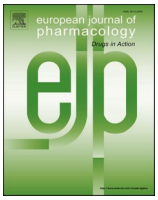
European Journal of Pharmacology 933 (2022) 175275

Contents lists available at ScienceDirect

European Journal of Pharmacology

journal homepage: www.elsevier.com/locate/ejphar



Potential roles of genistein in polycystic ovary syndrome: A comprehensive systematic review

Ramin Nasimi Doost Azgomi [a], Arezoo Moini Jazani [a,*], Arash Karimi [a,b,**], Sanaz Pourreza [c]

[a] Traditional Medicine and Hydrotherapy Research Center, Ardabil University of Medical Sciences, Ardabil, Iran
[b] Department of Clinical Nutrition, Faculty of Nutrition and Food Science, Tabriz University of Medical Sciences, Tabriz, Iran
[c] Department of Community Nutrition, School of Nutritional Sciences and Dietetics, University of Medical Sciences, Tehran, Iran

ARTICLE INFO

Keywords:
Genistein
Polycystic ovary syndrome
Systematic review

ABSTRACT

Polycystic ovary syndrome (PCOS) is one of the most prevalent polygenic endocrine disorders in reproductive-age women. Genistein is a soy-isolated phytoestrogen and isoflavone with antioxidant, anti-inflammatory, estrogenic, and antineoplastic activity. This systematic review aimed to investigate the therapeutic effects and mechanisms of actions of genistein in PCOS. The present study was conducted according to the guidelines of the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) protocol. We searched PubMed, Scopus, Embase, and Google Scholar databases up to February 2022 using relative keywords. Studies published in English evaluated genistein's effects on PCOS, and its related symptoms were considered. Out of 298 records screened, only 13 articles met the inclusion criteria: Nine animal and 4 human studies. The results of the current study indicated that genistein supplementation may effectively improve PCOS-related symptoms by decreasing insulin resistance and anthropometric indices, improving ovarian morphology and regulating reproductive hormones, and reducing oxidative stress and inflammation by influencing biological pathways. According to the current literature, genistein may diminish the dues of PCOS. Therefore, this study shows that genistein can be considered an effective agent. in reducing the complications of PCOS. However, further studies are recommended for a broad conclusion on the exact mechanism of genistein in PCOS patients.

1. Introduction

Polycystic ovary syndrome (PCOS) is a prevalent endocrine disorder among women in their reproductive years (Karimi et al., 2022). The prevalence of PCOS is estimated between 5 and 20% worldwide (de Medeiros et al., 2021). PCOS is diagnosed by clinical signs and symptoms such as hirsutism, dysmenorrhea, amenorrhea, oligomenorrhea, androgenic alopecia, hyperandrogenism, severe acne, and infertility (Rosenfield and Ehrmann, 2016). The pathophysiology of PCOS has not been fully understood since it is a complex and multiple disorder that is influenced by various factors, including lifestyle, environment, and genetics (Rosenfield, 2020).

Recently, several hypotheses have been proposed regarding PCOS etiopathogenesis (Fakhoury et al., 2012). A high percentage (55–75%) of women with PCOS have an elevated luteinizing hormone (LH)/follicle-stimulating hormone (FSH) ratio (De Leo et al., 2016). Gonadotropin-releasing hormone stimulation (GnRH) causes those women to produce excessive levels of LH (Barbieri, 2014). Under the influence of the pituitary LH, the ovaries release testosterone (T), androstenedione, and dehydroepiandrosterone (DHEA)(Ullah et al., 2017). Sex hormone binding globulin (SHBG) and albumin are each responsible for binding about 98–99% of the circulating T (Antoniou-Tsigkos et al., 2019; Hammond, 2016). Free testosterone (FT) is converted into dihydrotestosterone (DHT) in target tissues via 5-alpha-reductase enzymes (Ly and Handelsman, 2005). DHT stimulates the production of thicker and longer hair by binding to androgen receptors in dermal papilla cells and the outer root sheath of hair follicles (Azziz et al., 2016; Ceruti et al., 2018). This status may be determined by a higher amplitude or frequency of GnRH (Ye et al., 2021). Hypersecretion of LH in PCOS women may promote early luteinization of granulosa cells and contribute to early growth arrest of antral follicles (Azziz et al., 2016; van der Spuy and Dyer, 2004). LH may also activate premature meiotic processes that

* Corresponding author. Traditional Medicine and Hydrotherapy Research Center, Ardabil University of Medical Sciences, Ardabil, 5166614711, Iran.
** Corresponding author. Traditional Medicine and Hydrotherapy Research Center, Ardabil University of Medical Sciences, Ardabil, 5166614711, POBOX: 14711, Iran.
E-mail addresses: dr.moeeni@yahoo.com (A. Moini Jazani), karimi.ara1990@gmail.com (A. Karimi).

https://doi.org/10.1016/j.ejphar.2022.175275
Received 7 June 2022; Received in revised form 7 September 2022; Accepted 8 September 2022
Available online 13 September 2022

damage oocyte quality and contribute to the formation of embryonic aneuploidies (Sarhan et al., 2017). Also, the excessive number of growing follicles causes the ovary to overproduce anti-Mullerian hormone (AMH), a crucial characteristic of PCOS. (Victoria et al., 2019; Ye et al., 2021). Hyperandrogenism and hyperinsulinemia, which affect each other, lead to metabolic complications such as obesity, dyslipidemia, insulin resistance, increased inflammation, and oxidative stress (Palomba et al., 2015). Inflammatory factors, antioxidant imbalances, and a tendency to insulin resistance in this disease play an important role in causing long-term adverse outcomes such as the increased risk of cardiovascular disease (CVD), and type 2 diabetes (Hyderali and Mala, 2015; Zheng and Li, 2016). Women diagnosed with PCOS are often overweight and obese (Sendur and Yildiz, 2021; Vanky and Løvvik, 2020). PCOS's most common pregnancy complications are gestational diabetes, miscarriage and premature birth, high blood pressure, and preeclampsia (Boomsma et al., 2008). Lately, some human trials have shown an association between metabolic profiles and dietary patterns, oxidative stress biomarkers, and inflammatory factors in women with PCOS(Asemi and Esmaillzadeh, 2015; Hyderali and Mala, 2015; Patisaul et al., 2014). Conventional medical management in PCOS is hormonal contraceptives as the first line of treatment for menstrual irregularities and hirsutism/acne in PCOS. Currently, clomiphene is the first line of infertility treatment (Legro et al., 2013). Metformin is helpful for metabolic/glycemic abnormalities and for improving menstrual irregularities. It can also slow the progression of type 2 diabetes and aid in weight loss but has limited or no benefit in treating hirsutism, acne, or infertility (Harborne et al., 2003). There is no clear answer as to whether weight loss improves PCOS status, but lifestyle interventions are beneficial for overweight/obese people (Legro et al., 2013). Natural compounds and plant derivatives with diverse biological activities can be used as one of the treatment methods along with the primary treatment for reducing PCOS complications (Arentz et al., 2014). The use of soy isoflavones in diseases associated with metabolic syndrome is increasing (Behloul and Wu, 2013). Clinical and animal models' studies show that isoflavones have protective effects on hyperlipidemia, coronary heart disease, chronic renal disease, osteoporosis, menopausal symptoms and cancer (He and Sun, 2016; Javanbakht et al., 2014; Mulvihill and Huff, 2010). Phytoestrogens have a non-steroidal structure and a group of herbal compounds (Bhathena and Velasquez, 2002). Genistein has estrogenic properties and is structurally similar to 17β-estradiol (Fig. 1), an isoflavone found in soy (Thangavel et al., 2019). Genistein pleiotropic activity can be effective in helping treat prostate and breast cancer, wounds, osteoporosis, menopausal syndrome and type 2 diabetes (Ansor; Thangavel et al., 2019; Zaheer and Humayoun Akhtar, 2017). The present systematic review aims to evaluate the effect of genistein on metabolic and hormonal parameters of PCOS. In addition, the knowledge gap has been recommendations for future research have been provided.

2. Materials & methods

The search strategy applied in this systematic review is the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) protocol. Searching for related studies using keywords in PubMed, Google Scholar, SCOPUS, and Embase databases were conducted. Keywords were "genistein" [MESH] OR "Soy isoflavones" [Title/Abstract] OR "soy phytoestrogen" [Title/Abstract] AND "PCOS" [MESH] OR "polycystic ovary syndrome" [MESH] OR "sclerotic ovary syndrome" [Title/Abstract] OR "dysmetabolic syndrome" [Title/Abstract]. There were no limitations on the type of study and the time of publication of articles in the search, and only articles published in English journals up to February 2022 were reviewed. In this study, the PICO criterion was used for systematic review, which includes, Population (P): patient with PCOS, Intervention (I): genistein, Comparison (C): the group as control or administered placebo; and Outcome (O): altered inflammatory, glycemic, oxidative stress, lipid profile, ovarian function hormonal, and anthropometric parameters.

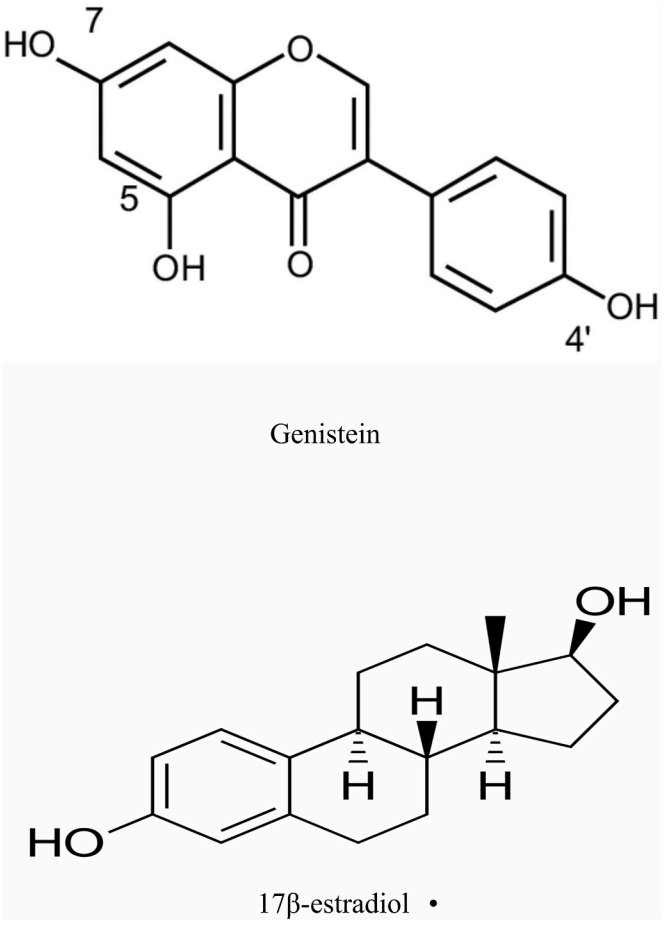


Fig. 1. Structures of genistein and 17β-estradiol.

2.1. Eligibility criteria

The eligibility studies included in this systematic review were as follows: (1) animal studies, (2) all clinical trials, (3) the intervention was genistein supplementation, and (4) it was compared without treatment with genistein or other drugs. Also, articles with insufficient information, review studies, and editorial comments were deleted. After extracting the data and receiving the full text of the articles, the studies were screened independently by the two authors.

2.2. Data extraction

The reviewed articles were saved in EndNote software (Thomson Reuters, Philadelphia, PA, USA). All relevant articles were extracted to screen for articles with a scope appropriate for this study, and duplicates were removed from the study. Two researchers (A.M.J and A.K) independently assessed the full text of the separate articles for eligibility and data extraction. A third author (R.N.D) evaluated the extracted information in terms of accuracy and quality. Any disagreements were referred to the principal investigator and resolved.

2.3. Risk of bias assessment

Two researchers (A.K and A.M.J) assessed the degree of bias for all chosen studies. The Cochrane Risk of Bias (ROB) tool was used to assess the overall bias risk of the randomized and controlled trials. The SYRCLE tool was used to assess the overall bias risk of the animal studies included (Hooijmans et al., 2014). Each domain was given a "high risk"

score if the study contained methodological defects, which might have affected the main findings, a "low risk" score if there was no defect for that domain, and an "unclear risk" score if numerous of the data was not adequate to determine the impact. If the trial had "low risk" for all domains, it was considered a high-quality study with a low risk of bias.

3. Results

The flowchart of the study process is presented in Fig. 2. Finally, after searching the electronic databases, 298 studies were included, 107 duplicate studies were excluded, and 191 studies were screened. Of these, 178 studies did not meet the inclusion criteria. Finally, 13 studies were included in this systematic review. Features and results are summarized in Tables 1 and 2. The present systematic review exhibits the beneficial impacts of genistein on improving the metabolic and hormonal disorders regarding PCOS. Of note, in animal studies, genistein improved the follicular distribution, morphology, and function of ovaries in PCOS (Ma et al., 2021). Almost all selected studies showed that genistein administration decreases the inflammatory cytokines such as tumor necrosis factor-alpha (TNF-α), interleukin (IL-6), and oxidative stress biomarkers, namely malondialdehyde (MDA) and 8-hydroxy-2′-deoxyguanosine (8-OHdG). Based on the current literature, genistein could ameliorate the metabolic disorders regarding lipid profiles, particularly triglyceride (TG), very-low-density lipoprotein (VLDL), and low-density lipoprotein (LDL).

3.1. Physical and chemical property

Genistein is a heterocyclic diphenol with three hydroxyl groups, and its chemical name is 4′,5,7-trihydroxy-isofavonethe or 5,7-dihydroxy-3-(4-hydroxyphenyl)-4H-1-benzopyran-4-one (Yang et al., 2012). Genistein has a very low permeability, so its absorption depends on the permeation of genistein after bacteria have hydrolyzed it. Genistein is a lipophilic compound with a low molecular weight. At 25 °C, it showed low solubility in water (5.3 mM) but high solubility in methanol (32.5 mM)(Ungar et al., 2003; Wu et al., 2010). One of the reasons for genistein's non-linear pharmacokinetic behavior may be its poor aqueous solubility, which may explain why increasing its dose cannot improve its bioavailability through saturation of metabolic enzymes (Zhou et al., 2008). Gensitein's antioxidant activity lasts more than 20 days at pH = 7 and 70 °C and is thermodynamically and chemically stable (Ungar et al., 2003).

3.2. Bioavailability of genistein

Numerous studies indicate genistein is poorly bioavailable, and its plasma or tissue concentrations are comparatively lower than its in vitro IC50, which may impact it's in vivo efficacy (Manach et al., 2005; Setchell et al., 2001). Genistein has favorable absorption properties in the intestine, but due to its poor solubility, larger doses may not be absorbed without proper formulation (Cassidy et al., 2006; Nielsen and Williamson, 2007). Genistein's low oral bioavailability can be mainly

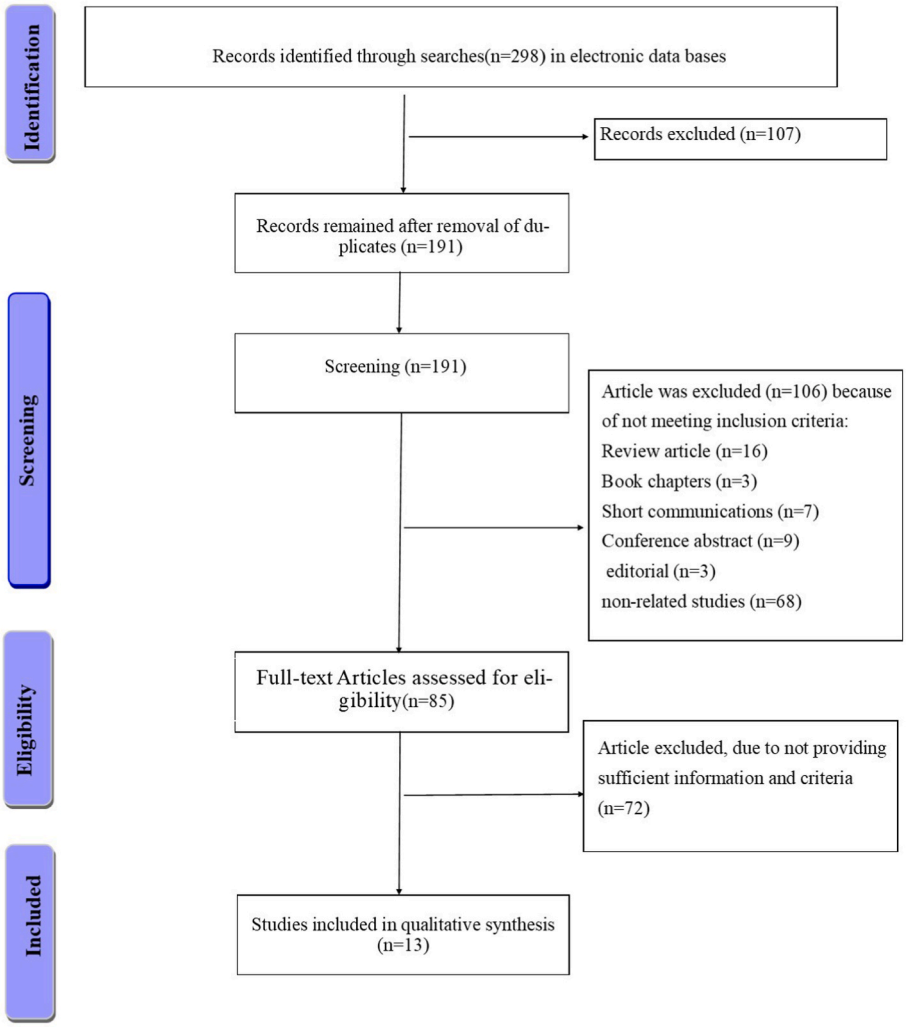


Fig. 2. Flowchart of study.

Table 1
Characteristics of studies investigating the potential roles of genistein on PCOS.

Authors name	Year	Compound	Study design	Number and type of subjects	Dosage and type of administration	Study duration	Route	Main results
Farkhad and Khazali	2019	isoflavone-aglycone	In vivo	Rats (n = 16)	50 and 100 mg/kg	21 days	Oral	Significant increase • TAC Significant decrease • IL-6, TNF-α, TOS, number of corpora luteal and granulosa cellsnumber of cystic follicles and thickness of the theca layer
Luo et al.	2021	Genistein	In vivo	Mice	8.75 mg kg	15 days	I.p	Significant increase • improved the ovarian secretion function, E2, FSH, Cyp17a1 and Cyp19a1, SOD, GPX, GR,CAT, GSH content and the GSH/GSSG ratio, Nrf2, Foxo1 Significant decrease • LH/FSH ratios, T, P4, LH, AMH, Cyp11a1, Hsd3b1, 8-OHdG, MDA
Zhang and Chi	2019	Genistein	In vivo	Rats (n = 5)	5, 10 and 20 mg/kg	-	Oral	Significant decrease • Progesterone, Testosterone, LH, LH/FSH, Average area of FSHR, Ovary wet weight Significant increase: • FSH, Average area of LHR
Ma et al.	2021	Soy isoflavone	In vivo	Rats (n = 7)	100 mg/kg	28 days	I.p	Significant increase • E2, FSH, SOD, GPX, estrous cycle Significant decrease • LH/FSH ratios, T, P4, LH, IL-6, TNF-α, IL-1β, MDA, body weight, ovarian volume and weight,
Amanat et al.	2021	Genistein	In vivo	Rats (n = 7)	20 mg/kg	42 days	oral	Significant decrease • TNF-α,MDA, TG, TC, LDL, Insulin, Glucose, HOMA-IR body and Ovary weight Significant increase • HDL, TAC, SOD
Chi et al.	2018	Genistein	In vivo	Rats (n = 30)	5, 10 and 20 mg/kg	15 days	oral	Significant increase • Bcl-2 Significant decrease • Bax No changes • Ovarian area, Ovarian volume
Rajaei et al.	2019	Genistein	In vivo	Rats (n = 5)	1 mg/kg/day	14 days	oral	Significant decrease • MDA, body and Ovary weight Significant increase • GPX, TAC, SOD No change • Body and Ovary weight
Patisaul et al.	2014	Soy isoflavone	In vivo	Rats (n = 23)	20 ng/ml	28 weeks	oral	• Endocrine active phytoestrogen • Not developmental exposure
Rajan and Balaji	2017	Soy isoflavone	In vivo	Rats (n = 12)	50 and 100 mg/kg	14 days	Oral	Significant increase • GPx, GSH, CAT, SOD Significant decrease • uterine weight, body weight, percentage diestrous phase, testosterone 3b-HSD and 17b-HSD, LPO, NO

Abbreviations: Akt; protein kinase B. AMH; anti-mullerian hormone, AMPK; AMP-activated protein kinase, BAX; BCL-2-associated X-protein, BMI; body mass index, CYP11A1, cytochrome P450 family 11 subfamily A member 1, CYP17A1; cytochrome P450 17A1, CYP21; cytochrome P450c21, CAT; catalase, COX; cyclooxygenase, ELISA; enzyme-linked immunosorbent assay, FBG; fasting blood glucose, FSH; follicle-stimulating hormone, GKG; glucokinase, GPX; glutathione peroxidase, HDL-C; high-density lipoprotein cholesterol, HMGCR; hydroxy-3-methylglutaryl-CoA, HOMA-IR; homeostasis model of assessment-insulin resistance, HSD3B1; hydroxy-delta-5-steroid dehydrogenase, 3 beta- and steroid delta-isomerase 1, ICP-OES; inductively coupled plasma optical emission spectrometry, IGF1; insulin like growth factor 1, IL; interleukin, IR; insulin resistance, LDL-C; low-density lipoprotein cholesterol, LH; luteinizing hormone, MDA; malondialdehyde, NF-Kb; nuclear factor-*κB*, PCOS; polycystic ovarysyndrome, PCR; Polymerase chain reaction, PI3K; phosphatidylinositol 3-kinase, SOD; super oxide dismutase, TAC; total antioxidant capacity, TGF-β; transforming growth factor β, TNF-α; tumor necrosis factor α, TG; triglycerides. TC; total cholesterol, VLDL; very low-density lipoprotein, VEGF; vascular endothelial growth factor, VEGF; vascular endothelial growth factor.

attributed to extensive metabolism, but high expression levels of efflux transporters may be responsible for its low oral bioavailability (Yang et al., 2012). A single oral dose of 50 mg of genistein resulted in 3.7% genistein aglycone uptake in the first 2 h and 1.6% at a steady state in women (Busby et al., 2002; Setchell et al., 2001). Additionally, Gu et al. discovered that genistein aglycone accounted for only 1% of the total genistein in plasma after consuming a soy beverage (Gu et al., 2006). In human urine, genistein aglycone was less than 1%. Genistein distribution and elimination highly depend on coupling metabolic enzymes and efflux transporters (Fischer et al., 2004). It enables enteric and enterohepatic recycling, remarkably lessens the genistein's exposure level, and prolongs its residence time in vivo (Guo et al., 2020). Due to dual recycling mechanisms in GI tracts and livers, genistein and its conjugates are mainly accumulated there, while their concentration in reproductive organs is less than in plasma (Yang et al., 2012). Therefore several factors, including metabolic enzymes and efflux transporters, have contributed to genistein's low oral bioavailability (Yang et al., 2012).

3.3. Genistein metabolism

The metabolism of genistein is diverse and extensive as it undergoes oxidation, reduction, and conjugation in vivo (Chang and Nair, 1995;

Table 2
Summary of the human studies on the effect of genistein on PCOS.

Reference	Year	Number and type of subjects	Sample Size	Age	Dosage and type of administration	sources	Study duration	Maine outcome
Jamilian and Asemi	2016	Human with pcos (n = 35)	70	18–40	50 mg/d	Iran	12 weeks	Significant decrease: Insulin, insulin resistance, MDA, NO, TC, VLDL, QUICKI, HOMA-B, HOMA-A significant increase insulin sensitivity, GSH, TAC, SHBG, Unsignificant change: hs-CRP, LDL,HDL,TG, DHEAS, BMI,WC,HC
Khani et al.	2011	Human with pcos (n = 68)	146	18–32	36 mg/day	Iran	3 months	Significant decrease: LH,TG, LDL, Testosterone Unsignificant change: HDL, FSH, DHEAS
Romualdi et al.	2008	Human with pcos (n = 12)	24	18–32	36 mg/d	Italy	6 months	Significant decrease: LH,TC,LDL, Testosterone, DHEAS Unsignificant change: BMI, TC, FSH, LH, TG,HDL.VLDL, NEFA
Haudum et al.	2020	Human with pcos (n = 24)	44	18–32	soy milk	Iran	3 days	Significant increase: HDL, FSH Significant decrease: HOMA-IR, Insulin,TG, LH, SHBG, AMH, Total testosterone, DHEA, Hirsutism, Oligo-/amenorrhea Unsignificant change: DHT,LDL, DHEAS, BMI

Abbreviations AMH; anti-mullerian hormone, BMI; body mass index, CYP11A1, cytochrome P450 family 11 subfamily A member 1, cytochrome P450c21, CAT; catalase, COX;, FBG; fasting blood glucose, FSH; follicle-stimulating hormone, GKG; glucokinase, GPX; glutathione peroxidase, HDL-C; high-density lipoprotein cholesterol HOMA-IR; homeostasis model of assessment-insulin resistance, interleukin, IR; insulin resistance,LDL-C; low-density lipoprotein cholesterol, LH; luteinizing hormone,MDA; malondialdehyde, NF-Kb; nuclear factor-κB,PCOS; polycystic ovarysyndrome, PCR; Polymerase chain reaction, SOD; super oxide dismutase, TAC; total antioxidant capacity,TNF-α; tumor necrosis factor α, TG; triglycerides. TC; total cholesterol, VLDL; very low-density lipoprotein.

Kulling et al., 2001). Genistein is metabolized via glucuronidation and sulfation, with limited Cytochromes P450 (CYPs) activity (Doerge et al., 2000). The glucuronidation levels in humans are much greater than those of sulfates or aglycones. Genistein has an extremely high metabolism rate in the intestine and liver (Yang et al., 2012). It has been shown that enterocytes extensively metabolize genistein because of the high activity of UDP-glucuronosyltransferases (UGTs) and sulfotransferases (SULTs) in the intestines (Riches et al., 2009; Strassburg et al., 1998). After entering the portal vein, the remaining genistein aglycone can be further separated by the liver and undergo hepatic metabolism (Yang et al., 2010). There is also evidence of UGT and SULT expression in several other tissues, namely kidney, heart, and lung, which could be able to metabolize genistein in the organs (Kurkela et al., 2003; Riches et al., 2009; Strassburg et al., 1998).

3.4. Beneficial effects of genistein on PCOS and its related disorders

The main mechanisms of function of genistein in PCOS and its associated complications are explained in 3 sections: inflammation and oxidative stress, reproductive hormones, and metabolic profile.

3.5. Inflammation and oxidative stress

It has been revealed that inflammatory agents like TNF-a and IL-6 play a vital role in the pathogenesis of PCOS (Hong et al., 2016), both of which are elevated in the follicular fluid and the blood of patients with PCOS (Fulghesu et al., 2011; Gao et al., 2016) via causing ovarian hyperstimulation, hypothalamic-pituitary-ovarian dysfunction, and anovulation (Ebejer and Calleja-Agius, 2013; Qiao and Feng, 2011). TNF-a could be associated with enhancing ovarian volume, cortex thickness, and hyperandrogenism by boosting the proliferation of the internal theca cells (Hong et al., 2016). Of note, PCOS could promote the overexpression of TNF-a by recruiting leukocytes into the ovary, which in turn causes apoptosis in granulosa cells of the antral follicles, consequently worsening PCOS. As an anti-inflammatory isoflavonoid, genistein has been shown to have protective effects on the reproductive system (Fanti et al., 2006). According to the existing data, genistein decrease inflammation and oxidative stress by prohibiting the production of TNF-a, IL- IL-1β, IL-12, and IL-6 via decreasing the expression of nuclear factor kappa B (NF-κB) pathway and adenosine monophosphate-activated protein kinase (MAPK) pathway (Danciu et al., 2017; Yu et al., 2016). Ma et al. (2021) found that soy isoflavones (100 mg/kg) significantly decreased levels of IL-6, TNF-α, IL-1β, and malondialdehyde (MDA) and increased superoxide dismutase (SOD) and glutathione peroxidase (GPx) after 28 days of therapy in PCOS model rats. Also, another study conducted by Farkhad and Khazali (2019) revealed that genistein, an abundant isoflavonoid in soybeans, can exhibit phytoestrogen-like properties and impact inflammatory and oxidative factors in PCOS rats. An in vivo study examined the effects of 50 mg and 100 mg genistein on 16 rats for 21 days. The findings were in line with previous studies, which suggested the beneficial impacts of genistein through decreasing inflammatory cytokines levels IL-6, TNF-α, total oxidative status (TOS) levels, and increasing total antioxidant capacity (TAC) level (Farkhad and Khazali, 2019).

An imbalance between the antioxidative capacity and the overproduction of the reactive oxygen species (ROS), which occurs in PCOS (Murri et al., 2013), causes oxidative stress (OS), which could be a potential factor in PCOS pathogenesis (Zuo et al., 2016). It has been suggested that genistein can reduce the levels of MDA and 8-OHdG as two main biomarkers of OS (Khan et al., 2018; Yu et al., 2019). On the other hand, genistein could promote the function of oxidant scavenging enzymes, including glutathione reductase (GR), SOD, catalase (CAT), and GSH-Px (Luo et al., 2021). Genistein could also improve the activity of GSH as an electron donor, which then leads to the activation of GSH-Px (Luo et al., 2021; Murri et al., 2013). In a clinical trial, Jamilian and Asemi (2016) showed that oral supplementation with 50 mg/day of genistein for 12 weeks significantly decreased MDA and nitric oxide (NO) serum levels in 35 PCOS patients. Moreover, genistein supplementation had not significantly decreased the serum level of high-sensitivity C-reactive protein (hs-CRP) in PCOS patients. In another study, Rajaei et al. (2019) revealed that administration of 1 mg/kg/day of genistein for 14 days increased the levels of GPX, TAC, and SOD and reduced the MDA levels in PCOS rats. Another possible mechanism of action of genistein in suppressing ROS is reversing the mitochondrial dysfunction via, at least in part, estrogen receptors (ER). Genistein also enhances the expression and translocation of nuclear factor erythroid

2–related factor 2 (Nrf2) via ER, which might improve the ovarian secretion of estrogen ($E_{2)}$ and pregestrone ($P_{4)}$, and mitochondrial function (Luo et al., 2021). The increased Nrf2 levels could then regulate the expression of several genes via binding to the antioxidant response element (ARE) regions like forkhead box protein O1 (Foxo 1) (Esfandyari et al., 2021; Wang et al., 2021). In an in vivo study on mice, administration of genistein for 15 days significantly decreased MDA and OHdG levels. Also, it increased the levels of E2, Nrf2, forkhead box protein O1 (Foxo1), antioxidant enzymes, namely catalase (CAT), SOD, glutathione reductase (GR), and the GSH/oxidized glutathione (GSSG) ratio. Luo et al. (2021) reported that administration of 8.75 mg/kg/day of genistein for 15 days significantly decreased levels of cytochrome P450 (Cyp)11a1 extracted from peripheral blood mononuclear cell (PBMC) and MDA extracted from serum and remarkably increased levels of SOD, GPx and GSH content extracted from whole blood and nuclear factor-erythroid factor 2-related factor 2 (Nrf2) and forkhead box protein O1 (Foxo1) extracted from PBMC in PCOS mice. Similarly, Amanat et al. (2021) examined the effects of genistein on inflammatory and oxidative stress factors in PCOS rats. The authors explained that genistein inhibited the production of TNF-α and MDA in serum and increased the production of TAC and SOD (extracted from whole blood) in PCOS rats. One of the prevalent features of PCOS is apoptosis, in which Bax and Bcl-2 signaling pathway is involved (Chi et al., 2018). Genistein could reduce apoptosis in ovarian granulosa cells by increasing the mRNA expression of Bcl-2 gene extracted from PBMC, resulting in decreased Bax protein level (Chi et al., 2018).

3.6. Reproductive hormones and ovarian function

One of the complications of PCOS is the decrease in the efficiency of different parts of the ovary, which can significantly affect reproductive hormones. In a study by Farkhad and Khazali (2019), cell granulosa cells, the number of corpora luteal, the thickness of the theca layer, and the number of cystic follicles were statistically considerably decreased after 21 days of oral genistein intervention (50 and 100 mg/kg) in PCOS rats. Another study by Chi et al. (2018) showed no significant effect on the ovarian area and ovarian volume after 15 days of oral administration of genistein (5, 10, and 20 mg/kg) in PCOS rats. One of the main features of PCOS is high levels of AMH, which could, in turn, suppress the function of FSH and ameliorate follicles' sensitivity to FSH(Teede et al., 2019). Moreover, LH/FSH ratio and LH levels increase in patients with PCOS. CYPs and hydroxysteroid dehydrogenases (HSD) are the two main classes of enzymes involved in steroid hormone synthesis (Ye et al., 2021). Several enzymes are involved in steroidogenesis, including aromatase, an enzyme that belongs to the CYPs (The first class of steroidogenic enzymes) family, involved in the conversion of steroids. Aromatase deficiency results in a defect in the pathway that stops the conversion of androgens to estrogens (Burger, 2002; Chaudhary et al., 2021). An important component of the androgen synthesis pathway is CYPs (CYP1A1, CYP11B2, CYP21A2, CYP11A1, CYP17A1, CYP19A1, and CYP3A7), which are dysfunctional in PCOS (Ashraf et al., 2019; Walters and Handelsman, 2018). PCOS progression is increased by any abnormality in Cytochrome P450. The second class of steroidogenic enzymes known as alcohol oxydoreductases catalyzes the dehydrogenation of hydroxysteroids (Chaudhary et al., 2021). The NIC adenine dinucleotide dioxydoreductases can accept and donate electrons by acting as oxydoreductases Rosenfield and Ehrmann (2016). HSDs are classified into four classes, namely, 3β-, 11β-, 17β-, and 20α-HSDs (Burger, 2002). The 3β-HSDs catalyze the conversion of pregnenolone, 7α-hydroxypregnenolone, and dehydroepiandrosterone into the respective androstenedione, progesterone, and 17α-hydroxyprogesterone. 11β-HSDs catalyze the interconversion between active cortisol and inactive cortisone (Rosenfield and Ehrmann, 2016). 17β-HSDs catalyze the interconversion between 17β-hydroxyl steroids and 17-ketoandrogens and estrogens. 20α-HSDs catalyze the conversion of progesterone into 20α-hydroxyprogesterone (Walters et al., 2008).

Genistein supplementation has been shown to correct LH/FSH ratio and the aberrant LH, FSH, and AMH secretions by regulating, Cyp17a1, hydroxy-delta-5-steroid dehydrogenase, 3 beta- and steroid delta-isomerase 1 (Hsd3b1), Cyp19a1, and Cyp11a1 via binding to the estrogen receptor (ER) (Miller, 2017). Romualdi et al. (2008) have found that oral genistein supplementation (36 mg/d) for six months in PCOS patients significantly decreased the serum levels of testosterone, while there were no alterations in FSH and LH serum levels. Another study by Khani et al. (2011) demonstrated that genistein supplementation at 36 mg/day significantly decreased testosterone serum levels in women with PCOS. However, its effect on FSH and DHEAS was marginal. In an animal study, Luo et al. (2021) found that administration of genistein (8.75 mg kg) for 15 days led to a significant reduction in serum levels of LH/FSH ratios, testosterone (T), progesterone (P4), LH, AMH and considerably increased serum levels of ovarian secretion function, E2, and FSH in PCOS model rats. In line with these findings, genistein could reduce testosterone levels (T) in PCOS women (Khani et al., 2011). One of the proposed mechanisms of function of genistein in the modulation of estrogen formation and reproductive endocrine disorders is by decreasing the expression of follicle-stimulating hormone receptor (FSHR) and increasing the expression of the luteinizing hormone receptor (LHR) (Zhang and Chi, 2019). In an in vivo study, Zhang and Chi (2019) indicated that oral administration of genistein with different doses (5, 10, and 15 mg/kg) for 15 days significantly decreased serum levels of progesterone, testosterone, LH, and LH/FSH and noticeably increased serum levels of FSH in PCOS model rat. They also reported an increase in the average area of LH receptor (LHR) and a decrease in the average area of FSH receptor (FSHR) and ovary wet weight. Consistent with the results of Zhang and Chi (2019), Ma et al. (2021) have investigated the effects of soy isoflavones in PCOS rats. This study supplemented PCOS rats with soy isoflavones for 28 days. The findings revealed that genistein significantly reduced serum levels of E2, FSH, and estrous cycle and statistically increased LH/FSH ratios, T, P4, LH, and ovarian volume. Another main mechanism in which genistein modulates reproductive hormones is through sex hormone-binding globulin (SHBG). Genistein increases the levels of SHGB via either enhancing the expression of mRNA levels of SHBG or its production (Low et al., 2007; Mousavi and Adlercreutz, 1993). The binding of genistein to the estrogen receptors leads to the suppression of the expression and activity of steroidogenic enzymes like 3b-HSD, p450 aromatase, and 17β-HSD, which consequently results in decreased levels of T (Lacey et al., 2005). Rajan and Balaji (2017) reported that administration of 50 and 100 mg/kg of genistein for 14 days significantly decreased uterine weight, percentage of diestrous phase, testosterone (in serum), and 3b-hydroxysteroid dehydrogenase (3b-HSD), 17b-HSD (extracted from PBMC) in PCOS rats.

3.7. Genistein and energy hemostasis

Based on epidemiological data, obesity and PCOS have an intense bidirectional relationship. In PCOS, weight gain occurs mainly due to the accumulation of fat, which is induced by hyperandrogenism, insulin resistance (IR), and hepatic lipogenesis (Sam, 2007). Obesity worsens the functions of the insulin resistance and reproductive system as the leading cause of PCOS by affecting the insulin pathways after the PI3-kinase receptor kinase and intact mitogen-activated protein (MAP) (Barber et al., 2006; Cusi et al., 2000). A successful weight loss program can improve metabolic health, insulin sensitivity, and hyperandrogenic features in obese and overweight women with PCOS (Barber et al., 2006; Holte et al., 1995). Amanat et al. (2021) demonstrated that genistein administration at a dose of 20 mg/kg body weight (b.w) for 42 days reduced body weight in PCOS rats. In another study, Ma et al. (2021) reported that genistein treatment at a dose of 100 mg/kg b.w for 28 days decreased statistically significantly in the b.w of PCOS rats. However, unlike animal studies, human studies showed that genistein did not significantly affect weight loss in women with PCOS. Jamilian and

Asemi (2016) did not observe significant changes in weight changes, waist circumstance (WC), body mass index (BMI), and hip circumference (HC) in overweight and obese women after the administration of 50 mg per day for 12 weeks. Moreover, a study by Romualdi et al. (2008) found that 36 mg/d genistein supplementation for six months did not cause a change in weight in patients with PCOS. These different results in the human study may be due to different types of genistein, different duration of the study, and amount of dosage.

3.8. Metabolic profile

3.8.1. Genistein and glucose metabolism

PCOS is commonly associated with insulin resistance as a reproductive hormonal abnormality. Several clinical studies have suggested that PCOS is associated with a higher risk of Type2 diabetes and impaired glucose tolerance. The origin of insulin resistance in PCOS and its related mechanisms are still not fully elucidated (Ouadda et al., 2009). Genistein increases insulin secretion through pancreatic islet cells (Nagaraju et al., 2013). Genistein also decreases IR by decreasing body weight, which leads to enhanced organ sensitivity to hormones. It has been suggested that genistein could ameliorate IR by reducing the levels of fasting insulin via acting in the phosphatidylinositol 3-kinase, protein kinase C, and 5′-adenosine monophosphate-activated protein kinase (AMPK) pathways (Choi et al., 2012; Ha et al., 2012). Of note, genistein has exerted regulatory effects on glucose metabolism, mainly through the increase in expression of glucose transporter type 4 (GLUT 4) (Ha et al., 2012). Jamilian and Asemi (2016), in a randomized clinical trial (RCT) study, researched the effects of genistein on metabolic status in patients with PCOS. Seventy PCOS patients contributed to this study. Thirteen-five subjects were given a placebo (group 1), and 35 subjects received 50mg/daily of soy isoflavones for 12 weeks. The researchers reported that genistein significantly decreased serum levels of quantitative insulin-sensitivity check index (QUICKI), homeostasis model assessment (HOMA)-A and B, and increased insulin sensitivity. In another study performed by Haudum et al. (2020), they demonstrated that supplementation of soy milk for three days (soya base 92% (water, soya beans 5,8%), sugar 0,6%, salt, stabilizers: gellan gum, carrageenan; flavoring) in PCOS patients significantly decreased serum levels of HOMA-IR and insulin. In an in vivo study, Choi et al. administrated genistein at a concentration of unit to 0.1 to mice for one month. The results showed that genistein reduced insulin resistance. It also inhibited the oxidation and synthesis of fatty acids (Choi et al., 2012). Also, Amanat et al. (2021) reported statistically considerable effects of 20 mg/kg of oral administration of genistein for 42 days on insulin, glucose, and homeostasis model assessment-estimated insulin resistance (HOMA-IR) levels in PCOS model rats.

3.8.2. Genistein and lipid metabolism

Women with PCOS usually have dyslipidemia, high TG, total cholesterol (TC), LDL, and low high-density lipoprotein (HDL) levels. Even so, many PCOS patients may still have normal lipid profiles. As a consequence of insulin resistance, catecholamine-induced lipolysis causes fatty acids to be released into the bloodstream from adipocytes. Very-low-density lipoprotein (VLDL) particles are assembled due to the liver's elevated free fatty acid flux, which results in the increased secretion of VLDL into the bloodstream, leading to hypertriglyceridemia. Thus, potential PCOS dyslipidemia treatments should target the mechanisms mentioned above. Another main complication of PCOS is dyslipidemia (Amanat et al., 2021). Genistein increases peroxisome proliferator-activated receptor (PPAR) alpha/gamma expression, two genes for lipid homeostasis such as the cluster of differentiation 36 (CD36) and lipoprotein lipase (LPL) in 3T3-L1 adipocytes, which improves lipid profile (Lee et al., 2021). In addition, genistein activates AMPK, which induces malonyl-CoA-dehydrogenase activation. Also, it represses acetyl-CoA-carboxylase in lipid metabolism, leading to a decline in fatty acid biosynthesis and an increase in beta-oxidation (Guevara-Cruz et al., 2020; Liu et al., 2017). Genistein can decrease the levels of LDL, which relies on the activation of peroxisome proliferator-activated receptor-alpha, which in turn leads to the decrease of sterol regulatory element-binding protein 2 (SREBP-2) expression and β-Hydroxy β-methylglutaryl-CoA (HMG-CoA) reductase activity (Kim et al., 2004). The lipid profile-lowering property of genistein, particularly TG and VLDL levels, might be due to its effect on decreasing the conversion of glucose into lipids, lipid synthesis, and increasing lipolysis (Jiang et al., 2015; Kim et al., 2004). For instance, Jamilian and Asemi (2016)revealed that genistein supplementation in 35 PCOS women for 12 weeks significantly decreased TG and VLDL serum levels. Contrary to the study of Jamalian et al., Romualdi et al. (2008) have found that oral supplementation with 36 mg/d genistein for six months in PCOS patients did no significant change in serum levels of HDL, TG, and VLDL in the genistein group compared to the control group. Also, Khani et al. (2011) found that treatment with genistein for three months on 68 PCOS women significantly changed the serum LDL level. However, it did not change the levels of HDL-C in PCOS patients. In another, in vivo study, Amanat et al. (2021) demonstrated that genistein administration at a dose of 20 mg/kg b.w for 42 days reduced serum levels of TG, TC, LDL, and increased HDL in PCOS rats.

3.9. Risk of bias assessment

All in vivo studies were assessed for risk of bias using SYRCLE's tool. The qualitative assessment indicated that most studies were rated as low risk of bias for the sequence generation category, selective outcome reporting, group similarities at baseline category, and other sources of bias category. Methods of allocation concealment were adequately reported in 66% of the included studies. In most of these studies, random outcome assessment and blinding of outcome assessor randomization in animal housing and blinding data were not reported (Fig. 3). The random sequence generation, allocation concealment, incomplete outcome data, and selective reporting were described adequately in this study. However, in three studies blinding of participants and researchers and blinding of outcome assessment were unclear (Fig. 4).

4. Discussion

To the best of our knowledge, the present study systematically reviewed the effects of genistein supplementation on PCOS for the first time. One of the study's main strengths is the sufficient sample size of the selected studies and the decent duration of interventions. Despite the strengths, there are some limitations, one of which is related to the heterogeneity of the studies, namely differences in genistein dosage and intervention duration. Of note, there was not enough information regarding the bioavailability of genistein. One of the main complications in PCOS patients is insulin resistance. Very few studies examined the potential effects of genistein on insulin levels and metabolic disorders. Furthermore, there is little data regarding the side effects of genistein, which are required to be mentioned in future investigations. Generally speaking, the selected studies demonstrated the beneficial effects of genistein on PCOS and its associated complications, yet the exact mechanisms have remained unclear. Thus, more studies are needed in the future to come up with a precise conclusion on the impacts of this natural compound on PCOS.

5. Conclusion

According to the results of this systematic study, genistein and isoflavones soy supplementation can decrease oxidative stress indices and inflammatory factors and increase antioxidant enzymes. However, due to the effect of several factors on weight and BMI, the results related to the effect of genistein on anthropometric indices in patients with PCOS were inconsistent. Genistein also has promising results on hormonal status indices, lipid, and glycemic profiles, but more studies are needed

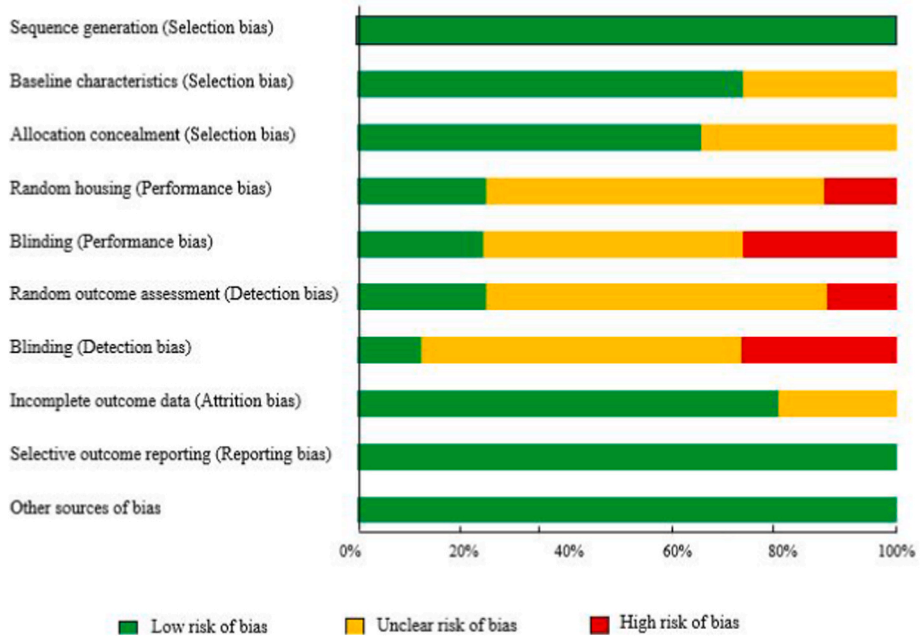


Fig. 3. Animal of assess of bias.

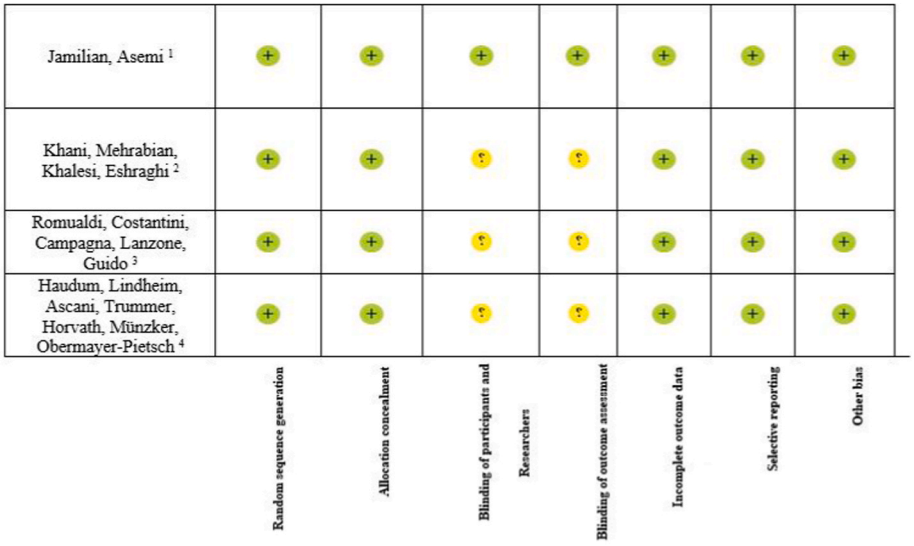


Fig. 4. Human assess of bias.

to determine this effect more accurately.

Author contributions

The authors' responsibilities were as follows: R.N.D, A.K, and S.P.R wrote the original paper; A.M.J contributed to data collection; provided advice and consultation and Contributed to the finalrevision of the manuscript. All authors read and approved the finalversion of the manuscript.

Availability of supporting data

Data that support the outcomes of this review are accessible upon request.

Declaration of competing interest

The authors declare that there are no conflicts of interest that may influence the results.

Data availability

The data that has been used is confidential.

Acknowledgments

The authors would like to thank the Ardabil University of Medical Sciences for their support

References

Amanat, S., Ashkar, F., Eftekhari, M.H., Tanideh, N., Doaei, S., Gholamalizadeh, M., Koohpeyma, F., Mokhtari, M., 2021. The effect of genistein on insulin resistance, inflammatory factors, lipid profile, and histopathologic indices in rats with polycystic ovary syndrome. Clin. Exp. Reproduct. Med. 48, 236.
Ansor, N.M., Wound Healing Properties of Phytoestrogens: in Vitro and in Vivo Studies.
Antoniou-Tsigkos, A., Zapanti, E., Ghizzoni, L., Mastorakos, G., 2019. Adrenal Androgens (Endotext [Internet]).
Arentz, S., Smith, C.A., Abbott, J.A., Bensoussan, A., 2014. A survey of the use of complementary medicine by a self-selected community group of Australian women with polycystic ovary syndrome. BMC Compl. Alternative Med. 14, 472.
Asemi, Z., Esmaillzadeh, A., 2015. DASH diet, insulin resistance, and serum hs-CRP in polycystic ovary syndrome: a randomized controlled clinical trial. Horm. Metab. Res. 47, 232–238.
Ashraf, S., Nabi, M., Rashid, F., Amin, S., 2019. Hyperandrogenism in polycystic ovarian syndrome and role of CYP gene variants: a review. Egypt. J. Med. Human Genet. 20, 1–10.
Azziz, R., Carmina, E., Chen, Z., Dunaif, A., Laven, J.S., Legro, R.S., Lizneva, D., Natterson-Horowtiz, B., Teede, H.J., Yildiz, B.O., 2016. Polycystic ovary syndrome. Nat. Rev. Dis. Prim. 2, 1–18.
Barber, T.M., McCarthy, M.I., Wass, J.A., Franks, S., 2006. Obesity and polycystic ovary syndrome. Clin. Endocrinol. 65, 137–145.
Barbieri, R.L., 2014. The Endocrinology of the Menstrual Cycle, Human Fertility. Springer, pp. 145–169.
Behloul, N., Wu, G., 2013. Genistein: a promising therapeutic agent for obesity and diabetes treatment. Eur. J. Pharmacol. 698, 31–38.
Bhathena, S.J., Velasquez, M.T., 2002. Beneficial role of dietary phytoestrogens in obesity and diabetes. Am. J. Clin. Nutr. 76, 1191–1201.
Boomsma, C.M., Fauser, B.C., Macklon, N.S., 2008. Pregnancy Complications in Women with Polycystic Ovary Syndrome, Seminars in Reproductive Medicine. © Thieme Medical Publishers, 072-084.
Burger, H.G., 2002. Androgen production in women. Fertil. Steril. 77, 3–5.
Busby, M.G., Jeffcoat, A.R., Bloedon, L.T., Koch, M.A., Black, T., Dix, K.J., Heizer, W.D., Thomas, B.F., Hill, J.M., Crowell, J.A., 2002. Clinical characteristics and pharmacokinetics of purified soy isoflavones: single-dose administration to healthy men. Am. J. Clin. Nutr. 75, 126–136.
Cassidy, A., Brown, J.E., Hawdon, A., Faughnan, M.S., King, L.J., Millward, J., Zimmer-Nechemias, L., Wolfe, B., Setchell, K.D., 2006. Factors affecting the bioavailability of soy isoflavones in humans after ingestion of physiologically relevant levels from different soy foods. J. Nutr. 136, 45–51.
Ceruti, J.M., Leirós, G.J., Balañá, M.E., 2018. Androgens and androgen receptor action in skin and hair follicles. Mol. Cell. Endocrinol. 465, 122–133.
Chang, Y.-C., Nair, M.G., 1995. Metabolism of daidzein and genistein by intestinal bacteria. J. Nat. Prod. 58, 1892–1896.
Chaudhary, H., Patel, J., Jain, N.K., Joshi, R., 2021. The role of polymorphism in various potential genes on polycystic ovary syndrome susceptibility and pathogenesis. J. Ovarian Res. 14, 1–21.
Chi, X.-X., Zhang, T., Chu, X.-L., Zhen, J.-L., Zhang, D.-J., 2018. The regulatory effect of Genistein on granulosa cell in ovary of rat with PCOS through Bcl-2 and Bax signaling pathways. J. Vet. Med. Sci. 80, 1348–1355.
Choi, J.S., Koh, I.-U., Song, J., 2012. Genistein reduced insulin resistance index through modulating lipid metabolism in ovariectomized rats. Nutr. Res. 32, 844–855.
Cusi, K., Maezono, K., Osman, A., Pendergrass, M., Patti, M.E., Pratipanawatr, T., DeFronzo, R.A., Kahn, C.R., Mandarino, L.J., 2000. Insulin resistance differentially affects the PI 3-kinase- and MAP kinase-mediated signaling in human muscle. J. Clin. Invest. 105, 311–320.
Danciu, C., Antal, D.S., Ardelean, F., Chiş, A.R., Şoica, C., Andrica, F., Dehelean, C., 2017. New Insights Regarding the Potential Health Benefits of Isoflavones. Flavonoids-From Biosynthesis to Human Health. Intech, Rijeka, Croatia, pp. 257–286.
De Leo, V., Musacchio, M., Cappelli, V., Massaro, M., Morgante, G., Petraglia, F., 2016. Genetic, hormonal and metabolic aspects of PCOS: an update. Reprod. Biol. Endocrinol. 14, 1–17.
de Medeiros, S.F., de Medeiros, M.A.S., Barbosa, B.B., Yamamoto, M.M.W., Maciel, G.A. R., 2021. Comparison of metabolic and obesity biomarkers between adolescent and adult women with polycystic ovary syndrome. Arch. Gynecol. Obstet. 303, 739–749.
Doerge, D.R., Chang, H.C., Churchwell, M.I., Holder, C.L., 2000. Analysis of soy isoflavone conjugation in vitro and in human blood using liquid chromatography-mass spectrometry. Drug Metabol. Dispos. 28, 298–307.
Ebejer, K., Calleja-Agius, J., 2013. The role of cytokines in polycystic ovarian syndrome. Gynecol. Endocrinol. 29, 536–540.
Esfandyari, S., Aleyasin, A., Noroozi, Z., Taheri, M., Khodarahmian, M., Eslami, M., Rashidi, Z., Amidi, F., 2021. The protective effect of sulforaphane against oxidative stress through activation of Nrf2/ARE pathway in human granulosa cells. Cell J. (Yakhteh) 23, 692.
Fakhoury, H., Tamim, H., Ferwana, M., Siddiqui, I.A., Adham, M., Tamimi, W., 2012. Age and BMI adjusted comparison of reproductive hormones in PCOS. J. Fam. Med. Prim. Care 1, 132.
Fanti, P., Asmis, R., Stephenson, T.J., Sawaya, B.P., Franke, A.A., 2006. Positive effect of dietary soy in ESRD patients with systemic inflammation—correlation between blood levels of the soy isoflavones and the acute-phase reactants. Nephrol. Dial. Transplant. 21, 2239–2246.
Farkhad, S.A., Khazali, H., 2019. Therapeutic effects of isoflavone-aglycone fraction from soybean (Glycine max L. Merrill) in rats with estradiol valerate-induced polycystic ovary syndrome as an inflammatory state. Gynecol. Endocrinol. 35 (12), 1078–1083.
Fischer, L., Mahoney, C., Jeffcoat, A.R., Koch, M.A., Thomas, B.F., Valentine, J.L., Stinchcombe, T., Boan, J., Crowell, J.A., Zeisel, S.H., 2004. Clinical characteristics and pharmacokinetics of purified soy isoflavones: multiple-dose administration to men with prostate neoplasia. Nutr. Cancer 48, 160–170.
Fulghesu, A.M., Sanna, F., Uda, S., Magnini, R., Portoghese, E., Batetta, B., 2011. IL-6 serum levels and production is related to an altered immune response in polycystic ovary syndrome girls with insulin resistance. Mediat. Inflamm. 2011.
Gao, L., Gu, Y., Yin, X., 2016. High serum tumor necrosis factor-alpha levels in women with polycystic ovary syndrome: a meta-analysis. PLoS One 11, e0164021.
Gu, L., House, S.E., Prior, R.L., Fang, N., Ronis, M.J., Clarkson, T.B., Wilson, M.E., Badger, T.M., 2006. Metabolic phenotype of isoflavones differ among female rats, pigs, monkeys, and women. J. Nutr. 136, 1215–1221.
Guevara-Cruz, M., Godinez-Salas, E.T., Sanchez-Tapia, M., Torres-Villalobos, G., Pichardo-Ontiveros, E., Guizar-Heredia, R., Arteaga-Sanchez, L., Gamba, G., Mojica-Espinosa, R., Schcolnik-Cabrera, A., 2020. Genistein stimulates insulin sensitivity through gut microbiota reshaping and skeletal muscle AMPK activation in obese subjects. BMJ Open Diabet. Res. Care 8, e000948.
Guo, T.L., Chen, Y., Xu, H.S., McDonough, C.M., Huang, G., 2020. Gut microbiome in neuroendocrine and neuroimmune interactions: the case of genistein. Toxicol. Appl. Pharmacol. 402, 115130.
Ha, B.G., Nagaoka, M., Yonezawa, T., Tanabe, R., Woo, J.T., Kato, H., Chung, U.-I., Yagasaki, K., 2012. Regulatory mechanism for the stimulatory action of genistein on glucose uptake in vitro and in vivo. J. Nutr. Biochem. 23, 501–509.
Hammond, G.L., 2016. Plasma steroid-binding proteins: primary gatekeepers of steroid hormone action. J. Endocrinol. 230, R13–R25.
Harborne, L., Fleming, R., Lyall, H., Sattar, N., Norman, J., 2003. Metformin or antiandrogen in the treatment of hirsutism in polycystic ovary syndrome. J. Clin. Endocrinol. Metab. 88, 4116–4123.
Haudum, C., Lindheim, L., Ascani, A., Trummer, C., Horvath, A., Münzker, J., Obermayer-Pietsch, B., 2020. Impact of short-term isoflavone intervention in polycystic ovary syndrome (PCOS) patients on microbiota composition and metagenomics. Nutrients 12, 1622.
He, X., Sun, L.-m., 2016. Dietary intake of flavonoid subclasses and risk of colorectal cancer: evidence from population studies. Oncotarget 7, 26617.
Holte, J., Bergh, T., Berne, C., Wide, L., Lithell, H., 1995. Restored insulin sensitivity but persistently increased early insulin secretion after weight loss in obese women with polycystic ovary syndrome. J. Clin. Endocrinol. Metab. 80, 2586–2593.
Hong, L., Zhang, Y., Wang, Q., Han, Y., Teng, X., 2016. Effects of interleukin 6 and tumor necrosis factor-α on the proliferation of porcine theca interna cells: possible role of these cytokines in the pathogenesis of polycystic ovary syndrome. Taiwan. J. Obstet. Gynecol. 55, 183–187.
Hooijmans, C.R., Rovers, M.M., De Vries, R.B., Leenaars, M., Ritskes-Hoitinga, M., Langendam, M.W., 2014. SYRCLE's risk of bias tool for animal studies. BMC Med. Res. Methodol. 14, 1–9.
Hyderali, B.N., Mala, K., 2015. Oxidative stress and cardiovascular complications in polycystic ovarian syndrome. Eur. J. Obstet. Gynecol. Reprod. Biol. 191, 15–22.
Jamilian, M., Asemi, Z., 2016. The effects of soy isoflavones on metabolic status of patients with polycystic ovary syndrome. J. Clin. Endocrinol. Metab. 101, 3386–3394.
Javanbakht, M.H., Sadria, R., Djalali, M., Derakhshanian, H., Hosseinzadeh, P., Zarei, M., Azizi, G., Sedaghat, R., Mirshafiey, A., 2014. Soy protein and genistein improves renal antioxidant status in experimental nephrotic syndrome. Nefrologia 34.
Jiang, G., Li, L., Fan, J., Zhang, B., Oso, A., Xiao, C., Yin, Y., 2015. Dietary soy isoflavones differentially regulate expression of the lipid-metabolic genes in different white adipose tissues of the female Bama mini-pigs. Biochem. Biophys. Res. Commun. 461, 159–164.
Karimi, A., Tutunchi, H., Naeini, F., Vajdi, M., Mobasseri, M., Najafipour, F., 2022. The therapeutic effects and mechanisms of action of resveratrol on polycystic ovary syndrome: a comprehensive systematic review of clinical, animal and in vitro studies. Clin. Exp. Pharmacol. Physiol. 49, 935–949.
Khan, M., Liu, X., Tang, Y., Liu, X., 2018. Ultra-sensitive electrochemical detection of oxidative stress biomarker 8-hydroxy-2′-deoxyguanosine with poly (L-arginine)/graphene wrapped Au nanoparticles modified electrode. Biosens. Bioelectron. 117, 508–514.
Khani, B., Mehrabian, F., Khalesi, E., Eshraghi, A., 2011. Effect of soy phytoestrogen on metabolic and hormonal disturbance of women with polycystic ovary syndrome. J. Res. Med. Sci.: the official journal of Isfahan University of Medical Sciences 16, 297.
Kim, S., Shin, H.-J., Kim, S.Y., Kim, J.H., Lee, Y.S., Kim, D.-H., Lee, M.-O., 2004. Genistein enhances expression of genes involved in fatty acid catabolism through activation of PPARα. Mol. Cell. Endocrinol. 220, 51–58.
Kulling, S.E., Honig, D.M., Metzler, M., 2001. Oxidative metabolism of the soy isoflavones daidzein and genistein in humans in vitro and in vivo. J. Agric. Food Chem. 49, 3024–3033.

Kurkela, M., Garcıa-Horsman, J.A., Luukkanen, L., Mörsky, S., Taskinen, J., Baumann, M., Kostiainen, R., Hirvonen, J., Finel, M., 2003. Expression and characterization of recombinant human UDP-glucuronosyltransferases (UGTs): UGT1A9 is more resistant to detergent inhibition than the other UGTs and was purified as an active dimeric enzyme. J. Biol. Chem. 278, 3536–3544.
Lacey, M., Bohday, J., Fonseka, S.M., Ullah, A.I., Whitehead, S.A., 2005. Dose–response effects of phytoestrogens on the activity and expression of 3β-hydroxysteroid dehydrogenase and aromatase in human granulosa-luteal cells. J. Steroid Biochem. Mol. Biol. 96, 279–286.
Lee, D., Kim, J.-Y., Kim, H.-W., Yoo, J.-E., Kang, K.S., 2021. Combined beneficial effect of genistein and atorvastatin on adipogenesis in 3T3-L1 adipocytes. Biomolecules 11, 1052.
Legro, R.S., Arslanian, S.A., Ehrmann, D.A., Hoeger, K.M., Murad, M.H., Pasquali, R., Welt, C.K., 2013. Diagnosis and treatment of polycystic ovary syndrome: an Endocrine Society clinical practice guideline. J. Clin. Endocrinol. Metab. 98, 4565–4592.
Liu, H., Zhong, H., Yin, Y., Jiang, Z., 2017. Genistein has beneficial effects on hepatic steatosis in high fat-high sucrose diet-treated rats. Biomed. Pharmacother. 91, 964–969.
Low, Y.-L., Dunning, A.M., Dowsett, M., Folkerd, E., Doody, D., Taylor, J., Bhaniani, A., Luben, R., Khaw, K.-T., Wareham, N.J., 2007. Phytoestrogen exposure is associated with circulating sex hormone levels in postmenopausal women and interact with ESR1 and NR1I2 gene variants. Cancer Epidemiol. Prevent. Biomark. 16, 1009–1016.
Luo, M., Zheng, L.-W., Wang, Y.-S., Huang, J.-C., Yang, Z.-Q., Yue, Z.-P., Guo, B., 2021. Genistein exhibits therapeutic potential for PCOS mice via the ER-Nrf2-Foxo1-ROS pathway. Food Funct. 12, 8800–8811.
Ly, L.P., Handelsman, D.J., 2005. Empirical estimation of free testosterone from testosterone and sex hormone-binding globulin immunoassays. Eur. J. Endocrinol. 152, 471–478.
Ma, X., Li, X., Ma, L., Chen, Y., He, S., 2021. Soy isoflavones alleviate polycystic ovary syndrome in rats by regulating NF-κB signaling pathway. Bioengineered 12, 7215–7223.
Manach, C., Williamson, G., Morand, C., Scalbert, A., Rémésy, C., 2005. Bioavailability and bioefficacy of polyphenols in humans. I. Review of 97 bioavailability studies. Am. J. Clin. Nutr. 81, 230S–242S.
Miller, W.L., 2017. Steroidogenesis: unanswered questions. Trends Endocrinol. Metabol. 28, 771–793.
Mousavi, Y., Adlercreutz, H., 1993. Genistein is an effective stimulator of sex hormone-binding globulin production in hepatocarcinoma human liver cancer cells and suppresses proliferation of these cells in culture. Steroids 58, 301–304.
Mulvihill, E.E., Huff, M.W., 2010. Antiatherogenic properties of flavonoids: implications for cardiovascular health. Can. J. Cardiol. 26, 17A–21A.
Murri, M., Luque-Ramírez, M., Insenser, M., Ojeda-Ojeda, M., Escobar-Morreale, H.F., 2013. Circulating markers of oxidative stress and polycystic ovary syndrome (PCOS): a systematic review and meta-analysis. Hum. Reprod. Update 19, 268–288.
Nagaraju, G.P., Zafar, S.F., El-Rayes, B.F., 2013. Pleiotropic effects of genistein in metabolic, inflammatory, and malignant diseases. Nutr. Rev. 71, 562–572.
Nielsen, I.L.F., Williamson, G., 2007. Review of the factors affecting bioavailability of soy isoflavones in humans. Nutr. Cancer 57, 1–10.
Ouadda, A.B.D., Levy, E., Ziv, E., Lalonde, G., Sané, A.T., Delvin, E., Elchebly, M., 2009. Increased hepatic lipogenesis in insulin resistance and Type 2 diabetes is associated with AMPK signalling pathway up-regulation in Psammomys obesus. Biosci. Rep. 29, 283–292.
Palomba, S., Santagni, S., Falbo, A., La Sala, G.B., 2015. Complications and challenges associated with polycystic ovary syndrome: current perspectives. Int. J. Wom. Health 7, 745.
Patisaul, H.B., Mabrey, N., Adewale, H.B., Sullivan, A.W., 2014. Soy but not bisphenol A (BPA) induces hallmarks of polycystic ovary syndrome (PCOS) and related metabolic co-morbidities in rats. Reprod. Toxicol. 49, 209–218.
Qiao, J., Feng, H.L., 2011. Extra-and intra-ovarian factors in polycystic ovary syndrome: impact on oocyte maturation and embryo developmental competence. Hum. Reprod. Update 17, 17–33.
Rajaei, S., Alihemmati, A., Abedelahi, A., 2019. Antioxidant effect of genistein on ovarian tissue morphology, oxidant and antioxidant activity in rats with induced polycystic ovary syndrome. Int. J. Reproduct. BioMed. 17, 11.
Rajan, R.K., Balaji, B., 2017. Soy isoflavones exert beneficial effects on letrozole-induced rat polycystic ovary syndrome (PCOS) model through anti-androgenic mechanism. Pharmaceut. Biol. 55, 242–251.
Riches, Z., Stanley, E.L., Bloomer, J.C., Coughtrie, M.W., 2009. Quantitative evaluation of the expression and activity of five major sulfotransferases (SULTs) in human tissues: the SULT "pie". Drug Metabol. Dispos. 37, 2255–2261.
Romualdi, D., Costantini, B., Campagna, G., Lanzone, A., Guido, M., 2008. Is there a role for soy isoflavones in the therapeutic approach to polycystic ovary syndrome? Results from a pilot study. Fertil. Steril. 90, 1826–1833.
Rosenfield, R.L., 2020. Current concepts of polycystic ovary syndrome pathogenesis. Curr. Opin. Pediatr. 32, 698–706.
Rosenfield, R.L., Ehrmann, D.A., 2016. The pathogenesis of polycystic ovary syndrome (PCOS): the hypothesis of PCOS as functional ovarian hyperandrogenism revisited. Endocr. Rev. 37, 467–520.
Sam, S., 2007. Obesity and polycystic ovary syndrome. Obes. Manag. 3, 69–73.
Sarhan, D., El Mazny, A., Taha, T., Aziz, A., Azmy, O., Fakhry, D., Torky, H., 2017. Estradiol and luteinizing hormone concentrations in the follicular aspirate during ovum pickup as predictors of in vitro fertilization (IVF) outcome. Middle East Fertil. Soc. J. 22, 27–32.
Sendur, S.N., Yildiz, B.O., 2021. Influence of ethnicity on different aspects of polycystic ovary syndrome: a systematic review. Reprod. Biomed. Online 42, 799–818.
Setchell, K.D., Brown, N.M., Desai, P., Zimmer-Nechemias, L., Wolfe, B.E., Brashear, W. T., Kirschner, A.S., Cassidy, A., Heubi, J.E., 2001. Bioavailability of pure isoflavones in healthy humans and analysis of commercial soy isoflavone supplements. J. Nutr. 131, 1362S–1375S.
Strassburg, C.P., Manns, M.P., Tukey, R.H., 1998. Expression of the UDP-glucuronosyltransferase 1A locus in human colon: identification and characterization of the novel extrahepatic UGT1A8. J. Biol. Chem. 273, 8719–8726.
Teede, H., Misso, M., Tassone, E.C., Dewailly, D., Ng, E.H., Azziz, R., Norman, R.J., Andersen, M., Franks, S., Hoeger, K., 2019. Anti-Müllerian hormone in PCOS: a review informing international guidelines. Trends Endocrinol. Metabol. 30, 467–478.
Thangavel, P., Puga-Olguín, A., Rodríguez-Landa, J.F., Zepeda, R.C., 2019. Genistein as potential therapeutic candidate for menopausal symptoms and other related diseases. Molecules 24, 3892.
Ullah, K., ur Rahman, T., Wu, D.-D., Lin, X.-H., Liu, Y., Guo, X.-Y., Leung, P.C., Zhang, R.-J., Huang, H.-F., Sheng, J.-Z., 2017. Phoenixin-14 concentrations are increased in association with luteinizing hormone and nesfatin-1 concentrations in women with polycystic ovary syndrome. Clin. Chim. Acta 471, 243–247.
Ungar, Y., Osundahunsi, O.F., Shimoni, E., 2003. Thermal stability of genistein and daidzein and its effect on their antioxidant activity. J. Agric. Food Chem. 51, 4394–4399.
van der Spuy, Z.M., Dyer, S.J., 2004. The pathogenesis of infertility and early pregnancy loss in polycystic ovary syndrome. Best Pract. Res. Clin. Obstet. Gynaecol. 18, 755–771.
Vanky, E., Løvvik, T.S., 2020. Polycystic ovary syndrome and pregnancy–From a clinical perspective. Curr. Opin. Endocrine Metabolic Res. 12, 8–13.
Victoria, M., Labrosse, J., Krief, F., Cédrin-Durnerin, I., Comtet, M., Grynberg, M., 2019. Anti Müllerian hormone: more than a biomarker of female reproductive function. J. Gynecol. Obstetrics Human Reproduct. 48, 19–24.
Walters, K., Allan, C., Handelsman, D., 2008. Androgen actions and the ovary. Biol. Reprod. 78, 380–389.
Walters, K., Handelsman, D., 2018. Role of androgens in the ovary. Mol. Cell. Endocrinol. 465, 36–47.
Wang, Y., Li, N., Zeng, Z., Tang, L., Zhao, S., Zhou, F., Zhou, L., Xia, W., Zhu, C., Rao, M., 2021. Humanin regulates oxidative stress in the ovaries of polycystic ovary syndrome patients via the Keap1/Nrf2 pathway. Mol. Hum. Reprod. 27 gaaa081.
Wu, J.-G., Ge, J., Zhang, Y.-P., Yu, Y., Zhang, X.-Y., 2010. Solubility of genistein in water, methanol, ethanol, propan-2-ol, 1-butanol, and ethyl acetate from (280 to 333) K. J. Chem. Eng. Data 55, 5286–5288.
Yang, Z., Kulkarni, K., Zhu, W., Hu, M., 2012. Bioavailability and pharmacokinetics of genistein: mechanistic studies on its ADME. Anti Cancer Agents Med. Chem. 12, 1264–1280.
Yang, Z., Zhu, W., Gao, S., Xu, H., Wu, B., Kulkarni, K., Singh, R., Tang, L., Hu, M., 2010. Simultaneous determination of genistein and its four phase II metabolites in blood by a sensitive and robust UPLC–MS/MS method: application to an oral bioavailability study of genistein in mice. J. Pharmaceut. Biomed. Anal. 53, 81–89.
Ye, W., Xie, T., Song, Y., Zhou, L., 2021. The role of androgen and its related signals in PCOS. J. Cell Mol. Med. 25, 1825–1837.
Yu, H.-F., Duan, C.-C., Yang, Z.-Q., Wang, Y.-S., Yue, Z.-P., Guo, B., 2019. HB-EGF ameliorates oxidative stress-mediated uterine decidualization damage. Oxid. Med. Cell. Longev. 2019.
Yu, J., Bi, X., Yu, B., Chen, D., 2016. Isoflavones: anti-inflammatory benefit and possible caveats. Nutrients 8, 361.
Zaheer, K., Humayoun Akhtar, M., 2017. An updated review of dietary isoflavones: nutrition, processing, bioavailability and impacts on human health. Crit. Rev. Food Sci. Nutr. 57, 1280–1293.
Zhang, T., Chi, X., 2019. Estrogenic properties of genistein acting on FSHR and LHR in rats with PCOS. Pol. J. Vet. Sci. 83–90, 83–90.
Zheng, S.-H., Li, X.-L., 2016. Visceral adiposity index as a predictor of clinical severity and therapeutic outcome of PCOS. Gynecol. Endocrinol. 32, 177–183.
Zhou, S., Hu, Y., Zhang, B., Teng, Z., Gan, H., Yang, Z., Wang, Q., Huan, M., Mei, Q., 2008. Dose-dependent absorption, metabolism, and excretion of genistein in rats. J. Agric. Food Chem. 56, 8354–8359.
Zuo, T., Zhu, M., Xu, W., 2016. Roles of oxidative stress in polycystic ovary syndrome and cancers. Oxid. Med. Cell. Longev. 2016.