozole injections and before treatment at age 9 weeks; **B.** Estradiol levels in pg/ml following letrozole injections and before treatment at age 9 weeks; **C.** Progesterone levels in ng/ml following letrozole injections and before treatment at age 9 weeks; **D.** Glucose serum levels mg/dl following letrozole injections and before treatment at age 9 weeks; **E.** Testosterone levels in ng/ml following treatment at age 12 weeks; **F.** Estradiol levels in pg/ml following treatment at age 12 weeks; **G.** Progesterone levels in ng/ml following treatment at age 12 weeks; **H.** Glucose serum levels mg/dl following treatment at age 12 weeks.

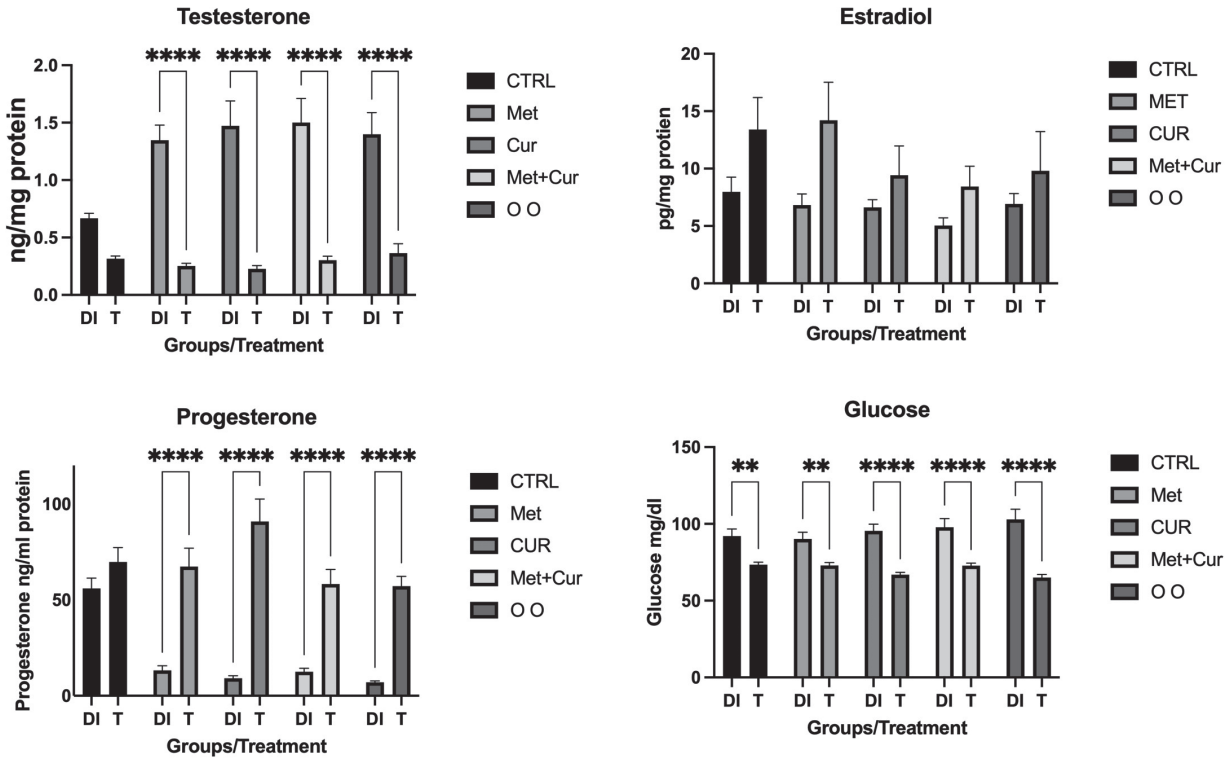

Figure 3. Hormonal and metabolic serum levels over time. Changes in serum levels of testosterone, estradiol, progesterone, and glucose before and after treatment with metformin, curcumin, metformin + curcumin, and olive oil compared to control rats' side by side. Data expressed as mean ± SEM (n = 16–17).

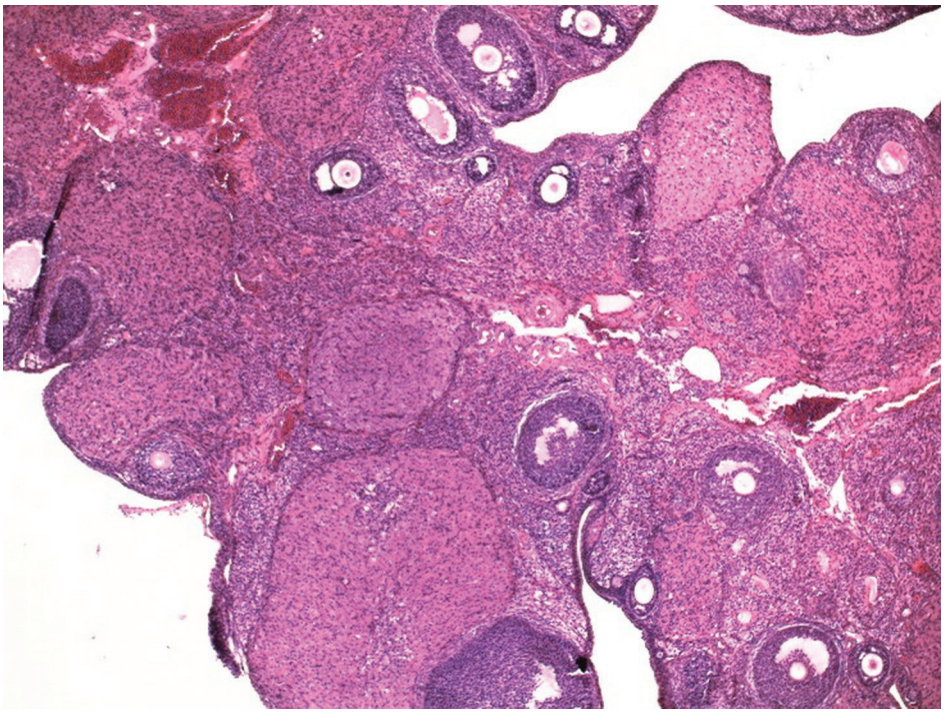

Figure 4. Ovarian section from control group showing different stages of follicular development and normal ovarian morphology.

Curcumin, metformin and their combination showed morphological changes in the ovaries indicative of ovulation and normal estrous cycle. Rats not treated with letrozole (control group) had follicles at different stages of development.

We opted to compare the therapeutic effects, if any, of curcumin with a standard PCOS symptomatic treatment widely used, metformin (Banaszewska et al. 2019; Tiwari et al. 2019; Xing et al. 2020). Metformin is widely used for symptomatic treatment of PCOS in women (Ayesha

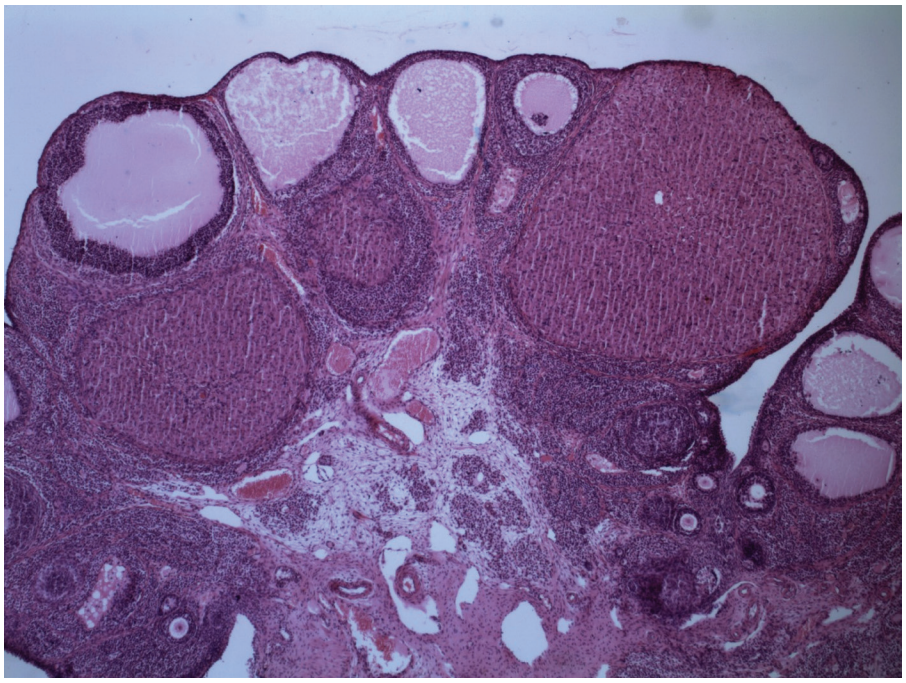

Figure 5. Ovarian section from G3 group showing multiple variably sized follicular cysts with disrupted normal follicular development.

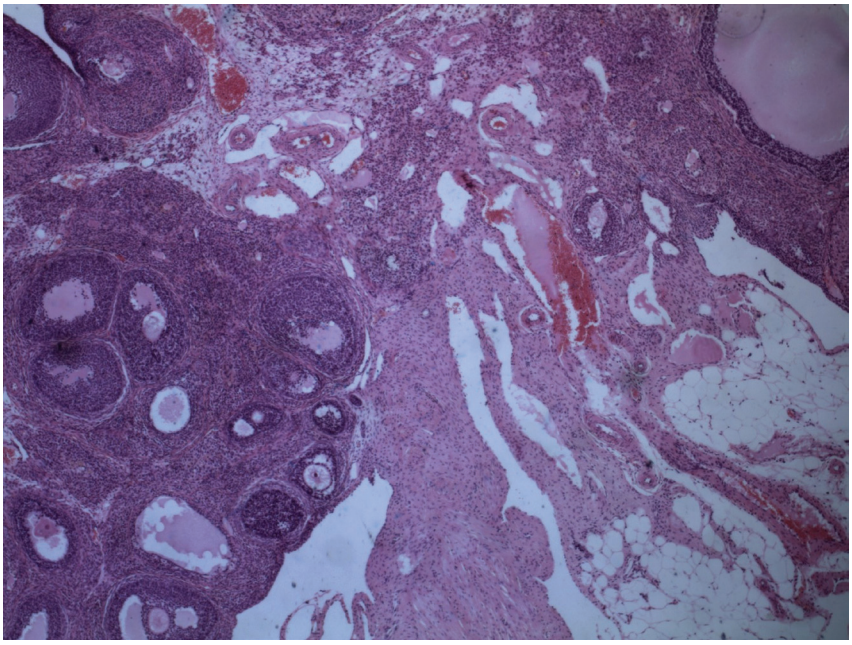

Figure 6. Ovarian section from G6 group showing disrupted follicular development, lack of corpora lutea and presence of follicular cysts.

Tariq et al. 2018; Tiwari et al. 2019). Amazingly, curcumin was comparable to metformin in reversing weight and hormonal changes that occurred due to letrozole induced PCOS.

Curcumin effects were expected as it had been shown to be effective for many disorders including gynecological disorders (Arablou and Kolahdouz-Mohammadi 2018). One possible mechanism for curcumin effects on PCOS signs might be its antioxidant powers as oxidative stress has a role in pathogenesis of PCOS (Ryu et al. 2019). Curcumin anti-inflammatory properties also most probably contribute to its effectiveness in disorders such as PCOS (Salehi et al. 2019). Also, the effect of curcumin could be through its beneficial effects on glucose tolerance and lipid profile which when improved can also improve symptoms of PCOS. Curcumin improved insulin sensitivity and lipid profiles in rats' models of type 2 diabetes (Francesca Pivari, Alessandra Mingione 2019). This study did not attempt to examine the exact mechanism of curcumin effects in this model of PCOS. Thus, we cannot be certain about the mechanism(s) of curcumin in improving symptoms of PCOS in this study.

Curcumin is not very palatable, thus, to enhance its palatability curcumin was dissolved in olive oil, a food ingredient very common in the Mediterranean diet.

Furthermore, lipid and oily substances enhance the absorption of otherwise not very bioavailable curcumin (Cui et al. 2009; Pawar et al. 2012; Prasad et al. 2014). The use of food ingredients to administer curcumin had been employed before; yoghurt enriched with curcumin was used in one study to enhance its pharmacokinetics parameters (Gutierres et al. 2015).

To our surprise, olive oil was also comparable to both curcumin and metformin. The Mediterranean diet is useful for cardiovascular health as well as PCOS, thus, olive oil, being an integral part of the Mediterranean diet could be valuable (Barrea et al. 2019). We embarked on this study in order to elucidate the effects of curcumin on letrozole-induced PCOS in rats, and to our surprise we found out that olive oil was also just as beneficial.

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