The effects of ginger extract on cyclooxygenase-2 gene expression in polycystic ovary syndrome rats

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Abstract

Introduction: Polycystic ovary syndrome (PCOS) is one of the most common endocrine disorders, can be associated with problems, such as hyperandrogenism, chronic anovulation and infertility. Ginger and its active components, have powerful anti-inflammatory and antioxidant effects. Cyclooxygenase-2 (COX-2) is the enzyme in the biosynthesis of prostaglandins and operates as an inducible enzyme with a number of inflammatory stimuli like PCOS. In this research, we evaluated the effects of ginger extract on ovarian COX-2 gene expression and reproductive improvement in PCOS rats.
Materials and methods: After induction of PCOS (by Estradiol Valerate injection), the rats divided into, control, PCOS control, PCOS treated with ginger extract (150 and 300 mg/kg) groups. At the end of treatment period, biochemical factors were measured by ELISA kits and histological assessment was done. Then RNA isolation and cDNA synthesis of ovarian tissues was performed. The data were analized by one way ANOVA ,followed by Tucky test and gene expression data were evaluated by using ΔΔCT method. Statistical significance level was set at $P<0.05$.
Results: Administration of ginger extract to PCOS treated groups, led to improved gonadotropin, sex steroids and ovarian functioning. In addition, treatment of the PCOS group with 300 mg/kg of ginger extract caused to reduce COX-2 gene expression significantly ($P<0.01$).
Conclusion: Ginger extract can act as a natural anti-inflammatory agent, and can use as a replacement of conventional synthetic anti-inflammatory drugs, in the chronic inflammatory conditions like PCOS.

Keywords: Ginger, Cyclooxygenase, PCOS,Gene expression

Introduction

Polycystic ovary syndrome (PCOS) is the most common endocrine disorder, affecting 6-10% of women in reproductive age. It is characterized by some clinical symptoms such as: hyperandrogenemia, menstrual irregularities, the presence of ovarian microcysts, hirsutism, hyperinsulinemia, chronic anovulation and infertility. PCOS is a multifactorial disorder, although insulin resistance, hypersecretion of LH and

increased levels of inflammatory markers are known to be responsible for the pathogenesis of the syndrome (1, 2).
Cyclooxygenase (COX) enzyme converts arachidonic acid to prostaglandin (PG) H2, a precursor for all prostanoids (3). Two distinct isoforms of COX have been identified. Cyclooxygenase – 1 (COX-1) is constitutively expressed in most tissues and it is critical for cytoprotective and homeostatic functions, whereas Cyclooxygenase - 2 (COX-2) is mainly induced by inflammatory stimuli and prostaglandins generated through COX-2 activation play a major role in proinflammatory reactions (4). Acute and chronic inflammations are two stages of inflammation. Acute inflammation persists only for a short time and mediated through the activation of the immune system. Chronic inflammation lasts for a longer time and it may initialize various chronic diseases such as diabetes, obesity, arthritis, cancer, PCOS, cardiovascular and neurodegenerative diseases (5, 6).
Ginger is the rhizome of the plant *Zingiber officinale* and one of the most widely consumed spices around the world (7). It is also widely used in the treatment of various disorders, including arthritis, nausea, catarrh, asthma and diabetes (8). It was reported that ginger also possessed anti-inflammatory, anti-cancer, analgesic, antimicrobial, gastroprotective, cardioprotective and antioxidant properties (9).
PCOS is associated with chronic inflammatory state and selective COX-2 inhibitors act as potential anti-inflammatory agents and there is convincing evidence that chronic inflammatory disorders have been successfully treated by them (10,11). Thus the present study was planned to evaluate the effects of ginger extract on COX-2 gene expression in PCOS rats.

Materials and methods

Chemicals

Estradiol valerate (EV) was obtained from Aburaihan Co. (Iran). Testosterone and gonadotropin hormones were evaluated by rat/mouse ELISA kits (Cosmo Bio Co. Japan). Rat Estradiol and Progesterone Elisa kits were purchased from Monobind Co. (USA).
Total RNA extraction reagent (Tripure, Roche – Germany), cDNA synthesis kit (Fermentas, Vilnius, Lithvania) and qPCR master mix (Eva green, Solis. Bio Dyne Co. Estonia) were purchased from Cinnagen company, Iran. The Glyceraldehyde-3-Phosphate Dehydrogenase (GAPDH) and COX -2 primers were obtained from Tag Copenhagen A/S, Denmark.

Ginger extract preparation

The fresh rhizomes of ginger were obtained from the local markets of Tehran, Iran. The ginger rhizomes were washed, dried and powdered by electric grinder. After grinding the dried rhizomes, to prepare hydro-alcoholic extract, 200 grams of ginger powder were soaked with 2000 ml of 70% ethanol and the solution was filtrated and evaporated to yield a dried extract. To prepare the treatment doses, the powdered extract was dissolved in distilled water.

Animals

Thirty five adult female Wistar rats (*Rattus norvegicus*) were obtained from the Pasteur Institute of Tehran, Iran. The rats were given adlibitum access to food and water, and they were kept on a 12h light/ dark cycle, at a room temperature of 23-24°C.

Experimental design

After 2 weeks of acclimatization to the environment, the rats were divided into 4 groups (n=8 per group) as follows:
- Control Group: without any treatment.
- PCOS Group: control PCOS group (rats with polycystic ovary).
- PCOS + ginger 150: PCOS rats received an intraperitoneal injection of 150 mg/kg ginger extract for 40 days.

- PCOS + ginger 300: PCOS rats received an intraperitoneal injection of 300 mg/kg ginger extract for 40 days.

Induction of the syndrome was done by subcutaneous injection of EV (4mg in 0.4 ml oil/rat) for 28 days. After 28 days and confirm the induction of PCOS in rats (by hormonal and morphological studies of three rats of PCOS groups), treatment with ginger extract was done for 40 days.

Histological and biochemical assessment

At the end of treatment period, the ovaries were removed and fixed in 10% formaldehyde buffer, and then the fixed samples were dehydrated by alcohol solutions, cleared in xylene, embedded in paraffin, sectioned in 6 µm thickness and finally stained with hematoxylin and eosin. The serum concentrations of Estradiol, Testosterone, Progesterone, Follicle stimulating hormone (FSH) and Luteinizing hormone (LH) were evaluated by ELISA kits, according to the manufacturer's instruction.

RNA isolation and cDNA synthesis

Tripure isolation reagent was used to extract total RNA from ovarian tissue samples and the isolated RNA was reverse transcribed using cDNA synthesis kit. 2 µL random hexamer (100 µM) and 8 µL nuclease free water was added to each 2 µL ovary RNA sample (50 ng/ µL). The samples were placed in the thermocycler for 5 min at 65°C to incubate. After this time the tubes were placed on ice, and 5X reaction buffer (4 µL), 200 U reverse transcriptase enzyme (1 µL), 10 mM dNTP (2 µL) and 20 U RNase inhibitor (4µL), was added to the reaction. Reverse transcription reaction was performed on an Applied Bio Rad thermocycler. The amplification program for the reverse transcription reaction was as follows: 42°C for 60 min, 25°C for 5 min, 42°C for 60 min and 70°C for 5 min.

Quantitative PCR analysis

In the present study COX-2 was target gene and GAPDH selected as an internal reference gene. The primer sequences of these genes were shown in Table 1.

The relative expression of COX-2 was performed using the ABI PRISM 7500 SDS software (ABI PRISM 7500 Sequence Detection System, Applied Biosystems). The PCR reaction mixture consisted of Evagreen supermix 1X (10 µL), cDNA (2 µL), forward primer (0.5 µL), reverse primer (0.5 µL) and nuclease free water (7 µL). The negative control with no template cDNA was used with each assay, and all amplification reactions were run in triplicate for each sample. The final reaction volume was 20 µL and the cycling parameters were 15 min at 95°C, 15 s at 95°C for denaturation, 20 s at 60°C and 20 s at 72°C for amplification, and 40 cycles of extension.

Table 1. Rat primer sequences used for real time PCR.

Gene		Primer sequences
Cyclooxygenase-2 (COX-2)	Forward	CAGACAACATAAACTGCGCCTT
	Reverse	GATACACCTCTCCACCAATGACC
Glyceraldehyde-3-Phosphate Dehydrogenase (GAPDH)	Forward	CCCATCACCATCTTCCAGGAGC
	Reverse	CCAGTGAGCTTCCCGTTCAGC

Statistical analysis

The data were analyzed using SPSS Ver.18 software. The means of biochemical parameters were compared by analysis of variance with Tukey HSD post-hoc test. The relative changes in gene expression were calculated using ΔΔCT method [(ΔCT (COX2) – ΔCT (GAPDH)], with REST 2009 software. P value < 0.05 was considered statistically significant.

Results

Our findings showed that, the PCOS rats which had been treated with 150 and 300 mg/kg of ginger extract, showed significant decrease in serum levels of LH, FSH, Estradiol and Testosterone as compared to PCOS control group. However the levels of Progesterone in the PCOS treated groups increased significantly. The results have shown in Table 2. In PCOS rats ovarian cysts increased and the number of corpora lutea markedly decreased, and reduction of corpora lutea leaded to decrease serum progesterone levels. In PCOS treated rats with ginger extract the ovarian corpora lutea and progesterone levels increased and ovarian cysts decreased significantly (Figure 1). The COX-2 gene expression increased significantly in EV-induced PCOS rats and treatment with ginger extract significantly decreased the mRNA levels of COX-2 in the PCOS groups. It would be worth that, this reduction was significant in a dose of 300mg/kg ginger extract (Figure 2).

Table 2. Effects of ginger extract on serum biochemical parameters.

Groups	LH (ng/ml)	FSH (ng/ml)	E2 (ng/ml)	P4 (ng/ml)	T (ng/ml)
Control	1.55 ±0.35	19.4 ± 1.42	0.0379± 0.002	2.34 ± 0.142	2.97 ± 0.13
PCOS control	*	*	*	*	*
	4.32 ± 0.68	21.52 ± 1.13	0.0487± 0.005	2.16 ± 0.137	4.14 ± 0.22
PCOS + ginger 150	*, ***	*, **	*, **	***	*, ***
mg/kg	1.55 ± 0.54	21.52 ± 1.13	0.0455± 0.002	2.31 ± 0.085	1.55 ± 0.16,
PCOS + ginger 300	*, ***	***	*, ***	***	*, ***
mg/kg	3.65 ± 0.44	18.71 ± 1.84	0.0436± 0.004	2.37 ± 0.028	3.55 ± 0.11

Values are means ± standard deviation (Mean ± SD). E2: Estradiol, P4: Progesterone, T: Testosterone, LH: Luteinizing hormone, FSH: Follicle stimulating hormone. * P < 0.001, differences between PCOS control group and PCOS treated groups with the control group. **P < 0.01, ***P < 0.001, differences between PCOS treated groups with ginger extract (150 mg/kg, 300 mg/kg) and PCOS control group.

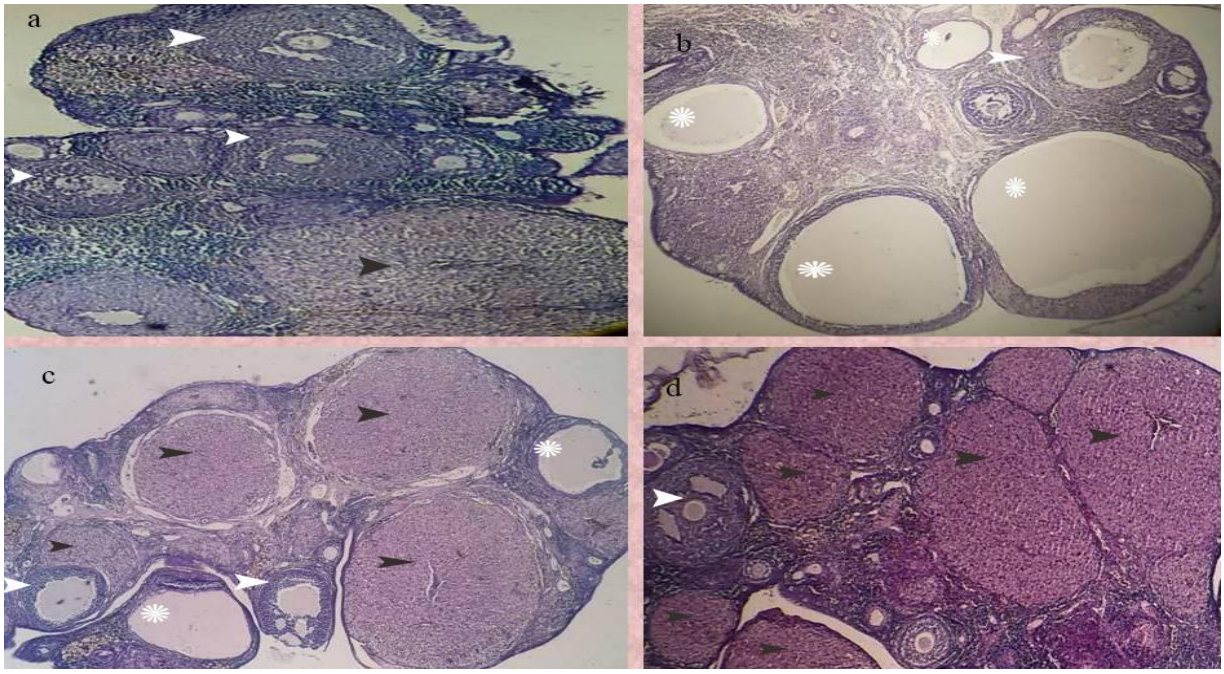

Figure 1. Histological evaluation of ovary in treated groups. Control (a), PCOS control (b), PCOS+ ginger 150 mg/kg (c), PCOS+ ginger 300mg/kg (d). White arrows indicate the 40raafian follicles, black arrows indicate the corpora lutea and star symbols indicate the cysts.

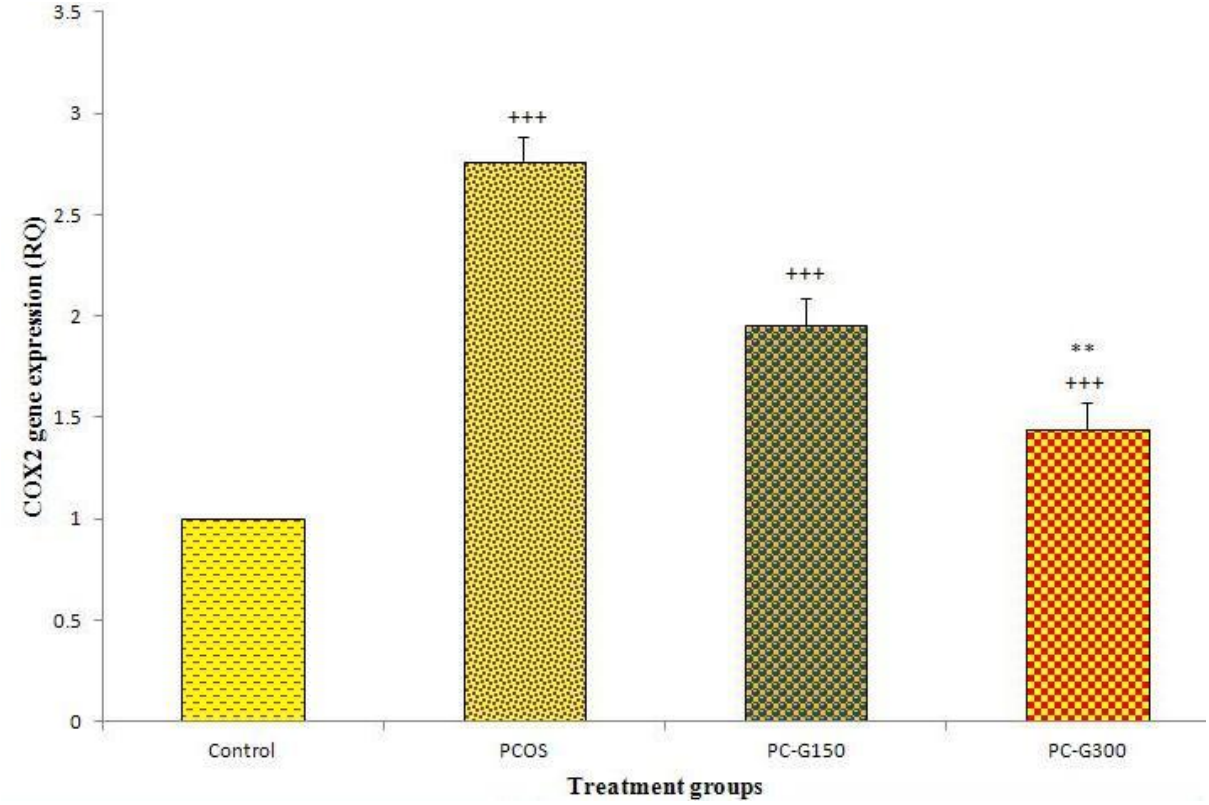

Figure 2. The effect of ginger extract on COX-2 gene expression. +++ P < 0.001, differences between PCOS control group and PCOS treated groups with the control group. **P < 0.01, differences between PCOS treated groups with ginger extract (300 mg/kg) and PCOS control group.

Discussion

In the present study, we examined the effects of hydroalcoholic extract of ginger on the serum levels of gonadotropin hormones, steroids, ovary histology and COX-2 gene expression in ovary tissue. Similar to our results Shalaby et al and Modaresi et al indicated that ginger have been improved sex hormones and serum antioxidant levels (12, 13).

Recent studies have reported that the major pharmacological activity of ginger is due to gingerols and shogaols. 6-gingerol reduces the secretion of LH and FSH via an effect on pituitary-gonadal axis (14). In another study Pournaderi et al showed that 6-gingerol cause to decrease gonadotropin hormones and Estradiol in PCOS rats (15). Prostaglandins have the main role in gonadotropin synthesis and ginger component (include gingerols) via inhibition of cyclooxygenase and lipooxygenase pathways cause to suppress prostaglandin synthesis and decrease gonadotropin levels in this way (16). Reduction of blood glucose and insulin levels by 6-gingerol cause to decrease Testosterone. 6-gingerol available in the ginger extract also inhibits the aromatase enzyme, and in this way cause to decrease biosynthesis of estrogens from androgens, thus leads to reduce the estrogen levels (17, 18).

Similar to our study, Karimzadeh et al (19) have shown that the COX-2 gene expression increased significantly in EV-induced PCOS rats. The inflammatory markers, such as tumor necrosis factor alpha (TNFα), COX-2, Interleukin 6 (IL-6) and Interleukin 8 (IL-8) increase in PCOS condition (6). In this syndrome hyperandrogenemia and insulin resistance disturbe the antioxidant defence, and cause to release proinflammatory factors (20). In the present study, treatment with ginger

extract caused to reduce the mRNA levels of COX-2 in the PCOS groups.

Studies in rats have revealed that daily oral or intraperitoneal administration of ginger extract caused to reduce blood concentrations of Prostaglandin E2 (PGE2) (21). Another investigation has shown that gingerols and shogaols in ginger, reduce prostaglandin synthesis through suppression of COX-1and COX-2 and also has been reported that ginger prevent leukotriene biosynthesis by inhibition of 5-lipoxygenase (22). Thus, experimental studies demonstrated that anti-inflammatory effects of ginger extracts are derived from an inhibition of arachidonic acid metabolism via the COX-2 (prostaglandins, thromboxanes) and lipoxygenase products (leukotriens) pathways (23).

In another study El-sharaki et al have shown that pretreatment of rats with 100mg/kg dose of ginger extract was completely abolished the increased activity of COX-2 by oxidative stress (24). Lentz et al have also reported that many gingerols and shogaols (active components of ginger) express anti-inflammatory activity in cultured human histiocytes (25). Another study showed that ginger extract suppressed cytokine production (associated with inhibition of NF-kβ and IL-α activation) in human synoviocytes (26). Rhod has revealed that treatment of cultured ovarian cancer cells with ginger causes to inhibit NF-kβ activation and decreases VEGF (Vascular endothelial growth factor) and IL-8 secretion (22). In fact secretion of angiogenic factors in ovarian cancer cells modulate by ginger components. Habib et al in another study has reported that ginger extract reduced elevated expression of Nuclear Factor kappa-β (NF-kβ) and Tumour Necrosis Factor-α (TNF-α) in liver cancer of rat (27). Breeman et al have reported that a chloroform extract of ginger root inhibited COX-2 approximately three-fold more than COX-1(28). In vitro investigations of Tjendraputra et al and Grzanna et al have shown anti-inflammatory effect of ginger, including inhibition of NF-kβ, inhibition of COX, and inhibition of 5-lipoxygenase (29,30). Thus the mechanisms involved in the chemopreventive effects of ginger in the inflammatory disorder are contributed by alteration of gene expression, antioxidant pathways and free radical scavenging (27).

Conclusion

Inflammation is a physiological response to infection and tissue injuries and is associated with alteration of signalling pathways, which results in increased levels of inflammatory markers and free radicals (10). On the other hand COX-2 is the target of non-steroidal anti-inflammatory drugs (NSAIDs) widely used in the inflammatory processes and NSAIDs prevent the enzymatic conversion of arachidonic acid to pro-inflammatory markers (17, 18). In this research, we proposed that the active components in ginger can act as the natural antioxidant and anti-inflammatory agents, it can use as replacement of conventional synthetic antioxidants and older NSAIDs with many side effects, and we can say ginger and its constituents through the specific inhibition of COX-2, may have anti-inflammatory activities.

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Conflict of interest

The authors state no conflict of interest regarding the content of this article: The effects of ginger extract on cyclooxygenase-2 gene expression in polycystic ovary syndrome rats.

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