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The effects of Curcumin supplementation on oxidative stress, Sirtuin-1 and Peroxisome proliferator activated receptor γ coactivator 1α gene expression in polycystic ovarian syndrome (PCOS) patients: a randomized placebo-controlled clinical trial

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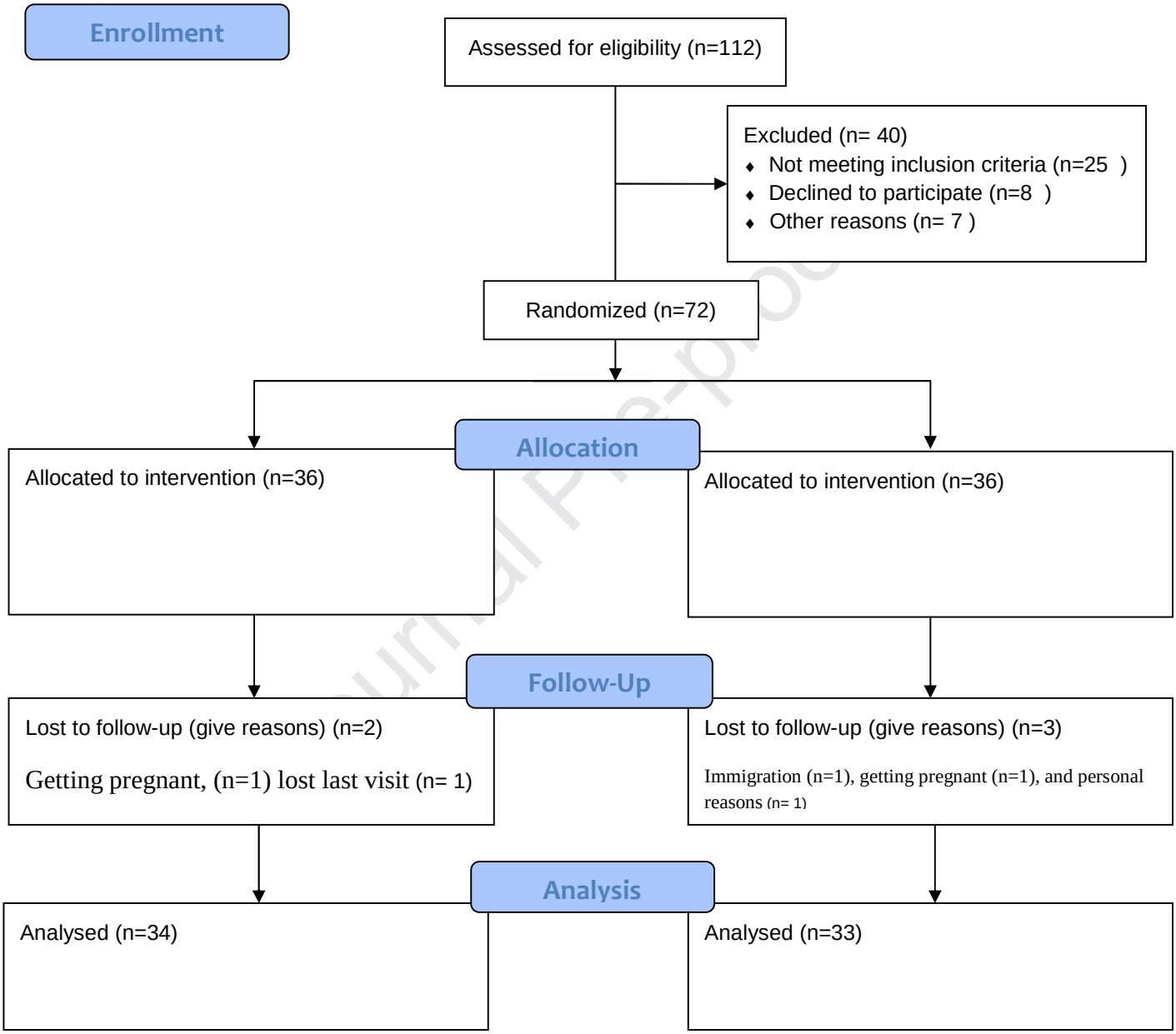

Figure 1. Flow diagram of the study

The effects of Curcumin supplementation on oxidative stress, Sirtuin-1 and Peroxisome proliferator activated receptor γ coactivator 1α gene expression in polycystic ovarian syndrome (PCOS) patients: a randomized placebo-controlled clinical trial

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Conflicts of Interest

The authors have no conflicts of interest to declare.

Highlights

- Polycystic ovary syndrome (PCOS) is a common gynecological disease
- Oxidative stress and imbalance between oxidants and antioxidants correlate with PCOS complications
- Curcumin is a potent antioxidant in turmeric and it has been determined to impact the expression of antioxidant related genes.
- Curcumin can improve body antioxidant enzymes by impacting related gene

expression in patients with PCOS

Abstract

Background & Aims: Curcumin is a biologically active phytochemical ingredient found in turmeric and has antioxidant pharmacologic actions that may benefit patients with polycystic ovarian syndrome (PCOS). The aim in this trial was to evaluate the efficacy of curcumin supplementation on oxidative stress enzymes, sirtuin-1 (SIRT1) and Peroxisome proliferator activated receptor γ coactivator 1α (PGC1α) gene expression in PCOS patients.

Methods: Seventy-two patients with PCOS were recruited for this randomized, double-blinded, clinical trial. Thirty-six patients received curcumin, 1500 mg (three times per day), and 36 patients received placebo for 3 months. Gene expression of SIRT1, PGC1α and serum activity of glutathione peroxidase (Gpx) and superoxide dismutase (SOD) enzymes were evaluated at the beginning of trial and at 3-month follow-up.

Results: Sixty-seven patients with PCOS completed the trial. Curcumin supplementation significantly increased gene expression of PGC1α (p=0.011) and activity of the Gpx enzyme (p=0.045). Curcumin also non-significantly increased gene expression of SIRT1 and activity of the SOD enzyme.

Conclusions: Curcumin seems to be an efficient reducer of oxidative stress related complications in patients with PCOS. Further studies on curcumin should strengthen our findings.

Keywords: Curcumin; Glutathione peroxidase; PGC1α; SIRT1; Superoxide dismutase

Background

Polycystic ovarian syndrome (PCOS) is considered to be the most common world-wide cause of infertility [1]. PCOS is also one of the major metabolic disorders in women of developing countries[2, 3]. Inflammation and oxidative stress may cause PCOS and related complications[4]. Oxidative stress refers to the imbalance between excessive production of reactive oxygen species (ROS) and a limited amount of body antioxidant defense [5]. Various factors are involved in oxidative stress control. The first defensive line against ROS are detoxifying enzymes like superoxide dismutase (SOD) and glutathione peroxidase (GPx). These enzymes are found in the mitochondria, cytoplasm and cell peroxisomes[6]. The next line of antioxidant defense is the limitation of ROS production by uncoupling proteins (UCPs)[7]. These UCPs reduce the production of ROS by decreasing the electrochemical potential within the inner layer of the mitochondria which shortens most of the steps in the electron transfer chain[8].

Peroxisome proliferator activated receptor γ coactivator-1α (PGC-1α), is an activator in the cell nucleus that regulates various biological actions, including: mitochondrial biogenesis; fatty acid oxidation and glucose metabolism. PGC-1α also has several roles in the metabolism of fatty acids and insulin sensitivity[9]. PGC-1α has been demonstrated to be an effective factor for increasing levels of SOD and Gpx enzymes and enhancing the antioxidant defense[10]. PGC-1α is mainly expressed in brown adipose tissue (BAT), and most other cells in the body, and is responsible for inducing the differentiation of BAT. The expression of this gene has been shown to increase the expression of UCP-1, which is the main thermogenesis inducer factor in BAT[11]. Studies in recent years have also demonstrated polymorphism of the PCG-1a gene in women with PCOS is different compared to healthy controls[12]. Other studies have shown that

PCG-1a gene expression alters the insulin resistance in PCOS[13]. It seems that increasing PCG-1a expression by increasing the expression of UCP-1 can elevate the oxidation of fats, induce thermogenesis, reduce lipogenesis, and decrease obesity in PCOS women.

Silent Information Regulator 1 (Sirt1) is also a NAD + dependent histone deacetylase in the pathway of insulin secretion. Sirt1 is involved in the expression of antioxidant defense factors and enzymes and also plays a role in apoptosis[14]. Sirt1 can regulate oxidative stress and prevent damage to DNA through its impact on the p53 protein[15]. Sirt1 contributes to the deacetylation of the PCG-1a gene, thereby increasing the rate of thermogenesis and oxidation of lipids by activating Peroxisome proliferator-activated receptor-α (PPAR-α). The effects of Sirt1 on glucose and insulin hemostasis are also applied through PCG-1a[16]..

Curcumin is a naturally active phytochemical derived from the traditional spice turmeric[17]. Curcumin has demonstrated anti-inflammatory and anti-oxidant activity through multiple mechanisms such as its impact on gene expression and cellular signaling [18, 19]. The antioxidant effects of curcumin are represented through increasing gene expression of SOD and GPx[20]. Curcumin has been shown to reduce blood glucose by inhibiting liver gluconeogenesis through affecting the 5' AMP-activated protein kinase (AMPK) signaling pathway[21]. The effect of curcumin on PGC-1a has been evaluated only in cellular studies in which curcumin has been shown to increase the expression of PGC-1a through its effect on AMPK[22].

Our hypothesis is that curcumin can help to improve the complications of PCOS through regulating the gene expression of antioxidant enzymes. Furthermore, only a limited number of cellular studies have investigated the effect of curcumin, or similar polyphenols, on Sirt1 gene expression. This study aims to investigate the effect of curcumin supplementation on the expression of PGC-1a and Sirt1 genes and oxidative factors in women with PCOS.

Methods and Design

Study Design

This was a randomized, double-blinded, clinical trial involving 72 overweight or obese [Body mass index (BMI), >25 kg/m^2] female patients with PCOS with impaired glucose tolerance at our Arash hospital in Tehran, Iran. PCOS was diagnoses according to the Rotterdam criteria[23]. After providing informed consent, the subjects were studied for a period between January 2019 and June 2019. The study was approved by the Iranian Registry of Clinical Trials on 2019-01-23 and our registration reference is IRCT20091114002709N50 (https://www.irct.ir/trial/35137). The subjects were divided into two groups (each group contain 36 subjects) by computer-based block randomization. The intervention group received 1500 mg/day (500 mg Three times daily) of curcumin, and the control group received 1500 mg/day of placebo (maltodextrin) for 12 weeks. Curcumin and placebo capsules were prepared by KAREN Pharma (Yazd, Iran), Shape, size, smell and color of placebo capsules were completely similar to the curcumin capsules. Personnel and patients was blinded to the treatment allocation. Height and waist circumference were measured before treatment and at 3-month follow-up to the nearest 0.1 centimeter, and bodyweight was measured to the nearest 100 grams. BMI was calculated as the weight (kg) divided by the square of height (meters). All patients were advised not to change their physical activity and dietary patterns during the intervention. Dietary intake was evaluated by 24h food recall two times, in beginning and end of the intervention. Patient's compliance were evaluated by weekly phone call of the researcher to each patients and monitoring for possible side effects assessed as well.

In the beginning of the intervention and after 12 weeks of study, blood samples of 10 ml were obtained after 12-14 hour of overnight fasting. Buffy coat of blood white cells was separated by centrifugation of blood samples. RNA was extracted using RNX- plus Sinacolon Kit and then cDNA was synthesized using SinaClon first strand cDNA synthesis kit. Real-time polymerase chain reaction (PCR) was carried out based on the protocols described on Sinacolon kit (SinaSYBR Blue HS-qPCRMix, 2x, Iran). GAPDH was used in real-time PCR as the housekeeping gene. The primer sequences that we used in real-time PCR are described in **Table 1**. Serum concentration of GPX and SOD activity were evaluated by the methods prescribed by Paglia et al. and Sun et al., respectively[24, 25].

Statistical analysis was performed using SPSS Software v.21. Data were shown as mean ± SE. Baseline characteristics were compared among the two intervention groups using an independent sample t-test for continuous data and a chi-square test for ordinal data. The magnitude of the effect is presented as mean difference and its 95% confidence interval. Final antioxidant enzymes and gene expression results was adjusted for possible confounders such as basic value of dependent variable, treatment type, age and BMI.

Results

One hundred and twelve women with PCOS were initially consented to participate in the trial, of which 40 subjects did not met the inclusion criteria. Seventy-two patients met inclusion criteria and were included in this study. Five patients stopped the supplement intake due to personal reasons, pregnancy, or immigration and were excluded from the trial. Therefore, at the end of treatment, 67 patients completed the study. **Figure 1** presents a flow diagram of the study.

There were no statistically significant differences between the mean values of participant's age at the beginning of the trial. **Table 2** presents the baseline and endpoints of anthropometric parameters of the participants. There were no significant differences between the anthropometric indices within the various groups at the beginning of the treatment. Neither curcumin nor placebo had significant impacts on anthropometric parameters. **Table 3** shows the dietary intakes of the trial groups at the beginning and end of the intervention according on their 24-hr recall analyses. As presented, there were no significant differences in energy, macronutrients, or main antioxidants of the diet between the study groups at the baseline and end-point of the intervention.

Serum antioxidant enzymes

As indicated in **Table 4**, curcumin supplementations significantly increased serum activity of GPx (p=0.041). This effect was still significant after adjustment for age, BMI and baseline values of GPx (p=0.010). Curcumin supplementation increased serum activity of SOD, but it was not statistically significant (p= 0.075), this impact also did not change after adjustment (p=0.0730).

Gene expression findings

Figures 2 and 3 show the effect of curcumin on gene expression of PGC-1a and SIRT1 respectively. Outcomes of this trial indicated that after adjustment for confounders like age, BMI and baseline values, the gene expressions of PGC-1a according to $2^{-\Delta\Delta ct}$ calculation were statistically increased in the curcumin group compared to the placebo (p =0.031). These results also indicated that curcumin supplementation compared to placebo increased SIRT1 gene expression, but after adjustment, this increase was not statistically significant (**Table 5**).

Discussion

This is the first randomized clinical trial to evaluate the impact of curcumin on gene expression of SIRT1 and PGC-1a in humans. We have demonstrated that curcumin, administered to women with PCOS, led to increased GPx and gene expression of PGC-1α, as well as a non-significant increase in SOD and gene expression of SIRT1, after 3 months compared to placebo. Curcumin supplementation in 1500 mg/d did not show any adverse events for participants and according to previous studies this dose is safe and effective in this regards [26, 27].

The pathogenesis of PCOS still remains unclear. Multiple features and complications of PCOS, including excessive androgens, insulin resistance, and abdominal adiposity, may lead to increase dlocal and systemic oxidative stress [28-30]. Reciprocally, oxidative stress also may worsen these metabolic abnormalities. Reducing oxidative stress in PCOS patients, or strengthening their antioxidant defense systems, can be helpful in decreasing and improving the complications of the disease[31].

PGC-1α expression increases antioxidant enzymes such as Gpx[32], and reduces oxidative stress. PGC-1α can also potentially decrease production of mitochondrial-driven ROS[33]. This randomized clinical trial has demonstrated that curcumin significantly increases gene expression of PGC-1α and Gpx activity. This likely occurs because the curcumin impacts Gpx activity through increased gene expression of PGC-1α. Previous cellular studies have indicated that curcumin stimulation increased gene expression of PGC-1α, and the impacts of curcumin on PGC-1α expression were associated with the activation of adenosine monophosphate-activated protein kinase (AMPK)[22]. It has been shown that curcumin also increased antioxidant enzymes

such as SOD transcriptions and activity by the AMPK/PGC-1α axis[34]. It has been demonstrated in several in vitro studies that curcumin performs its antioxidant actions through changes in several nuclear factors such as Nrf2, PPARγ, and NF-κB[35-37]. It has also been shown that these factors are regulated by the AMPK/PGC-1α axis[38]. PGC-1α enhancement also may have other beneficial effects on PCOS such as increased lipid oxidation through activation of PPAR-α[39]. Abdominal obesity is one of the major risk factors for PCOS, and PPAR-α is one of the main factors to increase fat oxidation and thermogenesis of fat tissues[40]. So it seems that curcumin may have beneficial effects to reduce fat mass and obesity complications in patients with PCOS through increased gene expression of PGC-1α and so PPAR-α.

In our study, curcumin supplementation non-significantly increased Sirt1 gene expression. Previous in vivo and in vitro studies indicated that curcumin treatment improved mitochondrial oxidative damage through the activation of SIRT1 signaling[41, 42]. In vitro studies also indicated that curcumin supplementation can attenuate down-regulation of SIRT1, which showed that the activation of SIRT1 might be due to the protective effect of curcumin[43]. Perhaps the low dose and short duration of our study due to human research considerations have led to the inability to draw significant results in this area. However, Sirt1 is in the signaling pathway of the AMPK/PGC-1α axis[44], and according to our results, curcumin can increase PGC-1α gene expression and this may indicate that the increase in Sirt1 was associated with an increase in PGC-1α. It has been suggested that SIRT1-mediated deacetylation of PGC-1α is attributed to the anti-oxidant activity of curcumin [45]. Insulin resistance is one of the main complications of PCOS. Growing evidence proposes that SIRT1 regulates glucose and insulin metabolism through its deacetylase function for several known substrates, and also it has been shown that SIRT1 has

a positive activity in the metabolic pathway by its direct or indirect impact on insulin signaling[46]. There is also evidence that SIRT1 upregulation induces a glucose-dependent insulin secretion from pancreatic β cells[47], and directly induces insulin sensitivity[48]. This is the first randomized clinical trial which evaluated the effect of curcumin on gene expression in human model, however this study also have some limitations. For example maybe higher dose, longer duration of supplementation could make us to draw more resolute conclusion in this regards.

In conclusion, the results of this randomized clinical trial indicate that curcumin supplementation significantly increases PGC-1α gene expression and serum enzyme activity of Gpx. We have also demonstrated that curcumin supplementation increases SIRT1 gene expression and SOD enzyme activity in non-significant manner. These results indicate that curcumin is potentially a supplementary medication for the management of PCOS. However, there is still a need for larger and longer randomized clinical trials on other clinical factors to draw a clear conclusion about the effect of curcumin on PCOS.

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Table 1 Primers used in the current study.

PGC1-α -Forward	GTCAACATTCAAAGCAGCAGAGAG
PGC1-α-Reverse	GACACATAATCATTACCTACTGGAAGC
SIRT1- Forward	TAGTAGGCGGCTTGATGGTAATC
SIRT1- Reverse	GGTTCTTCTAAACTTGGACTCTGG

Table 2. Baseline characteristics after random assignment

		Total	**Groups**		**P**
			Intervention(A)(n=34)	Control(B)(n=33)	
Age	Median (range)	30 (18 to 52)	31 (18 to 41)	29 (20 to 52)	0.897†
BMI	Median (range)	26.77 (17.75 to 39.56)	28.30 (19.05 to 39.56)	26.72 (17.75 to 36.00)	0.230†
Waist circumference	Median (range)	89.20 (61 to 117)	87.68 (61 to 117)	90.65 (70 to 114)	0.245†

† Based on t-test.

BMI: Body mass index

Table 3. Dietary intake of study participants throughout the study

		Intervention (n=34)	**Control (n=33)**	**95% CI**		**P value†**
		Mean ± SD	Mean ± SD	Lower	Upper	
Energy intake (kcal/day)	Pre	2396.08 ± 462.68	2361.93 ± 534.52	-205.97	274.25	0.777
	Post	2226.16 ± 443.30	2207.60 ± 383.78	-178.73	215.84	0.852
	Change	-169.91 ± 398.79	-154.33 ± 516.96	-238.30	207.13	0.889
Carbohydrate intake (gr/day)	Pre	360.75 ± 78.07	339.00 ± 78.67	-15.71	59.21	0.251
	Post	348.40 ± 87.89	328.02 ± 88.91	-21.88	62.65	0.339
	Change	-12.34 ± 62.76	-10.97 ± 75.80	-34.84	32.11	0.935

Protein intake (gr/day)	Pre	92.27 ± 21.50	82.75 ± 28.14	-2.56	21.60	0.120
	Post	95.34 ± 24.56	85.94 ± 25.30	-2.52	22.33	0.114
	Change	3.07 ± 14.14	3.18 ± 15.93	-7.34	7.11	0.975
Vitamin E (mg/day)	Pre	11.34 ± 7.06	11.62 ± 9.06	-4.19	3.64	0.887
	Post	10.82 ± 5.83	9.61 ± 5.33	-1.45	3.87	0.368
	Change	-0.51 ± 6.21	-2.00 ± 8.10	-1.99	4.97	0.396
Vitamin C (mg/day)	Pre	108.55 ± 51.69	94.86 ± 59.16	-12.97	40.37	0.309
	Post	94.29 ± 57.16	90.97 ± 44.30	-20.94	27.59	0.785
	Change	-14.26 ± 41.35	-3.88 ± 55.49	-34.01	13.26	0.383

Data are presented as mean±SD

† Based on independent t-test.

*statistically significant

Table 4. Comparison of Gpx and SOD enzyme activities between the two groups

		INTERVENTION (N=34)	**CONTROL (N=33)**	**95% CI**		**P VALUE†**	**ADJUSTED P VALUE§**
GPX	Pre	108.83 ± 7.19	113.54 ± 9.48	-28.49	19.07	0.694	
	Post	141.66 ± 11.08	109.37 ± 7.54	5.49	59.10	0.019	
	Change	32.83± 13.10	-4.17 ± 11.99	1.56	72.44	0.041	0.010
	P# within	0.063	0.889				
SOD	Pre	202.77 ± 10.23	206.80 ± 12.16	-35.75	27.71	0.801	
	Post	241.02 ± 13.88	205.40 ± 16.01	-6.67	77.93	0.097	
	Change	38.25± 16.19	-1.40 ± 14.83	-4.17	83.47	0.075	0.073
	P# within	0.152	0.730				

Data are presented as mean±SE

† Based on independent t-test.

§ Based on linear regression (the included variables were: basic value of dependent variable, treatment type, age and BMI)

P value within groups based on paired t-test

*statistically significant

GPX: Glutathione peroxidase, SOD: Superoxide dismutase

Table 5. Comparison of Peroxisome proliferator activated receptor γ coactivator 1α and Sirtuin-1 gene expression between the two groups

		Intervention (n=34)	**Control (n=33)**	**95% CI**		**P value†**	**Adjusted P value§**
Pgc1-α	Pre	1.123 ± 0.277	1.168 ± 0.140	-0.667	0.574	0.884	
	Post	2.686 ± 0.353	1.717 ± 0.281	0.072	1.867	0.034*	
	Change	1.563± 0.494	0.548 ± 0.299	-0.136	2.167	0.083	0.031*
	P# within	0.003*	0.074				
SIRT1	Pre	1.740 ± 0.636	0.904 ± 0.905	-1.483	3.154	0.549	
	Post	3.167 ± 1.0578	1.050 ± 0.826	-0.671	4.904	0.130	
	Change	1.427± 0.547	0.146 ± 0.145	0.061	2.499	0.048*	0.087
	P# within	0.023*	0.338				

Data presented as mean±SE

† Based on independent t-test.

§ Based on linear regression (the included variables were: basic value of dependent variable, treatment type, age and BMI)

P value within groups based on paired t-test

*statistically significant.

SIRT1: sirtuin-1, PGC1α: Peroxisome proliferator activated receptor γ coactivator 1α

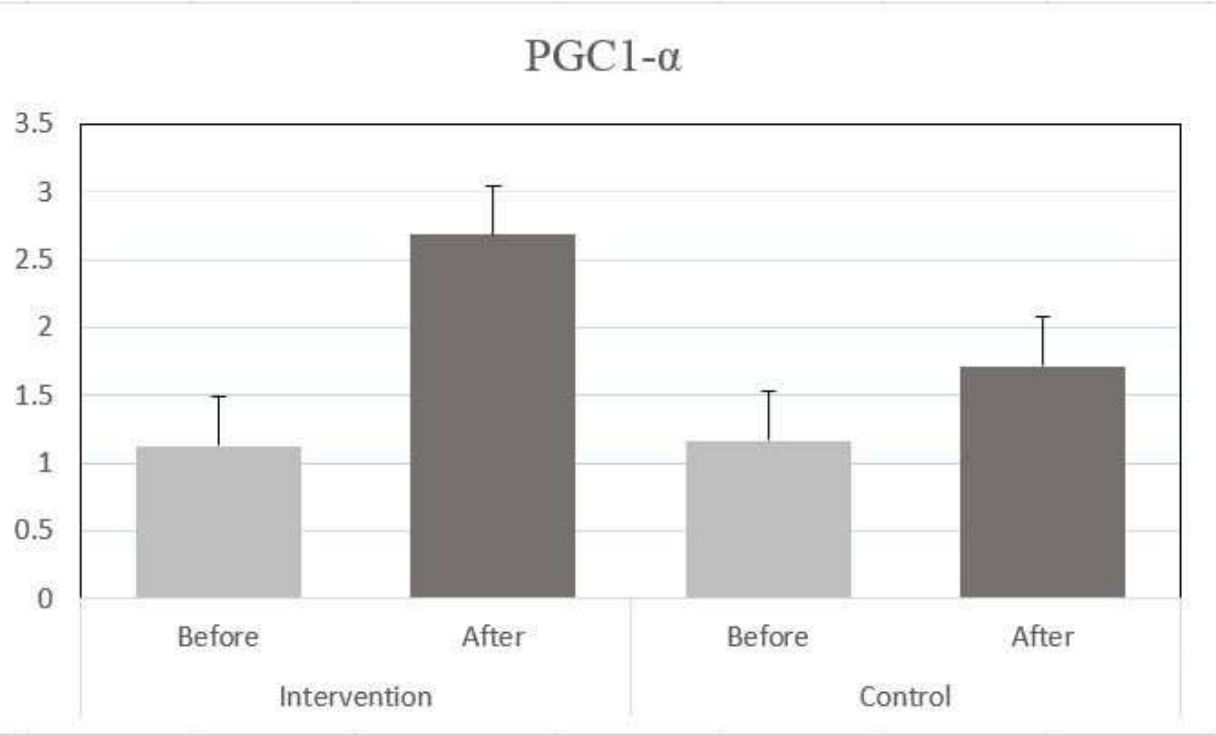

Figure 2- Effect of curcumin on gene expression of Peroxisome proliferator activated receptor γ coactivator 1α (PGC1-α) (data presented as $2^{-\Delta\Delta ct}$)

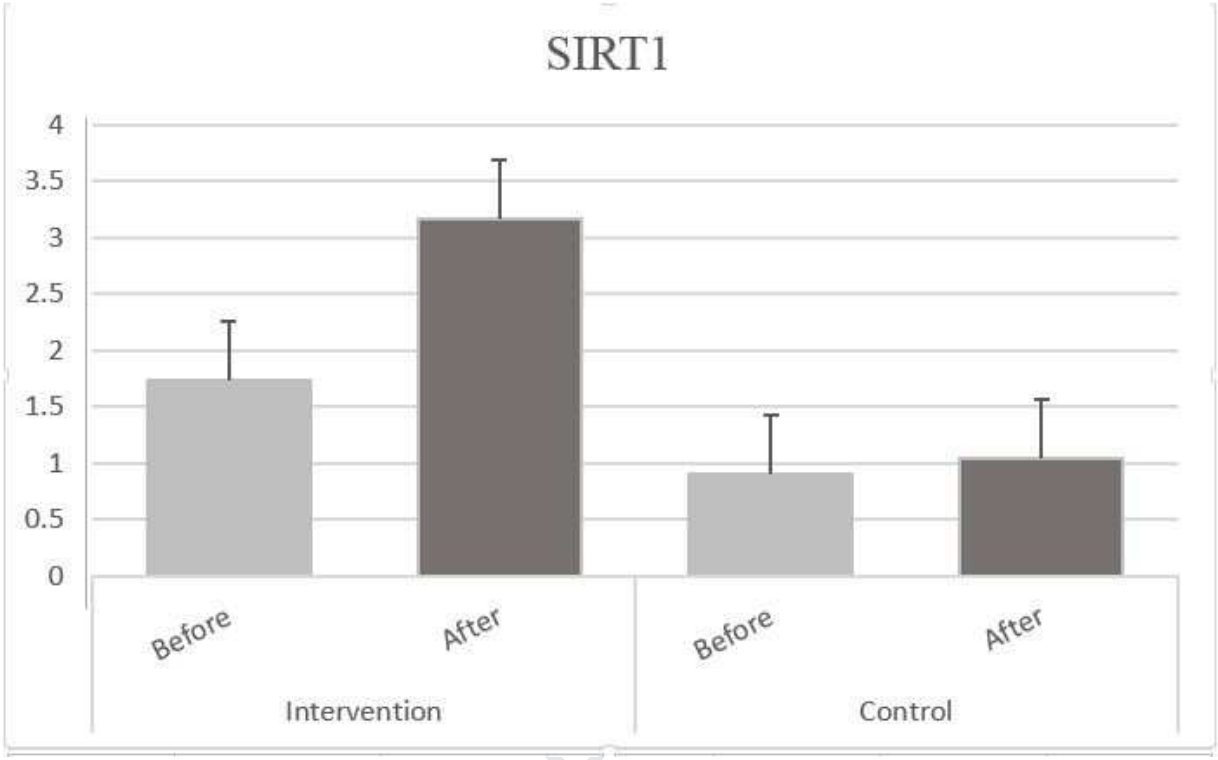

Figure 3- Effect of curcumin on gene expression of Sirtuin-1 (SIRT1) (data presented as $2^{-\Delta\Delta ct}$)

Iran University of Medical Sciences

Research Ethics Certificate

Approval ID:	IR.IUMS.REC.1397.690	Approval Date:	2018-10-28
Evaluated by:	Iran University of Medical Sciences		
Status:	Approved		
Approval Statement:	The project was found to be in accordance to the ethical principles and the national norms and standards for conducting Medical Research in Iran. Notice: 1. Although the proposal has been approved by the research ethics committee, meeting the professional and legal requirements is the sole responsibility of the PI and other project collaborators. 2. This certificate is reliant on the proposal/documents received by this committee on 2018-10-28. The committee must be notified by the PI as soon as the proposal/documents are modified.		
Proposal Title:	The effect of curcumin supplementation on PGC1 and SIRT1 gene expression in polycystic ovary syndromePCOS woman		
Principal Investigator:	Name: Farzad Shidfar Email: shidfar.f@iums.ac.ir		

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Permission letter from ethics committee