Activity of isoflavone in managing polycystic ovary syndrome symptoms (Review)

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Abstract. Polycystic ovary syndrome (PCOS), a hormonal and metabolic disorder manifested in women of reproductive age, is still being treated using drugs with side effects. As an alternative to these drugs, isoflavone, also identified as phytoestrogen, has anti-PCOS activity. Isoflavone can help relieve PCOS symptoms by lowering the level of testosterone, which causes hyperandrogenism, thereby normalizing the menstrual cycle and restoring normal ovarian morphology. Furthermore, isoflavone influences the improvement of the metabolic profile, which changes because of PCOS, as well as the reduction of inflammatory markers and oxidative stress. However, both significant and non-significant results have been generated on the activity of isoflavones in PCOS. The present review aims to discuss the existing literature on the effect of isoflavone on PCOS symptoms based on *in vivo* and clinical trial studies.

Contents

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1. Introduction

The most widespread hormonal and metabolic disorder affecting women of reproductive age is polycystic ovarian syndrome (PCOS), which manifests clinically as irregular menstruation, hyperandrogenism and polycystic ovaries (1). These females also have metabolic issues such as obesity, insulin resistance and dyslipidemia (2,3). Increased levels of androgen and lower levels of sex hormone-binding globulin are associated with irregular menstruation (4). Patients with PCOS have hyperandrogenism, which may include hirsutism, acne, androgenic alopecia, as well as high testosterone levels. Hyperandrogenism is caused by an increase in androgen production by the ovaries and adrenals (5). The Rotterdam criteria for PCOS define the polycystic ovary morphology as a follicle count per ovary 12 and/or an ovarian volume >10 cc in ≥1 ovary (6). Insulin resistance may influence various elements of ovarian shape by boosting theca cell proliferation, according to morphological anomalies in PCOS (7). The progression of PCOS symptoms over time is depicted in Fig. 1.

Combined oral contraceptives (COCs) contain two hormones, estrogen and progesterone that inhibit hypothalamic, pituitary and ovarian steroidogenesis; they consist the first-line treatment for monthly abnormalities of the menstrual cycle in patients with PCOS. Medication for PCOS-related hirsutism, acne and androgenic alopecia is just as successful as the COC treatment for monthly abnormalities. In addition, the metabolic characteristics of PCOS are aided by the anti-androgenic chemicals in third-generation combined oral contraceptive tablets. While this may be the case, oral contraceptives are used by women who are not actively trying to conceive (4,8). Metformin, spironolactone, clomiphene, thiazolidinediones and insulin-sensitizing agents are just a few of the synthetic medications used to treat PCOS and reduce insulin resistance, testosterone levels, inflammatory symptoms and menstrual cycle abnormalities. Congenital heart defects, nausea, vomiting and diarrhea are only some of the possible adverse reactions to these medications (9,10).

Several medicinal herbs, including *Cinnamomum verum*, *Trigonella foenum-graecum* and *Vitex agnus-castus*, were suggested as adjunctive treatments for PCOS (11,12). A varied effect of soy on PCOS was discovered, making it one of the medicinal plants with contentious results (12). Soy isoflavones may be useful in the treatment of PCOS as they enhance both

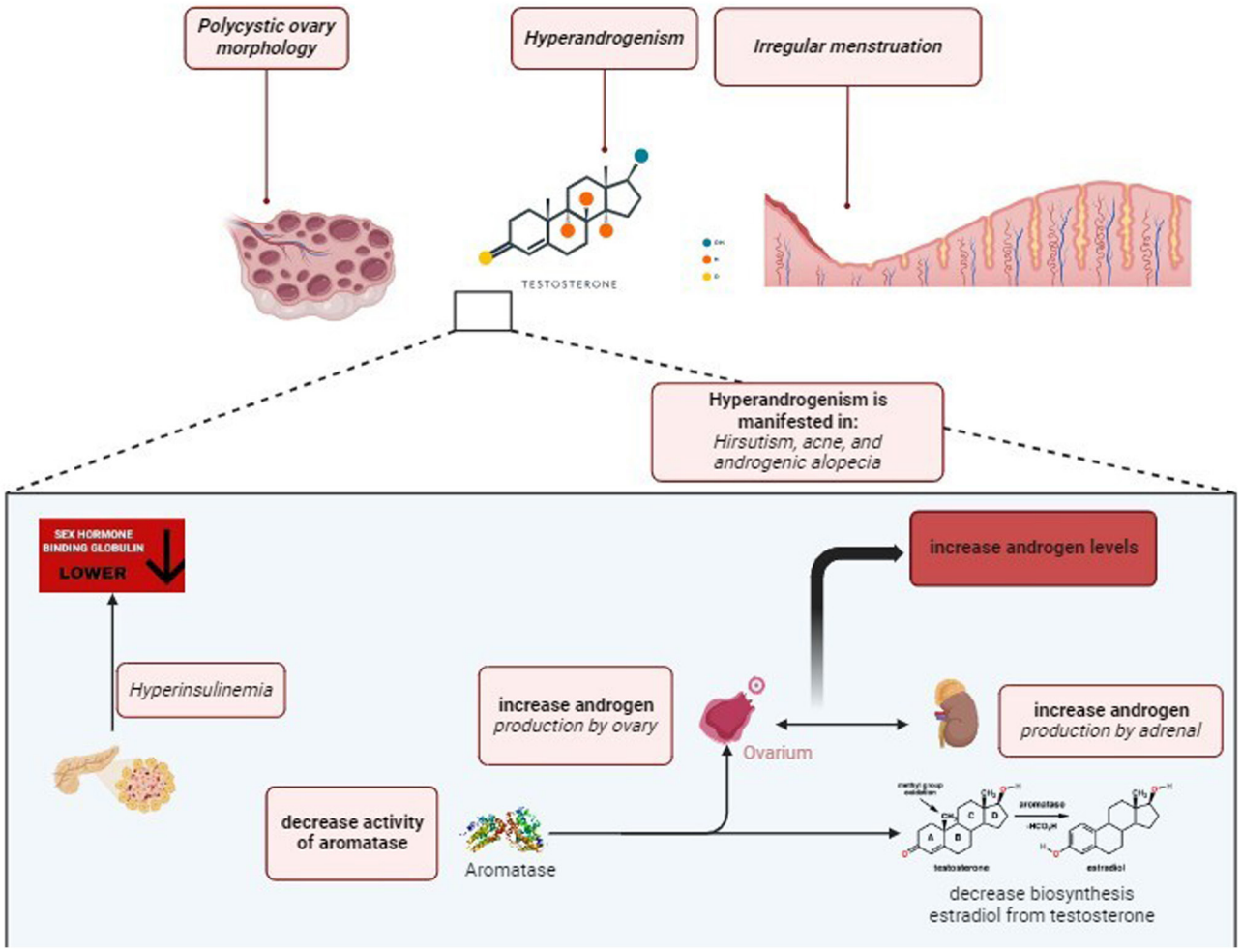

Figure 1. Pathophysiology of PCOS symptoms. Hyperandrogenism, a condition derived from both increasing the androgen level and lowering the sex hormone-binding globulin, is the main cause of other PCOS symptoms, including polycystic ovary morphology and irregular menstruation. The lowering of sex hormone-binding globulin is caused by hyperinsulinemia. Meanwhile, the increase in androgen levels is caused by the production of androgen in both the ovary and adrenal gland as a result of decreasing the activity of aromatase, which decreases the biosynthesis of oestradiol from testosterone. PCOS, polycystic ovary syndrome. The different colours in the testosterone structure refer to the OH bond (green), H bond (orange) and O bond (yellow). The structure at the right bottom refers to the process of converting testosterone to oestradiol by an aromatase catalyst. Steroids are composed of four fused rings (labelled A-D). Aromatase converts the ring labeled 'A' into an aromatic state.

metabolic conditions and reproductive hormones. The simple glucosides daidzin, genistin and glycitein, as well as the acylated glucosides genistin, glycitein and glycidyl isoflavone, are all soybean isoflavones (13,14). Isoflavones bind to the estrogen receptor (ER)-α and ER-β because of their chemical structure, which is comparable to that of the estrogen-like substance 17β-estradiol (15-17). Either an estrogenic or an anti-estrogenic effect follows. This effect is useful in the treatment of PCOS and other estrogen-related disorders (18,19). Previous research on the role of isoflavones in PCOS produced both positive and negative results (12). The present review aimed to analyze *in vivo* and clinical trial studies related to the activities of isoflavone in treating PCOS symptoms.

2. Model of *in vivo* and clinical studies of PCOS symptoms

The gold standard for *in vivo* PCOS research is the letrozole-induced PCOS rat model, in which female Sprague-Dawley rats (4-6 weeks old) received letrozole (1 mg/kg PO once daily) for 21 days (3,20,21). Letrozole, an aromatase inhibitor, boosts androgen levels in PCOS by preventing the conversion of androstenedione to estrone, and testosterone to estradiol (22,23). Female Wistar rats (8-10 weeks old) may receive subcutaneous injections of 2 mg/kg (24) or 4 mg/kg (25) estradiol valerate for 60 consecutive days to establish a PCOS model. This PCOS model, which is similar to PCOS in humans and can be generated in rats by a single dose of estradiol valerate, is characterized by infertility, irregular reproductive cycles and polycystic ovaries with a high number of atretic follicles and cysts (26).

A randomized, double-blind, placebo-controlled trial design is typically used when conducting a clinical experiment. To participate in a clinical trial, participants should have two of the following characteristics and match the Rotterdam criteria (27) for PCOS: i) Individuals must have irregular menstruation, hyperandrogenism or polycystic ovarian changes; ii) individuals who have not taken oral contraceptives within the last 3 months; and iii) individuals who have not taken antioxidant-related drugs, medications affecting reproductive endocrine functions or the metabolism of glucose and

Table I. Effects of isoflavone administration in patients with polycystic ovary syndrome.

First author/s, year	Isoflavone source	Subjects, n	Dose and duration	Effects	(Refs.)
Li *et al*, 2021	*Pueraria lobate* (Willd.) Ohwi Soy	36	150 mg/day for 3 months	Increased SOD and SHBG, and decreased TC, T and SBP	(28)
Jamilian and Asemi, 2016		35	50 mg/day for 3 months	Decreased levels of lipids, FAI, insulin, HOMA-IR, HOMA-B, T and MDA in the blood, and increased QUICKI, SHBG and GSH levels	(41)
Karamali *et al*, 2018	Soy	30	0.28 g/kg for 2 months	Decreased body weight, HOMA-IR, FPG, T, TG, VLDL and MDA levels, and increased QUICKI, NO and GSH	(42)
Khani *et al*, 2011	Genistein	69	18 mg twice a day for 3 months	Reduced LDL	(47)
Jamilian and Sahebkashaf, 2017	Soy isoflavone supplements	35	50 mg for 3 months	Reduced T and MDA levels, and increased plasma GSH	(43)

SOD, superoxide dismutase; SHBG, sex hormone binding globulin; TC, total cholesterol; T, testosterone; SBP, systolic blood pressure; FAI, free androgen index; HOMA-IR, homeostatic model assessment for insulin resistance; HOMA-B, homeostatic model assessment of β-cell function; MDA, malondialdehyde; QUICKI, quantitative insulin-sensitivity check index; GSH, glutathione; FPG, fasting plasma glucose; TG, triglyceride; VLDL, very low-density lipoprotein; NO, nitric oxide; LDL, low-density lipoprotein.

lipids, with high prolactin levels, thyroid disorders, endocrine diseases, gastrointestinal issues, diseases that cause elevated testosterone levels and those who have contracted any of these conditions (28). Tables I and II summarize clinical trials and *in vivo* studies looking at isoflavone therapy for PCOS.

3. Effect of isoflavone on body weight and testosterone level

Abdominal obesity is linked to high plasma androgen levels in patients with PCOS. Obesity is associated with high plasma levels of total or free testosterone (29). Increased testosterone levels are linked to abdominal obesity and fat accumulation because of its anti-lipolytic effect on abdominal subcutaneous preadipocytes due to the selective inhibition of catecholamine-induced lipolysis, which in turn can decrease the expression of β2-adrenergic receptor and hormone-sensitive lipase protein (30,31). PCOS may decrease lipolytic activity, which would increase the lipid content of fat cells and result in obesity.

Isoflavone treatment led to weight loss in PCOS-induced rats (3,20,24). Isoflavone was shown to reduce peripheral blood testosterone levels, which in turn reduces fatty production, hence leading to weight loss (20,24). The chemical structure of isoflavones, which contains a phenolic B ring in the third position of the pyran ring, may impede the action of steroidogenic enzymes like 3β-hydroxysteroid dehydrogenase (HSD) and/or 17β-HSD, hence lowering testosterone levels (20). Androgens are synthesized using both enzymes (32,33).

4. Effect of isoflavone as an antioxidant

The chemical structures of isoflavones are essentially linked to their ability to reduce oxidative stress and increase antioxidative levels (20). Advanced glycosylated end products (AGEs), nitric oxide (NO), malondialdehyde (MDA) and xanthine oxidase (XO) are markers of oxidative stress and some studies (34-37) indicated that their levels are higher in patients with PCOS than in women without the condition. Nonetheless, another study showed that patients with PCOS had reduced levels of the antioxidants glutathione (GSH), and the vitamins C and E (38). The level of reactive oxygen species is higher in the ovarian follicle of patients with PCOS than in normal subjects, which results in increased tissue damage (39). Moreover, MDA-induced polyunsaturated lipid peroxidation acts as a recognizable hallmark of the wounded tissue. MDA may also result from excessive androgen production, which affects insulin resistance and hyperglycaemia in patients with PCOS (40). Through a variety of mechanisms, antioxidants serve a key role in preventing, treating and eradicating oxidative damage. The free radicals formed by superoxide anion radicals and hydrogen peroxide are neutralized by the antioxidant enzymes superoxide dismutase (SOD), GSH peroxidase (GPx) and catalase (CAT) (21,24).

Human study. Regarding antioxidant status, isoflavone therapy was linked to an increase in SOD and GSH, while MDA was decreased (28,41-43). Karamali *et al* (42) reported that the level of NO was notably increased after 8 weeks of treatment with soy in patients with PCOS likely due to the L-arginine found in soy that can act as a NO precursor. Consequently, an increase in the NO level was observed in that study. NO in PCOS could play a role in determining polycystic characteristics (38) and further research on soy intake in patients with PCOS is required.

Animal study. Ma *et al* (3) discovered that soy isoflavones have an antioxidant effect on rats by increasing GPx and

Table II. Effects of isoflavone treatment in PCOS rats.

A, Isoflavone from soy

First author/s, year	Induction	Doses and duration of treatment	Effective dose	Effects	(Refs.)
Ma *et al*, 2021	Letrozole	20 mg/200 g for 4 weeks	-	Notable reductions in body weight, number of atretic follicles in ovarian tissue, serum T, LH, LH/FSH ratio, MDA, TNF-α, IL-1β and IL-6 levels Enhanced estrus cycle, granulosa cell layer in the ovary and elevated levels of FSH and estradiol Ovarian corpus luteum and healthy follicles. Decreased p-p65/p65 and p-IκBα/IκBα ratios in ovarian tissues	(3)
Manzar *et al*, 2021	Estradiol valerate	0.02/200, 0.03/200 and 0.04/200 g for 12 weeks	0.04/200 g	Higher level of progesterone and HDL, and lower FSH/LH ratio Decreased triglycerides, cholesterol, LDL and body weight	(14)
Rajan *et al*, 2017	Letrozole	50 and 100 mg/kg for 2 weeks	100 mg/kg	Reduced body weight, diestrus phase %, T levels, 3β and 17β-HSD enzyme activity and oxidative stress (LPO and NO) Increased antioxidant activity in the ovary (SOD, CAT, GPx and GSH) Rat ovary with well-formed antral follicles, a typical granulosa cell layer and a distinct theca layer	(20)
Rajaei *et al*, 2019	Estradiol valerate	1 mg/kg for 2 weeks	-	Decreased plasma and ovarian MDA levels, body weight, and the overall population of ovarian atretic follicles Increased plasma and ovarian TAC, SOD, and GPx activities Well-developed antral follicles, a normal granulosa cell layer, a distinct theca layer and few corpora lutea	(24)
Alivandi Farkhad and Khazali, 2019	Estradiol valerate	50 and 100 mg/kg for 3 weeks	100 mg/kg	Fully-grown antral follicles, healthy granulosa and theca cells in the ovary, normalized theca follicle thickness and enhanced granulosa layer thickness Reduced TNF-α, IL-6 and overall oxidative state levels Increased capacity of all antioxidants	(25)
Amanat *et al*, 2021	Letrozole	20 mg/kg for 6 weeks	-	Decreased body mass, LDL, MDA and cyst count Increased TAC and SOD Wholesome corpora lutea and follicles	(45)
Teixeira *et al*, 2019	-	150 mg/kg daily for 8 weeks	-	Low number of growing follicles, and corpora lutea and atretic follicles predominate Reduced percentage of BCL-2 and cleaved caspase-3^{+} cells Lowered lipid peroxidation and reactive oxygen species Increased capacity of all antioxidants	(55)

Table II. Continued.

A, Isoflavone from soy

First author/s, year	Induction	Doses and duration of treatment	Effective dose	Effects	(Refs.)
Geethika and Okada, 2018	Letrozole	0.05% for 2 weeks	-	Decreased number of cysts Enhanced the estrous cycle Lower number of gut microbiota granulicatella	(58)
Isoflavone from red clover (*Trifolium pratense*)					
Abbasian *et al*, 2020	Letrozole	100 mg/200 g or 150 mg/200 g) for 4 weeks	150 mg/200 g	Decreased levels of LDL, MDA, NO and T Elevated levels of CAT, GSH, SOD, HDL and estrogen Increased number of atretic follicles and decreased number of antral follicles	(21)

B, Isoflavone from chickpea (*Cicer arietinum*)

First author/s, year	Induction	Doses and duration of treatment	Effective dose	Effects	(Refs.)
Ali *et al*, 2021	Letrozole	50 mg/200 g and 100 mg/200 g for 4 weeks	100 mg/200 g	Both doses reduced the levels of MDA, triglycerides, LDL, glucose, testosterone and ovarian cysts Both doses increased GSH and granulosa cell thickness	(57)

T, testosterone; LH, luteinizing hormone; FSH, follicle-stimulating hormone; p-, phosphorylated; TAC, total antioxidant capacity; SOD, superoxide dismutase; MDA, malondialdehyde; GSH, glutathione; CAT, catalase; NO, nitric oxide; LDL, low-density lipoprotein; HDL, high-density lipoprotein; GPx, glutathione peroxidase; HSD, hydroxysteroid dehydrogenase; LPO, lipid peroxidation; PCOS, polycystic ovarian syndrome.

SOD. According to previous research results, the antioxidant effect of isoflavone via MDA also decreased (21,24,41,42). Isoflavone also affects the reduction of NO levels reported by Abbasian *et al* (21), who evaluated the treatment of isoflavone from red clover.

Total antioxidant capacity (TAC) levels in patients with PCOS were observed to be inconsistently higher or lower compared with those in controls. An elevation in TAC in PCOS is thought to act as a defence mechanism against the negative consequence of increased oxidative stress in immature ovum destruction (40). Further study on the effects of isoflavone on TAC and the relationship between TAC and PCOS is required.

5. Effect of isoflavone as an anti-inflammatory agent

The adipose tissue of patients with PCOS and obesity produces proinflammatory cytokines such as TNF-α and IL-6 in response to hypoxia (44).

In vivo study on rats with PCOS treated with soybean isoflavone-aglycone fraction demonstrated decreased IL-6 and TNF-α levels in ovarian tissue (25). Those findings were comparable with those of Amanat *et al* (45), who identified reduced levels of TNF-α in PCOS rat model compared with those in healthy rats. Theca cells, which make androgens, had increased proliferation due to increased TNF-α levels, which also contributed to hyperandrogenism (25). The ability of isoflavone to reduce inflammation by decreasing TNF-α also makes it an anti-androgenic and antioxidant agent.

Moreover, soy isoflavone treatment was reported to reduce the expression levels of NF-κB p65, phosphorylated (p)-NF-κB p65 and p-IκBα in ovarian tissues (46), suggesting a decrease in TNF-α, IL-1β and IL-6 (3).

6. Effect of isoflavone in the improvement of metabolic profile and reproductive hormone

Human study. Jamilian and Asemi (41) discovered that in patients with PCOS, blood levels of insulin, triglycerides and very low-density lipoprotein (VLDL) cholesterol were considerably reduced throughout the course of a 12-week soy isoflavone therapy compared with those in the placebo group. The homeostasis model of assessment (HOMA) for β cell function and that for insulin resistance (HOMA-IR) were shown to be lower in that study, and the quantitative insulin sensitivity check index (QUICKI) was also shown to be higher. In a 3-month clinical trial of the drug genistein, patients with PCOS demonstrated a notable reduction in LDL and triglycerides compared with that in the placebo group (47). Over the course of 8 weeks, patients with PCOS who had a soy diet high in unsaturated fat and low in saturated

fat had decreased insulin levels, HOMA-IR, triglycerides and VLDL. Moreover, the soy diet improved QUICKI scores compared with a control diet (42). Clinical trial outcomes demonstrated that 3 months of soy isoflavone (genistein) treatment notably decreased luteinizing hormone (LH) and testosterone levels in patients with PCOS compared with the placebo group (47). These findings imply that genistein may directly influence the hypothalamic-pituitary-gonadal or hypothalamic-pituitary-adrenal (HPA) axis, improving the production pattern function of gonadotrophin-releasing hormone (GnRH). To demonstrate that these systems do function, further studies are required.

Animal study. In an *in vivo* study, red clover isoflavone was shown to be more efficient than the PCOS group in reducing LDL while increasing high-density lipoprotein levels (21). The soy isoflavone genistein treatment was demonstrated to considerably lower the LDL level in the PCOS group and improve insulin resistance and dysregulated glucose haemostasis (45). However, plasma glucose and cholesterol levels were not substantially different between PCOS vehicle-treated rats and soy isoflavone-treated rats (20).

The hormonal disturbance observed in PCOS is caused by modifications to the hypothalamic-pituitary-ovarian or HPA axis, which originates from an abnormality in the GnRH production pattern. As a result, LH levels increased, follicle-stimulating hormone (FSH) levels decreased and thereby the LH/FSH ratio increased (48). The unfavourable feedback of oestradiol influences the generation of FSH as well (49). The activation of androgen synthesis by LH release in endometrial cells results in an increase in androgen levels in the body. Contrary to the PCOS group, rats treated with soy isoflavones exhibited considerably higher levels of FSH and oestradiol, and notably lower levels of LH, testosterone and LH/FSH ratio (3). Treatment with 50 and 100 mg/kg of soy isoflavones did not markedly raise estradiol levels (20).

7. Effect of isoflavone in normalizing the oestrous cycle

Similar to the menstrual cycle (ovarian and uterine cycles) in humans, rodents such as rats have a reproductive cycle known as the oestrous cycle (50). Dioestrus, proestrus, oestrus and metestrus are the four stages of the cycle, and each of them lasts 4-5 days (51). The cycle of letrozole-induced PCOS rats was studied for 42 days. Between day 9 and 30, the cycle was in the dioestrus phase. On days 31 and 32, it entered the proestrus phase. Subsequently, it went back into the dioestrus phase for another 10 days (52). Compared with the group demonstrating a protracted dioestrus phase due to letrozole-induced PCOS, rats that were given soy isoflavone progressively returned to a normal oestrous cycle. Changes in the levels of sex hormones and gonadotrophins such as testosterone, LH, FSH and oestradiol in the bloodstream may be responsible for that result (3,20).

Nonetheless, the menstrual cycle in humans consists of three distinct stages: Luteal, follicular and menstrual (53). From day 1 of one period to day 1 of the next, this cycle typically lasts ~28 days (51). To the best of our knowledge, there has been no clinical investigation of the influence of isoflavone on the menstrual cycle of patients with PCOS. Further research into the therapeutic effects of isoflavone in controlling the menstrual cycle of patients with PCOS is warranted.

8. Effect of isoflavone on restoring the ovarian morphology

Letrozole induction stimulated the development of cysts while suppressing oocyte, granulosa and theca layer hyperplasia, as well as follicular atresia (54). In the ovary histology of soy isoflavone-treated letrozole-induced PCOS rats, distinct theca layer, well-developed antral follicles and a normal granulosa cell layer were present (20). In a study by Ma *et al* (3), soy isoflavone was found to increase the granulosa cell layer, encourage healthy follicle and corpus luteum development, and reduce the number of atretic follicles compared with the control group. Teixeira *et al* (55) also discovered that the soy isoflavone group had fewer atretic follicles compared with the placebo group. According to a study by Amanat *et al* (45), genistein treatment in PCOS rats was reduced the number of atretic follicles; however, this number was markedly higher than that in the control group. There were fewer cysts in the follicles and corpora lutea of the healthy group than in the PCOS group (45).

In vivo research of isoflavone from red clover extract, compared with study of soy isoflavone (3,45,55), found a rise in atretic follicles and a reduction in antral follicles (21). Full-grown antral follicles, a few corpora lutea, normally functioning granulosa cells and theca cells were observed in the ovaries of soybean isoflavone-aglycone fraction-treated PCOS rats. These findings were in agreement with those predicted by the action of genistein, the primary component of the isoflavone-aglycone (24,56).

Contrary to the PCOS group, chickpea (*Cicer arietinum*) isoflavones were shown to decrease the prevalence of fluid-filled sac in the ovary, restore granulosa cell diameter and enhance the existence of corpus luteum (57).

9. Conclusions

Isoflavones were shown to effectively treat PCOS syndrome symptoms by targeting several interconnected pathways. The primary benefit of isoflavones in treating PCOS symptoms is the reduction of androgen levels, which are the main symptoms of PCOS. This is achieved by inhibiting the activities of 3β-HSD and/or 17β-HSD enzymes, reducing oxidative stress levels (MDA, NO, AGEs and XO), and increasing antioxidative levels (SOD, GPx, TAC and CAT) and anti-inflammatory activity (TNF-α, IL-6 and NF-κB). Additionally, several other symptoms of PCOS such as obesity, insulin resistance, metabolic dysfunction, impaired folliculogenesis, low-grade chronic inflammation, reproductive hormone imbalance, irregular monthly cyclicity, aberrant gut flora and ovarian characteristics can be treated effectively with isoflavones. The recommended dosage of isoflavone supplementation for patients with PCOS is 50 mg/day, which has shown promising results (41,43).

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Authors' contributions

CR collected raw data for analysis, performed critical analysis of the literature and drafted the manuscript. SS and NFK organized the framework of this paper, supervised the work and revised the manuscript. All authors have read and approved the final version of the manuscript. Data authentication is not applicable.

Ethics approval and consent to participate

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Patient consent for publication

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Competing interests

The authors declare that they have no competing interests.

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