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Effects of Zinc Supplementation on Endocrine Outcomes in Women with Polycystic Ovary Syndrome: a Randomized, Double-Blind, Placebo-Controlled Trial

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Abstract The current study was conducted to evaluate the effects of zinc supplementation on endocrine outcomes, biomarkers of inflammation, and oxidative stress in patients with polycystic ovary syndrome (PCOS). This study was a randomized double-blind, placebo-controlled trial. Forty-eight women (18–40 years) with PCOS diagnosed according to Rotterdam criteria were randomly assigned to receive either 220 mg zinc sulfate (containing 50 mg zinc) (group 1; $n = 24$) and/or placebo (group 2; $n = 24$) for 8 weeks. Hormonal profiles, biomarkers of inflammation, and oxidative stress were measured at study baseline and after 8-week intervention. After 8 weeks of intervention, alopecia (41.7 vs. 12.5 %, $P = 0.02$) decreased compared with the placebo. Additionally, patients who received zinc supplements had significantly decreased hirsutism (modified Ferriman-Gallwey scores) (-1.71 ± 0.99 vs. -0.29 ± 0.95, $P < 0.001$) and plasma malondialdehyde (MDA) levels (-0.09 ± 1.31 vs. $+2.34 \pm 5.53$ μmol/L, $P = 0.04$) compared with the placebo. A trend toward a significant effect of zinc intake on reducing high-sensitivity C-reactive protein (hs-CRP) levels ($P = 0.06$) was also observed. We did observe no significant changes of zinc supplementation on hormonal profiles, inflammatory cytokines, and other biomarkers of oxidative stress. In conclusion, using 50 mg/day elemental zinc for 8 weeks among PCOS women had beneficial effects on alopecia, hirsutism, and plasma MDA levels; however, it did not affect hormonal profiles, inflammatory cytokines, and other biomarkers of oxidative stress.

Keywords Zinc · Supplementation · Polycystic ovary syndrome · Reproductive outcomes · Inflammation · Oxidative stress

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Introduction

Polycystic ovary syndrome (PCOS) is a common endocrinopathy among reproductive-aged women with various prevalences from 5 to 21 % [1]. Previous studies have reported that PCOS is associated with wide-spectrum aberrations in different aspects of health, including reproductive outcomes (hyperandrogenism, hirsutism, anovulation, infertility, and menstrual disturbance); metabolic disorders such as obesity, type 2 diabetes mellitus (T2DM) and cardiovascular disease (CVD); and psychological features (mood and eating disorders) [2–4]. In addition, clinical studies have exhibited that abdominal obesity and hyperandrogenism are closely correlated with chronic inflammation and oxidative stress among patients with PCOS [5–7].

Recently, zinc administration is proposed for improving clinical and biochemical features of PCOS patients. Zinc is involved as a basic element for many vital functions including fertility and reproduction [8], inflammation [9], and oxidative stress [10]. Few studies have demonstrated that patients with

PCOS had lower zinc than in the controls [11, 12]. In addition, some investigators have shown an association between low circulating levels of serum zinc and acne [13], while others have not found the same [14]. Administration of supplemental zinc (30 mg/day) as zinc gluconate for 8 weeks among obese women resulted in a significant decrease of inflammatory markers such as high sensitivity C-reactive protein (hs-CRP), tumor necrosis factor-alpha (TNF-α), and interleukin (IL)-6 [15]. However, no significant effect in plasma malondialdehyde (MDA) levels was seen following the administration of zinc supplements (22 mg of zinc as zinc picolinate daily) for 8 weeks among patients with chronic obstructive pulmonary disease (COPD) [16].

Zinc deficiency may act as either an initiator or promoter of the underlying mechanisms and metabolic features of PCOS via causing insulin resistance, decreasing antioxidant capacity, and inducing apoptosis [17]. These lines of evidence emphasize the importance of zinc nutritional status on endocrine outcomes, inflammation, and oxidative stress suggesting that zinc supplementation may have beneficial effects on endocrine outcomes, biomarkers of inflammation, and oxidative stress. Therefore, we hypothesized that zinc supplementation might influence endocrine outcomes, biomarkers of inflammation and oxidative stress among women with PCOS. This study aimed to investigate the effect of zinc administration on endocrine outcomes, biomarkers of inflammation and oxidative stress of PCOS women.

Subjects and Methods

Participants

Forty-eight patients with PCOS from June 2014 to August 2014 were assigned to this randomized double-blind, placebo-controlled trial (Fig. 1). The protocol study was approved by the Ethical Clearance Committee on Human Rights Related to Researches Involving Human Subjects, Arak University of Medical Sciences (AUMS), Iran. All women with PCOS signed informed consent agreements. The current study was done based on the guidelines laid down in the Declaration of Helsinki. This trial and its progress were registered in the Iranian website (http://www.irct.ir) for registration of clinical trials (IRCT code: IRCT201407115623N24). To calculate the sample size, we used the standard formula suggested for parallel clinical trials by considering type one error (α) of 0.05 and type two error (β) of 0.20 (power = 80 %). Based on a previous study [18], we used 8.0 nmol/L as SD and 7.0 nmol/L as the difference in mean (d) of testosterone levels as key variable. Based on this, we reached to 20 patients in each group. Assuming a dropout of four patients per group, the final sample size was determined to be 24 patients per group. Patients were enrolled in the study if they met these inclusion criteria: aged 18–40 years old and have PCOS according to the 2003 Rotterdam criteria [19]. Patients who met two out of three of the following criteria were diagnosed with PCOS: (1) oligo- and/or anovulation as a menstrual cycle length of <21 or >35 days or more than 4 days of variation between cycles; (2) clinical signs (modified Ferriman-Gallwey score of ≥6, presence of acne or seborrhea) or biochemical hyperandrogenism (testosterone >0.4 ng/mL); and/or (3) polycystic ovaries by ultrasound, defined as 12 or more small follicles [19]. To fill mF-G score, we used a form validated for the Iranian population [20]. Exclusion criteria were hyperprolactinemia, thyroid disorders, liver or kidney diseases, T2DM, pregnancy and lactation, the use of medications such as insulin sensitizers, insulin, and diuretics. In addition, during the last 3 months before treatment, none of the study patients took any form of oral contraceptives (OCPs), other steroid hormones, or any other drugs probably to affect ovarian action. Enrolled study participants were assessed during the early follicular phase (day 3–7) after a spontaneous or induced menses.

Study Design

Before treatment and after stratification for pre-supplementation BMI (<25 and ≥25 kg/m^2) and age (<30 and ≥30 years), PCOS women were randomly divided into two groups to consume either 220 mg zinc sulfate (containing 50 mg zinc) supplements as tablet (n = 24) or identical placebo (n = 24) per day for 8 weeks. Zinc supplements and its placebos (starch) were produced by Alhavi Pharmaceutical Company (Tehran, Iran) and Barij Essence Pharmaceutical Company (Kashan, Iran), respectively. Moreover, all women with PCOS took metformin tablet at the initial dose of 500 mg, which was elevated in a stepwise manner during the first 3 weeks to incorporate the side effects until the patients were taking a total of 1500 mg/day. At before treatment, women were advised to keep their usual diet and levels of physical activity throughout the study period as well as not to receive any anti-inflammatory medications, supplements, and other medications that might affect their reproductive physiology during the 8-week intervention. Compliance to supplements and placebos was monitored by (1) follow-up by frequent short message service and (2) bringing the medication containers. All patients completed 3-day food records (2 weekdays and 1 weekend) and three physical activity records at weeks 2, 4, and 6 of intervention. To obtain nutrient intake, we used Nutritionist 4 software (N-Squared Inc., San Bruno, CA, USA) modified for Iranian foods.

Randomization and Blinding

Participant allocation and block size were obtained using random number tables. A trained midwife at gynecology clinic

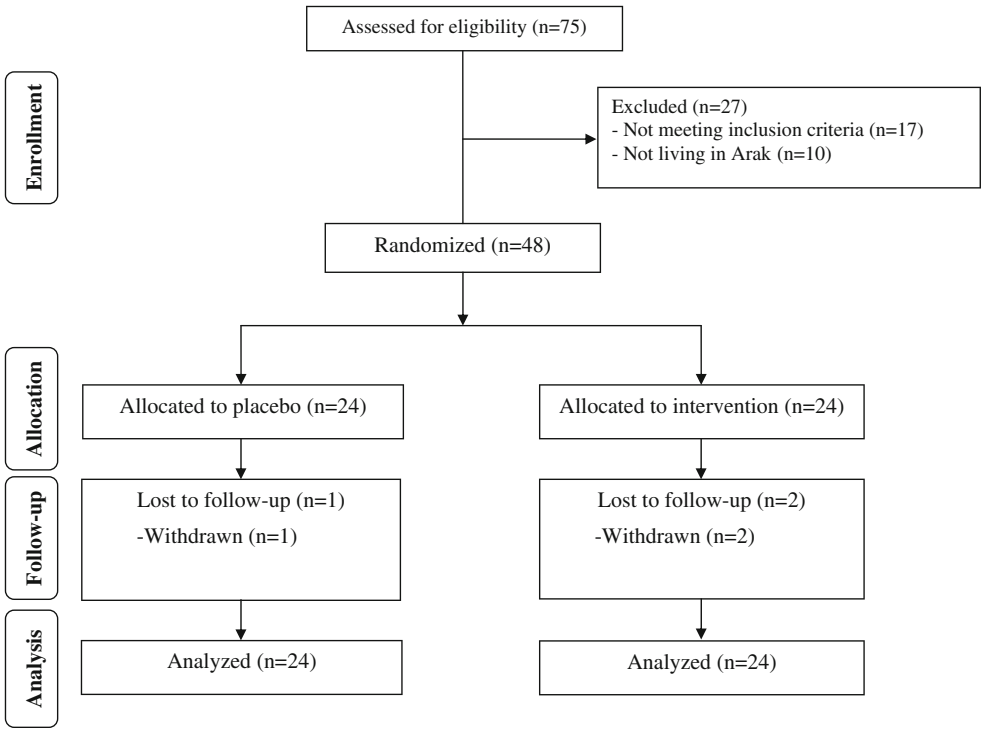

Fig. 1 Summary of patient flow diagram

carried out the randomized allocation sequence, enrolled subjects, and assigned participants to intervention and placebo groups. All patients, clinical investigators, and other health care personnel were blinded to treatment assignment.

Assessment of Variables

All participants were assessed at before treatment on the third day of a spontaneous or progesterone-induced menstrual cycle. Anthropometric measurements included determinations of height, weight (Seca, Hamburg, Germany), and body mass index (BMI). Waist circumference at the minimum circumference between the iliac crest and the last rib and hip circumference at the maximum protuberance of the buttocks were determined, respectively. All anthropometric measures were done by a trained midwife who was blinded to the randomization assignments.

Outcomes

As hyperandrogenism, alopecia, and hirsutism are common features of PCOS, primary outcomes such as free testosterone, dehydroepiandrosterone (DHEA), alopecia, and hirsutism were considered in the current study. Secondary outcomes were serum prolactin, follicular-stimulating hormone (FSH), luteinizing hormone (LH), 17-OH progesterone, hs-CRP, plasma nitric oxide (NO), total antioxidant capacity (TAC), glutathione (GSH), and MDA levels.

Clinical evaluations included were determinations of hirsutism using modified Ferriman-Gallwey scores [21], of acne score [22], and of alopecia based on assessment guidelines collated by Olsen et al. [23]. Acne was evaluated by a four-point scale: 0, no acne; 1, minor acne on the face; 2, moderate acne on the face only; and 3, severe acne on the face and back or chest. Alopecia evaluated by a five-point scale: S0 = no hair loss, S1 = <25 % hair loss, S2 = 25–49 % hair loss, S3 = 50–74 % hair loss, and S4 = 75–99 % hair loss. Fasting blood samples (10 mL) were obtained before and 8 weeks after treatment at Arak reference laboratory in an early morning after an overnight fast. Blood was collected in two separate tubes: (1) one without EDTA to separate the serum in order to determine serum zinc, prolactin, FSH, LH, free testosterone, DHEA, 17-OH progesterone, and hs-CRP levels and (2) another one containing EDTA to assess plasma NO and biomarkers of oxidative stress. To separate the serum, we centrifuged (Hettich D-78,532, Tuttlingen, Germany) the blood at 3500 rpm for 10 min and then stored at −70 °C until being analyzed at the AUMS reference laboratory. Available autoanalyzer kits (Elitech, Puteaux, France) were applied to determine serum zinc using enzymatic method. To determine serum prolactin, FSH, and LH levels, we applied commercial kits (Pars Azmun, Tehran, Iran). All inter- and intra-assay CVs for prolactin, FSH and LH measurements were less than 7 %. To quantify serum free testosterone, DHEA, and 17-OH progesterone concentrations, we used available kits (Monobind, CA, USA). Serum hs-CRP was quantified by an ELISA kit (LDN, Nordhorn, Germany). The plasma NO concentration

by the Griess method [24], TAC by the use of the ferric reducing antioxidant power (FRAP) method developed by Benzie and Strain [25], GSH using the method of Beutler et al. [26] and MDA levels by the thiobarbituric acid reactive substance spectrophotometric test [27] were determined.

Statistical Methods

The normal distribution of all studied variables was checked with Kolmogorov-Smirnov test. Intention-to-treat (ITT) analysis of the primary study end-point was done for all the randomly assigned participants. Missing data from dropped out participants were imputed using the method of "Last Observation Carried Forward (LOCF)." To compare qualitative variables between groups, Pearson's chi-square test was performed. To determine differences in general characteristics and dietary intake between the two groups, we used independent sample Student's *t* test. To identify within-group differences (end-point minus baseline values), we used paired-samples *t* tests. To determine the effects of zinc administration on hormonal profiles, biomarkers of inflammation, and oxidative stress, two-way repeated-measures ANOVA was used to evaluate the between-group changes in variables during the study. In this analysis, the treatment (zinc vs. placebo) was regarded as between-subject factor and time with two time-points (study baseline and after 8 weeks of intervention) was considered as within-subject factor. To control for several confounders including baseline values, age, and baseline BMI, we applied analysis of covariance (ANCOVA). *P* values less than 0.05 were considered significant. All statistical analyses of data were done using SPSS version 17 software (version 17; SPSS Inc., Chicago, IL).

Results

In the current study, 48 patients with PCOS met the inclusion criteria based on Rotterdam criteria and were enrolled in the study. Among participants in the zinc group, two women [withdrawn due to personal reasons ($n = 2$)] and in the placebo group, 1 patient [withdrawn due to personal reasons ($n = 2$)] did not complete the trial. However, as the analysis was done based on ITT protocol, all 48 PCOS women were included in the end analysis. On average, the rate of compliance in our study was high, such that higher than 90 % of tablets were taken throughout the study in both groups. In the current study, no side effects were reported following the administration of zinc in patients with PCOS throughout the study.

Participants' mean age and height were not significantly different between zinc and placebo groups. Baseline and end-point means of weight and BMI of patients in the two groups were not statistically different (data not shown).

There was no significant statistical difference in terms of daily dietary energy, carbohydrates, proteins, fats, saturated fatty acids (SFA), polyunsaturated fatty acids (PUFA), monounsaturated fatty acids (MUFA), cholesterol, total dietary fiber (TDF), zinc, magnesium, and manganese between the two groups throughout the intervention (Table 1).

After 8 weeks of the intervention, alopecia (41.7 vs. 12.5 %, $P = 0.02$) decreased compared with the placebo. However, we did not observe any significant effect of zinc supplementation on acne (12.5 vs. 8.3 %, $P = 0.63$) compared with the placebo (data not shown).

Women who received zinc supplements had significantly decreased hirsutism (-1.71 ± 0.99 vs. -0.29 ± 0.95, $P < 0.001$) and plasma MDA levels (-0.09 ± 1.31 vs. $+2.34 \pm 5.53$ μmol/L, $P = 0.04$) compared with the placebo (Table 2). A trend toward a significant effect of zinc intake on reducing hs-CRP levels ($P = 0.06$) was also observed. We did observe no significant changes of zinc supplementation on hormonal profiles, inflammatory factors, other biomarkers of oxidative stress, and waist and hip circumference. Within-group differences indicated a significant decrease in hirsutism ($P < 0.001$) in the zinc group. In addition, within-group changes demonstrated a significant rise in serum hs-CRP ($P = 0.04$) and plasma MDA levels ($P = 0.04$) in the placebo group.

We were controlled for baseline levels, age, and baseline BMI in the analyses. However, after adjustment, no significant changes in our findings occurred, except for TAC ($P = 0.02$) and 17-OH progesterone levels ($P = 0.008$) (Table 3).

Table 1 Dietary intakes of study participants throughout the study

	Placebo group ($n = 24$)	Zinc group ($n = 24$)	P^a
Energy (kcal/day)	2366 ± 178	2425 ± 178	0.26
Carbohydrates (g/day)	330.6 ± 46.6	338.7 ± 42.1	0.52
Protein (g/day)	83.1 ± 18.4	87.9 ± 11.4	0.27
Fat (g/day)	83.0 ± 15.4	83.7 ± 12.3	0.85
SFA (g/day)	23.9 ± 6.9	25.1 ± 5.2	0.50
PUFA (g/day)	27.7 ± 6.0	26.2 ± 5.4	0.35
MUFA (g/day)	21.9 ± 6.2	22.2 ± 4.6	0.85
Cholesterol (mg/day)	179.5 ± 124.4	201.3 ± 70.1	0.45
TDF (g/day)	18.5 ± 4.7	18.1 ± 4.0	0.74
Zinc (mg/day)	9.4 ± 2.9	10.2 ± 2.4	0.31
Magnesium (mg/day)	274.9 ± 78.4	279.8 ± 47.1	0.79
Manganese (mg/day)	2.1 ± 0.8	2.2 ± 0.6	0.83

Data are means ± SDs

SFA saturated fatty acid, *PUFA* polyunsaturated fatty acid, *MUFA* monounsaturated fatty acid, *TDF* total dietary fiber

[a] Obtained from independent *t* test

Table 2 The effect of zinc supplementations on hormonal status, biomarkers of inflammation, and oxidative stress

	Placebo group (n = 24)			Zinc group (n = 24)			P^a		
	Week 0	Week 8	Change	Week 0	Week 8	Change	Time	Group	Time × group
Zinc (mg/dL)	102.66 ± 13.71	99.32 ± 10.31	−3.34 ± 15.83	111.89 ± 20.90	128.87 ± 30.91	16.98 ± 22.30	0.01	0.001	0.001
Prolactin (mIU/L)	649.97 ± 417.40	505.37 ± 216.34	−144.60 ± 404.62	577.37 ± 512.07	453.46 ± 269.55	−123.91 ± 344.59	0.01	0.50	0.85
FSH (IU/L)	7.16 ± 1.92	8.05 ± 1.84	0.89 ± 2.28	12.94 ± 8.52	11.87 ± 7.44	−1.07 ± 5.16	0.87	0.004	0.10
LH (IU/L)	14.00 ± 14.70	11.14 ± 5.74	−2.86 ± 14.08	7.14 ± 3.61	10.49 ± 14.82	3.35 ± 15.02	0.90	0.12	0.14
Free testosterone (pg/mL)	3.51 ± 1.86	3.41 ± 1.93	−0.10 ± 0.96	2.65 ± 1.15	2.51 ± 1.09	−0.14 ± 0.62	0.32	0.04	0.86
DHEA (μg/mL)	1.67 ± 0.75	1.60 ± 0.73	−0.07 ± 0.40	1.72 ± 0.65	1.67 ± 0.64	−0.04 ± 0.50	0.40	0.75	0.87
17-OH progesterone (ng/mL)	2.10 ± 1.23	1.92 ± 1.35	−0.18 ± 1.78	1.13 ± 0.99	1.00 ± 0.61	−0.13 ± 1.20	0.47	<0.001	0.94
mF-G scores	8.20 ± 6.05	7.91 ± 5.94	−0.29 ± 0.95	9.37 ± 5.50	7.66 ± 4.88*	−1.71 ± 0.99	<0.001	0.77	<0.001
hs-CRP (ng/mL)	2042.14 ± 1633.48	2496.95 ± 1980.58*	454.81 ± 1020.90	2088.31 ± 1733.37	1865.68 ± 1469.46	−222.63 ± 1391.56	0.51	0.53	0.06
NO (μmol/L)	51.64 ± 13.01	57.92 ± 14.80	6.28 ± 19.30	51.02 ± 7.80	50.05 ± 15.00	−0.97 ± 13.61	0.27	0.14	0.13
TAC (mmol/L)	718.20 ± 138.32	666.85 ± 135.70	−51.35 ± 182.51	801.74 ± 210.59	781.66 ± 142.26	−20.08 ± 156.82	0.15	0.01	0.52
GSH (μmol/L)	435.55 ± 108.31	485.78 ± 124.55	50.23 ± 140.07	472.89 ± 84.71	498.74 ± 113.16	25.85 ± 111.22	0.04	0.32	0.50
MDA (μmol/L)	4.96 ± 2.97	7.30 ± 4.37*	2.34 ± 5.53	4.37 ± 1.04	4.28 ± 0.65	−0.09 ± 1.31	0.06	0.001	0.04
Waist circumference (cm)	85.79 ± 9.56	85.45 ± 10.35	−0.32 ± 1.40	88.00 ± 12.63	87.70 ± 12.53	−0.30 ± 0.62	0.05	0.50	0.89
Hip circumference (cm)	102.25 ± 9.88	101.62 ± 9.94	−0.63 ± 1.58	106.16 ± 13.54	105.70 ± 13.27	−0.46 ± 0.77	0.004	0.24	0.64

All values are means ± SDs

DHEA dehydroepiandrosterone, *FSH* follicle-stimulating hormone, *GSH* glutathione, *Hs-CRP* high-sensitivity C-reactive protein, *LH* luteinizing hormone, *mF-G* modified Ferriman-Gallwey, *MDA* malondialdehyde, *NO* nitric oxide, *TAC* total antioxidant capacity

*Significant difference with baseline study

[a] Obtained from repeated measures ANOVA test (time × group interaction)

Table 3 Adjusted changes in hormonal status, biomarkers inflammation, and oxidative stress in PCOS patients

	Placebo group (n = 24)	Zinc group (n = 24)	P^a
Zinc (mg/dL)	−4.53 ± 4.05	18.17 ± 4.05	<0.001
Prolactin (mIU/L)	−119.66 ± 39.57	−148.83 ± 39.57	0.60
FSH (IU/L)	0.02 ± 0.76	−0.21 ± 0.76	0.83
LH (IU/L)	0.14 ± 2.30	0.34 ± 2.30	0.95
Free testosterone (pg/mL)	−0.06 ± 0.16	−0.16 ± 0.16	0.67
DHEA (μg/mL)	−0.07 ± 0.09	−0.03 ± 0.09	0.75
17-OH progesterone (ng/mL)	0.31 ± 0.22	−0.62 ± 0.22	0.008
mF-G scores	−0.35 ± 0.18	−1.64 ± 0.18	<0.001
hs-CRP (ng/mL)	437.66 ± 244.80	−205.48 ± 244.80	0.07
NO (μmol/L)	6.41 ± 3.02	−1.09 ± 3.02	0.08
TAC (mmol/L)	−79.95 ± 25.81	8.53 ± 25.81	0.02
GSH (μmol/L)	42.43 ± 23.11	33.64 ± 23.11	0.79
MDA (μmol/L)	2.68 ± 0.66	−0.44 ± 0.66	0.002
Waist circumference (cm)	−0.32 ± 0.22	−0.30 ± 0.22	0.95
Hip circumference (cm)	−0.62 ± 0.25	−0.45 ± 0.25	0.63

All values are means ± SEs adjusted for baseline values, age, and baseline BMI

DHEA dehydroepiandrosterone, *FSH* follicle-stimulating hormone, *GSH* glutathione, *Hs-CRP* high-sensitivity C-reactive protein, *LH* luteinizing hormone, *mF-G* modified Ferriman-Gallwey, *MDA* malondialdehyde, *NO* nitric oxide, *TAC* total antioxidant capacity

[a] Obtained from repeated measure analysis of variance (time × group interaction)

Discussion

Our study revealed zinc supplementation for 8 weeks significantly decreased alopecia, hirsutism, and plasma MDA levels compared with the placebo.

Patients with PCOS are sensitive to wide-spectrum complications in different aspects of health, including reproductive outcomes, increased inflammatory cytokines, and increased biomarkers of oxidative stress [28]. Our findings demonstrated that taking zinc supplements for 8 weeks in women with PCOS resulted in significant decreases in hirsutism and alopecia, but did not affect acne and hormonal profiles compared with the placebo. Data regarding favorable effects of zinc supplementation on female fertility and hormonal pictures are scarce. Consistent with our findings, a 12-week supplementation with 50 mg/day zinc resulted in positive therapeutic effects among 9 out of 15 alopecia areata patients, although this was not significant [29]. Moreover, several previous studies have reported that the administration of zinc compounds improved hair growth [30–32]. However, Ead et al. [33] demonstrated that taking zinc supplements did show no positive therapeutic effect on hair loss. Zinc element is a main cofactor for various enzymes and it is involved with significant functional activities in the hair follicle [34]. In addition, previous studies have revealed that zinc inhibits hair follicle regression as well as zinc precipitates hair follicle recovery [35]. As this study is among the first that evaluated the beneficial effects of zinc intake on hormonal profiles among women with PCOS, we did not compare findings of the current study with similar studies. Although few studies assessed zinc levels in PCOS women. For instance, Zheng et al. [11] exhibited that zinc levels were significantly lower in patients with PCOS compared with the control group.

We found that zinc supplementation in patients with PCOS for 8 weeks did not influence serum hs-CRP and plasma NO concentrations. Supporting our results, the administration of rosuvastatin with or without zinc supplements (30 mg/day) in patients with atherosclerosis for 4 months did not affect hs-CRP levels [36]. Furthermore, no significant change in exhaled nitric oxide levels was observed following the administration of a nutritional supplement containing beta-carotene, vitamin C, vitamin E, zinc, selenium, and garlic compared with the placebo. However, some researchers have reported beneficial effects of zinc supplementation on inflammatory cytokines. For instance, Kelishadi et al. [37] demonstrated that hs-CRP levels significantly decreased after receiving supplemental zinc (20 mg/day) for 8 weeks among obese Iranian children with metabolic syndrome. In addition, nitric oxide concentrations increased in pregnant zinc-treated animals than in their untreated counterparts after 14 days [38]. Different study designs, lack of considering baseline levels of dependent variables along with characteristics of study subjects, different dosages of zinc administration as well as duration of the study might provide some reasons for our discrepant findings with other studies.

Findings from the present study revealed that zinc supplementation was associated with a significant decrease in plasma MDA, but did not affect TAC and GSH levels. This would mean that zinc administration significantly decreased lipid peroxidation in PCOS patients. In line with our study, decreased levels of MDA in diabetic patients have been reported following the administration of 30 mg/day zinc supplements after 6 months [39]. In addition, in an experimental study on zinc-deficient rats, zinc administration decreased MDA levels [40]. However, zinc supplementation did not influence MDA levels in rats with severe acute pancreatitis [41]. In addition, long-term supplementation with two moderate doses of 15 and 30 mg/day zinc for 6 months among a healthy elderly population did not affect biomarkers of oxidative stress including GSH, thiol groups (RSH), and MDA concentrations [42]. Recent studies have reported that oxidative stress and increased production of lipid hydroperoxides including MDA in female reproduction are associated with polycystic ovarian syndrome and endometriosis [43, 44]. MDA is one of the most frequently used to assess overall lipid peroxidation, which indicates the formation and decomposition of peroxidized fatty acids present in triglycerides, phospholipids,

cholesterol esters, and apolipoproteins [45]. The antioxidative action and decreasing MDA levels of zinc element may be divided into acute and chronic effects. The acute effect may result from decreased formation of ˙OH from hydrogen peroxide through the antagonism of redox-active transition metals including iron and copper [46]. The chronic effect of zinc intake on reduced production of lipid peroxidation may due to exposure of an organism to zinc on a long-term basis, resulting in induction of some other substances that are the ultimate antioxidant such as metallothioneins [47].

The principle limitations of the current study were the relatively small number of patients and the short follow-up time. In addition, future studies that evaluate the beneficial effects of oral zinc administration according to treatment dose and duration, and provide long-term maintenance treatment, will test the proposed therapeutic role of oral zinc supplementation in patients with PCOS. Moreover, the effect of zinc intake on the post-prandial glycemia and triglyceridemia would be of interest, may be as a topic for a future study. We calculated the sample size based on the primary outcome variable (testosterone). Therefore, the study had enough power to detect differences for this parameter. However, we did not consider secondary outcome variables such as other hormonal profiles or biomarkers of inflammation and oxidative stress in the sample size calculation. As the study is not powered to detect differences in these outcome measures, one cannot make any conclusions about them. Therefore, further large-scale studies are needed to examine the effect of zinc supplementation on other hormonal profiles or biomarkers of inflammation and oxidative stress. In addition, due to limited funding, we did not determine the effects of zinc supplementation on superoxide dismutase (SOD) concentrations as antioxidant enzyme catalyzed by zinc.

Taken together, the results of the current study indicated that taking elemental zinc (50 mg/day) for 8 weeks among PCOS women had beneficial effects on alopecia, hirsutism, and plasma MDA levels; however, it did not affect hormonal profiles, inflammatory cytokines, and other biomarkers of oxidative stress.

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Conflict of Interest The authors declare that they have no competing interests.

Authors' Contributions Z.A. contributed in the conception, design, statistical analysis, and drafting of the manuscript. M.J., F.F., F.B., R.T., and M.M. contributed in data collection and manuscript drafting. Z.A. supervised the study. All authors approved the final version for submission.

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