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Original Research Article

Effect of Curcumin (Standard and Supplement) with Zinc on Reproductive Hormones in Polycystic Ovary Syndrome (PCOS) Rats

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ABSTRACT

Polycystic ovary syndrome (PCOS) is a complex and heterogeneous disorder that affects a large percentage of women worldwide. The pathophysiology is not fully explained. In recent years, complementary and alternative medicine has emerged as a viable treatment option. This study examined how curcumin and zinc affected on reproductive hormones in rats with polycystic ovary syndrome (PCOS). It was induced by oral administration of letrozole (1mg/kg/day) for 21 days. PCOS groups were treated over 14 days with curcumin standard 200 mg/kg, curcumin supplement 200 mg/kg, and zinc 30 mg /kg (separately or in combination with two forms of curcumin), and metformin 50 mg/kg as standard treatment. The blood biochemistry of these groups was compared to those of the groups of healthy, PCOS, and those who received metformin. The immunoassay ELISA technique was employed for measuring the concentration of reproductive hormones. The results manifested that the administration of letrozole resulted in a significant elevation ($p \leq 0.05$) of luteinizing hormone, follicle-stimulating hormone, testosterone, and prolactin associated with a significant decrease ($p \leq 0.05$) of estrogen and progesterone levels in the PCOS-designed model. The results of effect of curcumin supplement alone and with zinc showed a significant improvement in all parameters by reducing the luteinizing hormone, follicle-stimulating hormone, testosterone, and prolactin. Estrogen and progesterone elevated significantly for all groups, while the elevation was highly significant in both curcumin supplement alone and curcumin supplement with zinc. The treatment with curcumin supplement and their combination with zinc showed promising results in restoring reproductive hormones to the normal level.

***Keywords*:** Curcumin, Follicle-stimulating hormone, Luteinizing hormone, Letrozole, Metformin, Polycystic ovary syndrome, Zinc.

Introduction

Polycystic Ovarian Syndrome, also known as PCOS, is the endocrine condition that affects reproductive aged-women the most frequently. The prevalence of PCOS ranges from 5% to 15%, depending on the diagnostic criteria used .[1] Stein and Leventhal were the first who mentioned and explained the association between amenorrhea, hirsutism, and enlarged PCO .[2] Polycystic ovary syndrome is accompanied by hyperandrogenism, menstrual disorder, infertility, and hirsutism.[3] A diagnosis of polycystic ovary syndrome (PCOS) is made when a woman has abnormal menstrual cycles, abnormal ultrasound findings of ovarian size and morphology, and clinical or laboratory evidence of hyperandrogenism.[1] The exact pathophysiology of PCOS is not yet completely explained.[4] PCOS leads to many health disorders that affect women's lives. These include dyslipidemia, type 2 diabetes, high blood pressure, and cardiovascular disease.[5,6] There are many hypotheses to detect the pathological physiology of PCOS. An altered hypothalamus secretion affects gonadotropin secretion and insulin resistance which leads to excess secretion of androgen from theca cells in the ovaries and causes hyperandrogenism oxidative stress and genetic factors, which contribute to the pathophysiology of PCOS.[4,5]

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Polycystic ovary syndrome is a common reason for Infertility.[7] Also, fatness has been shown to get irregular reproductive function in women leading to infertility. In addition, it has been presented that insulin resistance and increased testosterone level are enhanced in PCOS women who reduce 5% of their original weight, which appear to be essential relatives to PCOS pathogenesis.[8] Polycystic ovarian syndrome and hormonal disorders are common reasons for infertility,[9] PCOS can be induced in female rats by the administration of letrozole (a non-steroidal aromatase inhibitor). Letrozole causes biochemical changes in the aromatase enzyme, which converts androgen to estrogen in normal conditions. Inhibited aromatase enzyme causes the androgen to accumulate and aggregate.[10] The traditional treatment of PCOS generally includes insulin-sensitizing drugs and anti-androgen contraceptives. These drugs are beneficial but cause some health problems.[11] The study aims to describe the effect of the natural product of the plant that rises ovulation and diminishes the side effect of these medications and to explain the role of zinc alone and when combined with curcumin (standard and supplements). Curcumin is a polyphenolic ingredient with a lipophilic environment. The extraction of curcumin started from the root of turmeric rhizomes of a plant related to ginger origin, usually famous as Indian turmeric, which gives a typical yellow color.[12] Curcumin is a natural plant with many biological active uses for medicine,[13] as an antioxidant,[14] anti-tumor,[15] hypoglycemic factor,[16] and neuroprotective.[17] Zinc is implicated in all processes of an insulin-like organization, storage, and excretion. The direct activity of zinc in the body's metabolism depends on its enzymatic attraction and the way of a zinc-enzyme complex or metalloenzyme.[18] The deficiency of zinc leads to impair to T and B cell progress and modifies cytokine secretion.[19] Zinc has great work on the etiology of PCOS and other complications accompanied by this syndrome.[20] Several studies had revealed that curcumin has a very low bio-availability, which reduces

its benefits due to low absorption and rapid metabolism in the intestines and liver.[21,22] Peperine is usually added to increase the bio-availability of curcumin to reach a factor of 2000%.[23] Due to side effects associated with medications, the current study was conducted to find a safe and effective treatment for polycystic ovaries.

Material and Methods

Chemical and drugs

Curcumin supplement as curcumin 95% with piperine was purchased from Avaalab company (Germany), and Letrozole and curcumin standard was purchased from Sigma-Aldrich (Germany). Metformin was acquired from the Merck trademark (France). Zinc Sulphate tablets were gotten from a Winzor-Nutrocare trademark (England) .

Animals

Healthy female (Wister albino) rats 12 weeks old, weighing (160-200) g were used for the study. The rats were allowed to acclimatize for three weeks at the animal house of the College of the Medicine / University of Basrah at the control room temperature (22 ±2 C°) 12:12 hours (hr.) with a Light and dark cycle .[24]

Ethical approval

The study was approved by the Department of Chemistry in the College of Science at the University of Basrah, (APPROVAL NUMBER K 7/54/1995in 17/2/2021).

Induction of PCOS

Induction of polycystic ovaries for (Wister albino) female rats was done using letrozole at a dose of 1 mg/ kg/day dissolved in 0.5 % Carboxy Methyl Cellulose (CMC) for 21 days. All female rats received letrozole by gavage except the control groups received only CMC. The smear vaginal was examined daily for the estrous cycle and was also measured by the microscopic presence of the predominant cell type for induction of PCOS after staining the slide of the smear in Giemsa stain.[24]

Study design and treatments

The study included (56) virgin female (Wister albino) rats who were randomly assigned into eight sub-groups each group contained 7 rats. Group one included 7 healthy female rats as the healthy control group, while 49 remainder female rats suffering from PCOS induced by letrozole were classified into seven groups as follows: PCOS -control group, PCOS-treated rats with 30 mg/kg zinc alone, PCOS-treated rats with 200 mg/kg curcumin standard, PCOS treated rats with 200 mg/kg curcumin supplement, PCOS treated rats with 200 mg/kg Curcumin standard plus 30 mg/kg Zinc, PCOS treated rats with 200 mg/kg Curcumin supplement plus 30 mg/kg Zinc and PCOS treated rats with metformin at dose 50 mg/kg. After fourteen days of treatment, all female rats were slaughtered and blood was extracted from the inferior vena cava by sterile syringe then serum was separated by centrifugation at 3000 rpm for 15 minutes and stored at -20 Cº for biochemical analysis.

Body weight

The difference between the rat's body weights of the healthy control group and the PCOS group was determined before and after the period of PCOS induction. The weights were taken for both the healthy control group and the second group (PCOS group) on zero days and the 21st day of the PCOS-inducing period for statistical analysis later.

Estimation of Biochemical Parameters

Sex hormones quantification

The current study used a commercial test kit to determine levels of serum luteinizing hormone, follicle stimulating hormone, prolactin, and testosterone (code 625-300), (425-300), (Code No:725-300), and (Code No:3725-300) respectively. To measure LH, FSH, Testosterone, and Prolactin, this kit employs enzyme-linked immunosorbent assay (ELISA) utilising biotin antibody sandwich technology. Briefly, 10 µL of the serum samples and standard were added (LH: 10 µL, FSH: 10 µL, Testosterone: 10 µL, Prolactin: 10 µL) into respective pre-coated ELISA plates. Sixty minutes were spent incubating the plates. The plates were washed three times for two minutes each with 350 µL of 50 µL washing solution after incubation. After adding 100 µL of the working substrate to each well, they were gently shaken and left to incubate at 37°C for 15 minutes in the dark. Finally, an aliquot of Stop solution was added to each well to halt the reaction, which was shown by a transition from blue to yellow. The resulting optical density at 450 nm was measured after only 15 minutes. By plotting the absorbance of each standard against its concentration, a standard curve was created that allowed the hormone concentrations to be determined.[25- 28]

Using the ELISA method and a commercial test kit, we determined the progesterone and oestrogen hormone levels in the serum as expressed in ng/ml. The testing procedure was carried out in accordance with the instructions provided by the manufacturer of the test kits for progesterone (Code No. Eu 0398) and estrogen (Code No: Eu 0390). As a method, Competitive-ELISA is used in this ELISA kit. For quick and easy hormone determination, 50 µL of standard and sample were added to each well of the pre-coated microtiter plate included in the kit. After incubating at 37°C for 45 minutes, we added 50 µL of Biotin-detection antibody to each well and washed the plate three times. Following that, a total of 100 µL of the working solution was poured into each of the wells. Following an incubation period of 30 minutes at 37°C, the plates were aspirated, and then five times they were washed. To the substrate, an additional 90 µL was added. Incubate for 15-20 minutes at 37°C. In the end, 50 µL of the stop solution was added, and after 15 minutes, a calculation of the optical density was read at a wavelength of 450 nm. The absorbance of each standard was compared to its concentration, which allowed for the calculation of each hormone's specific concentration.

Statistical analysis

Biochemical measurements were analyzed by one-way ANOVA test and Fisher Pairwise LSD Comparison and 95% confidence tests, expressed by mean standard deviation. The statistical difference was tested by Minitab for a level of probability at ($p \leq 0.05$).

Results and Discussion

Table 1 shows the effect of letrozole on the average weight of rats before and after dosing, and comparison with a healthy control group of rats not dosed with letrozole. A non-significant difference ($P \geq 0.05$) in the body weight of healthy control group rats' during the study period, while a significant difference ($P \leq 0.05$) was observed between the initial rats' weights and the eventual weights of the PCOS group compared with the healthy control group. Results have shown a significant increase ($p \leq 0.05$) in the final body weight between the PCOS-induced and healthy control group because of the induced increments of body weight in the PCOS group. The 21 days of administrating letrozole for inducing PCOS in rats resulted in a significant increase in body weight in agreement with previous research due to the elevation in testosterone levels associated with PCOS.[29] The increase in testosterone could also be accompanied by an increased mass of adipose tissue.[30] Obese and non-obese PCOS patients had shown to increase in the visceral adipose tissue when compared with healthy control related to elevated androgen.[31] Accumulation of adipose tissue in the abdominal cavity is strongly associated with PCOS.[32] The results presented in table 2 showed the serum concentration of FSH, LH, Testosterone, and Prolactin hormones in PCOS induced female rats and a healthy control group. Indicate that PCOS induced group showed a significantly increased ($P \leq 0.05$) in serum levels of LH, FSH, testosterone, and prolactin in comparison with the healthy control group. While a significant increase ($P \leq 0.05$) in serum concentration of LH (13.63 ± 0.83) and FSH (8.08 ±0.41) of the PCOS group when compared with the healthy control group (4.03 ± 0.47) and (3.91 ± 0.29) respectively. The results of the study are in agreement with the previous study, that patients with PCOS had a significantly higher concentration of LH, FSH, testosterone, and prolactin, which found a significant increase in LH, FSH, and Testosterone concentration, and the concentration of estrogen and progesterone decreased significantly in PCOS.[10,33] The administration of letrozole a non-steroidal aromatase inhibiter blocks the conversion of androgen transformation to estrogen the decrease of estrogen level weakens the negative feedback on LH secretion from the

pituitary gland resulting in elevated LH, which further stimulates theca cell in the ovary to secrete testosterone. In women with PCOS of elevated LH negative correlation with progesterone during the luteal phase, it may be possible elevated LH-induced hyperandrogenism suppresses progesterone production.[10]

Serum testosterone levels increased significantly in PCOS group when compared with the healthy control group, this result matches with the line of reddy et. al. 2016 who found that curcumin can normalize serum testosterone levels just like clomiphene citrate.[24]

Results in table (3) indicate that the serum concentration of progesterone (9.45±0.76) and estrogen (13.579±0.575) in the PCOS induced group was significantly less than its level in the healthy control group.

In PCOS induced rats, letrozole showed reduced concentrations of estrogen and progesterone,[34] these results because of high androgen levels and ovarian cysts can induce by inhibiting androgen conversion to estrogen and promoting alteration of the hypothalamic–pituitary–gonadal axis in addition to that reduced progesterone are also an indication of anovulation.[24]

The results are shown in Table 2 and Figures 1 and 2 reveals serum levels of LH and FSH of treated groups were significantly lower ($P \leq 0.05$) than in the PCOS control. No significant difference in serum FSH concentrations between the two groups treated with (curcumin standard) and (curcumin standard plus zinc). Also, there was a significant increase in testosterone and prolactin (10.81 ± 0.96) and (29.52 ±1.13) in the PCOS-control group when compared with the control group (3.25 ± 0.22) and (17.82±0.64) respectively as shown in table 2 and figures 3 and 4. Serum testosterone concentration of PCOS-control was significantly higher than its levels in all treated groups except those treated with (curcumin supplement) and (curcumin supplement plus zinc) where it returned to normal value. The statistical analysis reported that the prolactin concentration of all treated groups was significantly lower ($p \leq 0.05$) than that of the PCOS-control group.

The serum concentration of LH, FSH, testosterone, and prolactin hormones in PCOS treated group with zinc 30 mg/Kg decreased significantly when compared with the PCOS control group, this result agrees with Fazel et al. 2020, who studied the effectiveness of zinc methionine at different doses on PCOS female rats and concluded the use of high concentration as treatment.[3]

Particularly in women with dysregulated insulin resistance and lipid balance, zinc supplementation reduces PCOS symptoms. Zinc can promote antioxidant action and protective effects in contradiction of reactive oxygen species (ROS) types that are synergistic with other antioxidants like vitamin E. The activity of numerous antioxidant enzymes, including Cu/Zinc Superoxide dismutase (SOD1), which protects against DNA damage and other forms of oxidative stress, is influenced by the level of zinc.[35]

Table (2) indicates that the treatment of the PCOS group with metformin (50 mg/kg) can effectively reduce the high serum concentration of LH, FSH, prolactin, and testosterone but not to a normal level of LH, FSH, testosterone, and prolactin. Our result agreed with previous studies.[11, 36]

Metformin is now recommended as an oral hypoglycemic agent in the treatment of type 2 diabetes. Metformin is an effective ovulation-induction factor for non-obese women with PCOS or is used with clomiphene to treat ovulation infertility. Women with PCOS usually suggested using metformin to decrease their risk of ovarian hyperstimulation syndrome. Metformin can reduce serum testosterone concentration by causing the ovaries to diminish the androgen biosynthesis, and improve the secretion of LH by the pituitary most likely by estrogen, which alters the negative feedback to LH.[37]

Metformin works on a variety of tissues, such as the liver, skeletal muscles, fatty tissues, and ovaries.[38]

Zinc and metformin treatments revealed a significant increase in estrogen and progesterone. But other treatments showed a higher level of these two hormones ($p \leq 0.05$). The administration of standard curcumin showed a significant ($p \leq 0.05$) reduction in the serum levels of LH, testosterone, and prolactin in good agreement with the previous study.[39]

Table 1: Effect of letrozole on Body Weight in healthy control and PCOS

	Parameters			
Groups	**B.W. (g) Before (mean ± SD)**	**B.W. (g) After (mean ± SD)**	**p Value**	**Significance**
Control	A174.43 ± 9.38	A184.57 ± 6.21	0.441	Non-Significant
PCOS-Induced	A175.14 ± 7.22	B198.14 ± 8.89	0.0002	Significant
p-value	0.875	0.001		

Different letters: Significant difference ($P \leq 0.05$) between groups.

Table 2: Effect of zinc, curcumin (standard and supplement) and metformin on serum concentrations of reproductive hormones in PCOS-induced female rats

	Parameters			
Groups	FSH mIU/ml **(mean ± SD)**	LH mIU/ml **(mean ± SD)**	Testosterone. ng/ml **(mean ± SD)**	Prolactin ng/ml **(mean ± SD)**
Healthy-control	D3.91 ± 0.29	G4.03 ± 0.47	F3.25±0.221	G17.82 ± 0.64
PCOS-control	A8.08 ± 0.41	A13.63 ± 0.83	A10.81 ± 0.96	A29.52 ± 1.13
Zinc only	B7.24 ± 0.45	B12.34 ± 0.45	B8.03 ± 0.36	B26.26 ± 1.02
Metformin	C5.41 ± 0.43	C10.29 ± 0.70	C7.54 ± 0.31	C24.65 ± 1.15
Cur. Standard	C5.48 ± 0.42	D9.25 ± 0.61	D6.16 ± 0.28	D22.79 ± 0.80
Cur.Standard +Zinc	C5.40 ± 0.47	D9.2 ± 0.54	E5.015 ± 0.21	E21.58 ± 1.01
Cur.Suppl	D4.37 ± 0.41	E7.34 ± 0.32	F3.25 ± 0.16	FG18.76 ± 0.83
Cur.suppl+Zinc	D4.02 ± 0.55	F5.28 ± 0.54	F3.13 ± 0.12	F18.85 ± 0.86
p-value	0.00051	0.00006	0.00022	0.00008

Different letters: Significant difference ($P \leq 0.05$) between groups.

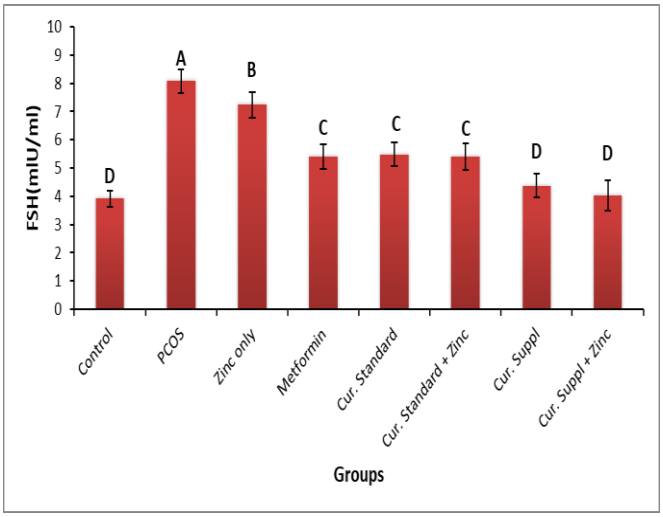

Figure 1: Serum FSH concentrations in treated groups with Zinc and Curcumin (standard and supplement), healthy control and PCOS control

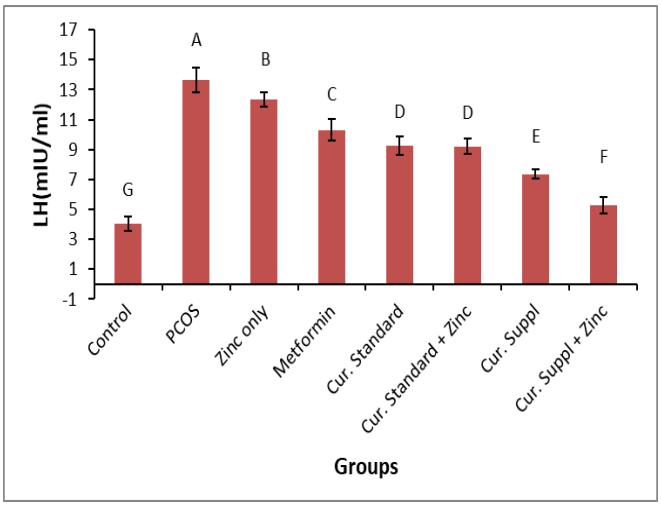

Figure 2: Serum LH concentrations in treated groups with Zinc and Curcumin (standard and supplement), healthy control and PCOS control

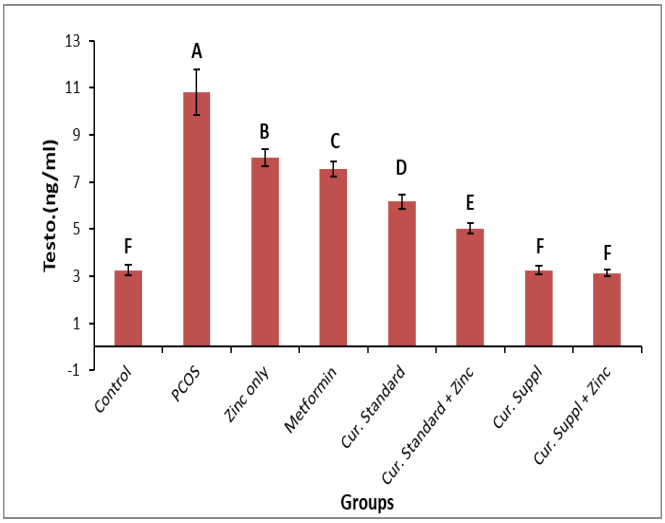

Figure 3: Serum Testosterone concentrations in treated groups with Zinc and Curcumin (standard and supplement), healthy control and PCOS control

A further reduction in these levels was obtained when groups consumed curcumin supplements (with or without zinc). Curcumin is an antioxidant, and anti-inflammatory.[40, 41] Studies showed that curcumin can suppress pro-inflammatory transcription factors, and decrease the pro-inflammatory cytokines anti-apoptotic, anti-bacterial, and anti-cancer.[42] The role of curcumin in the treatment of polycystic animals comes from its antioxidant activity in the ovary tissues.[9] Curcumin might regulate the reproductive endocrine function and enhance follicle primordial follicle development and maintenance. Yan *et al*. 2018 explained the protective effect of curcumin on premature ovarian failure by reducing LH, FSH, and increased estrogen in D-galactose -induced mice.[43]

The statistical tests showed that the curcumin supplement (with and without zinc) was capable of restoring the level of testosterone to normal. This is due to curcumin having anti-oxidant effects and that zinc is involved in many enzyme reactions. Need to estimate the effect of curcumin or zinc co-supplementation on glycemic level lipid profile, anti-inflammatory and anti-oxidants.[44]

The endoplasmic reticulum stress pathway is activated by reactive oxygen species, which can lead to cell death via apoptosis. After being treated with (20 μM), granulosa cells showed a decrease in hyperandrogenism caused by reactive oxygen species (ROS). According to the literature, granulosa cells exposed to dihydrotestosterone also experience increased oxidative stress and mitochondrial dysfunction. Curcumin was found to alleviate granulosa cell endoplasmic reticulum stress and lower granulosa oxidative stress. Since curcumin is both an antioxidant and a stress-altering emergency treatment, it has the potential as a PCOS medication.[45]

All treated groups showed significantly higher concentrations of serum estrogen compared to the PCOS control group. Our result as shown in table 3 and figures 5 and 6 demonstrated a highly significant decrease ($p \leq 0.05$) in estrogen and progesterone levels in PCOS female rats when compared with healthy control. This result agrees with previous studies.[24,33] The administration of zinc 30mg/kg caused PCOS groups to have significantly higher serum progesterone and estrogen concentrations but didn't attain normal levels. A further elevation of these two hormones was obtained from metformin treatment, a result that agreed with related research .[36]

Groups treated with curcumin supplement (with and without zinc) returned almost to normal serum progesterone levels. The fact that curcumin supplement contains piperine improves bioavailability and absorption. The combination of curcumin supplement plus zinc has a powerful effect as an anti-oxidant. Curcumin restores the level of estrogen because of its earlier reported phytoestrogens activity.[46,47] Recently many studies describe the special effects of curcumin on the ovarian failure of mice induced with D-galactose treated with 100 mg/kg/day curcumin intraperitoneally for 42 days. This study discovered that curcumin is proficient in inhibiting galactose-induced oxidative stress, apoptosis, and ovarian harm. Treatment with curcumin showed an increased SOD and decreased MDA levels.[43] Curcumin study also showed reduced apoptosis in the granulose cells of PCOS-induced rats after treatment with curcumin. [48]

Patients with polycystic ovary syndrome (PCOS) often suffer from insulin resistance due to the fact that hyperinsulinemia can increase steroidogenesis and stimulate the hypothalamus to release luteinizing hormone (LH) in PCOS patients.[3] In patients with PCOS, insulin further stimulates the production of ovarian androgen by activating its homologous receptor and or hypersensitivity to it.[49]

Some long-term metabolic effects of polycystic ovary syndrome have been linked to zinc deficiency, which has been found to play a potential primary role in the pathogenesis of PCOS.[49,50] Zinc's significance in insulin synthesis and action was reported well, both in healthy people and those with diabetes.[51] Zinc's ability to facilitate insulin binding to hepatocyte membranes plays a crucial role in the stabilisation of insulin hexamers and the pancreatic storage of insulin.[52] Reportedly, zinc can block the enzyme 5 -reductase from working, preventing the formation of the non-amortizable form of testosterone known as di-hydro testosterone (DHT). Therefore, preventing testosterone from being converted into its active form DHT may help to alleviate PCOS-associated hyperandrogenism. However, zinc is essential because it is a

co-factor in the production of antioxidant enzymes like catalase and superoxide dismutase (SOD).[53]

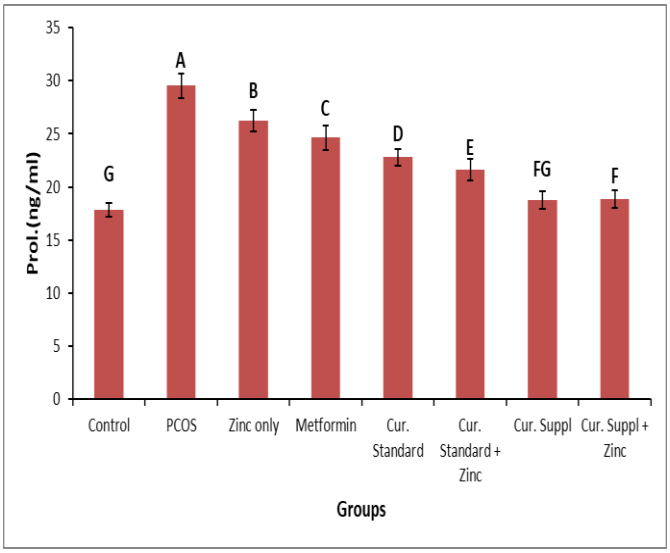

Figure 4: Serum Prolactin concentrations in treated groups with Zinc and Curcumin (standard and supplement), healthy control and PCOS control

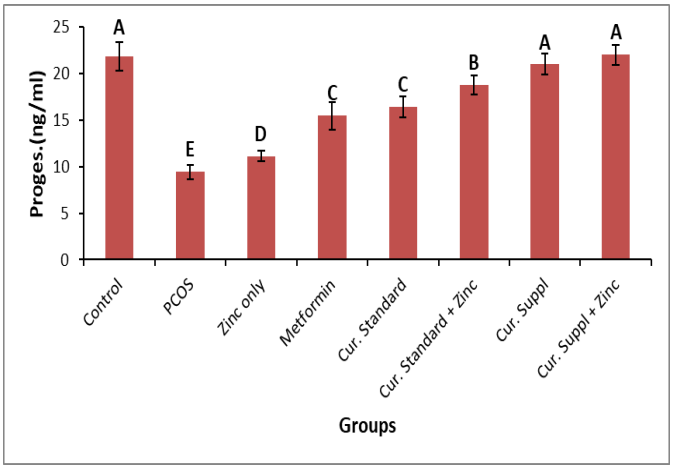

Figure 5: Serum Progesterone concentrations in treated groups with Zinc and Curcumin (standard and supplement), healthy control and PCOS control

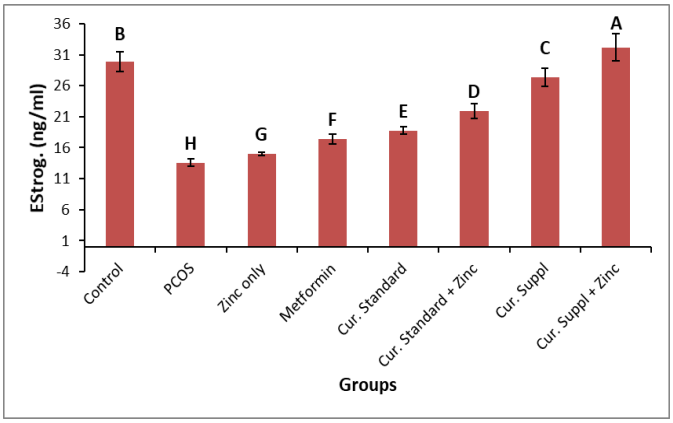

Figure 6: Serum Estrogen concentrations in treated groups with Zinc and Curcumin (standard and supplement), healthy control and PCOS control

Table 3: Effect of zinc, curcumin (standard and supplement) and metformin on serum concentrations of progesterone and estrogen in PCOS-induced female rats

Groups	Parameters	
	Progesterone ng/ml (mean ± SD)	Estrogen ng/ml (mean ± SD)
Healthy-control	A21.85 ± 1.53	B29.87 ± 1.61
PCOS-control	E9.45 ± 0.76	H13.57 ± 0.57
Zinc only	D11.12 ± 0.57	G15.00 ± 0.28
Metformin	C15.47 ± 1.48	F17.40 ± 0.79
Cur. Standard	C16.43 ± 1.13	E18.77 ± 0.58
Cur.Standard+Zinc	B18.78 ± 1.02	D21.90 ± 1.13
Cur.Suppl	A20.97 ± 1.12	C27.33 ± 1.42
Cur.suppl+Zinc	A22.02 ± 1.08	A32.19 ± 2.17
p-value	0.00047	0.00011

Different letters: Significant difference (P<0.05) between groups.

Conclusion

The present study shows the therapeutic value of curcumin supplement and zinc for rats with induced PCOS. This value is represented by reversing the changes associated with PCOS. Significant return to the levels of FSH, LH, and testosterone to their normal levels. As well as, its ability to reduce prolactin and increase levels of estrogen and progesterone.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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