Phytoestrogenic Effects of Various Levels of Soy Isoflavones in Polycystic Ovarian Syndrome

Nishaf Mazhar
Government College University Faisalabad
Mehr Un Nisa
Government College University Faisalabad
Muhammad Sajid Arshad (Sajid_ft@yahoo.com)
Government College University Faisalabad https://orcid.org/0000-0001-9564-886X

Research

Keywords: Soy Isoflavones, Polycystic Ovarian Syndrome, Lipid profile, Digestibility

Posted Date: May 11th, 2020

DOI: https://doi.org/10.21203/rs.3.rs-27247/v1

Abstract

Background: Soy isoflavones (SI) has estrogenic effect in tissues by binding to estrogen receptors and might be beneficial for women with Polycystic Ovarian Syndrome (PCOS) by reduction in testosterone, cholesterol , Insulin , weight gain, inflammatory markers and oxidative stress.

Methods: The study was plane to examine the effect of various levels of SI on nutrient intake, digestibility, lipid profile, insulin and reproductive hormones of estradiol-valerate (4mg/rat/IM) PCOS induced rat models. Thirty-Six Wistar 45 days old rats weighing 95 ± 5g were divided into 4 groups, each having 9 rats: C (control: without SF), SF10 (SF 0.10g/Kg BW), SF15 (SF 0.15g/Kg BW) and SF20 (SF 0.20g/Kg BW). SF was given through the oral gavage. Food and water were offered ad libitum and intake was recorded daily. Body weight was recorded on weekly basis. During last week of trial, collected feces by total collection method and blood samples were used to calculate nutrient digestibility and biochemical analysis respectively. The Completely Randomized Design and LSD test were used to analyze the data.

Results: The significant results were observed in female rats fed SF10, SF15 and SF20 compared to C. Estrogen, progesterone and prolactin were ($P<0.05$) high in rats fed SF diet that was 4 and 30% increase from C respectively. Insulin, Testosterone, FSH and LH were lowest in rats fed diet SF20. Significant ($P<0.05$) reduction in cholesterol was observed in rats fed SF15 and SF20 as compared to C. Serum HDL was improved ($P<0.05$) in all SIF 0.10 , SIF 0.15 and SIF 0.20 as 26.88±1.59 (mg/dl), 32.40±1.42 (mg/dl) and 53.44±1.50 (mg/dl) in comparison to PC. Serum LDL was significantly reduced to 68.89±4.36 (mg/dl) in SIF 0.20 , and to 108.20±4.14 (mg/dl) in SIF 0.15 whereas, insignificant reduction was observed in SIF 0.10 (127.75±4.62 mg/dl) as compared to PC. Highly significant reduction was noted in triglycerides level in SIF 0.20 as 115.00±2.04 (mg/dl), however, significant reduction was observed in both SIF 0.10 and SIF 0.15 as compared to PC after three months of treatment with soy isoflavones.

Conclusions: It is concluded that soy isoflavones have potential role on reproductive hormones, insulin levels, weight, lipid profile and nutrient digestibility in PCOS induced rats.

Introduction

In assessing the therapeutic effects of phytoestrogens, a clear dissimilarity must be made between foods having phytoestrogens and dietary supplements, as bioavailability, dosage and physiological effect may vary critically [1]. The majority of phytoestrogens belong to a large group of substituted phenolic compounds known as flavonoids. Isoflavones are polyphenolic compounds that are almost exclusively produced by the members of the Fabaceae family. The major bioactive isoflavones are genistein and daidzein, which are derived mainly from soy and red clover[2]. In particular soybeans, are the richest sources of isoflavones in the human diet.

Isoflavones are identified to exibit weak estrogen hormone-like activity. Because isoflavones are not synthesized by the human endocrine system and can only be ingested or consumed in a diet or as a supplement they are also called as “dietary estrogens”[3]. In human body, estrogen is secreted primarily

by the ovaries and in lesser quantities by the adrenal glands. The most active form of estrogen in the body is called estradiol. Estrogen put its effects by binding to estrogen receptors within cells. These estrogen-receptor complexes then combines with DNA to change the expression of estrogen-responsive genes. For simulating the effects of estrogen in tissues, soy isoflavones as phytoestrogens can bind to estrogen receptors [4].

As isoflavones and estradiol are contending for their binding on ERs, the effect of isoflavones also dependent on the level of endogenous estradiol. When there is high levels of endogenous estrogens, isoflavones may hinder full estrogen activity by conquering a part of the ERs. Alternatively, when there is low levels of endogenous estrogens the estrogen activity of isoflavones may become evident [5]. As compared to 17-β- estradiol the affinity of other isoflavones is 100–500 times lower. Isoflavones act against ERs, but their activity is lesser than that of 17-β-estradiol. At suitably high levels, the effect of isoflavones may approach the effect of endogenous 17-β-estradiol at its physiological level [6]. For this reason, there is emerging trend of using isoflavones as an alternative or complement of HRT in postmenopausal women, particularly when long-term administration is required [7]. Number of studies indicate that, soy being high in isoflavones, can prevent illness and promote good health.

Soy isoflavones have number of benefits for women with PCOS such as reduction in testosterone, Total cholesterol and LDL cholesterol, Insulin, inflammatory markers and oxidative stress. Polycystic ovary syndrome is the most common endocrine disorder among the women of reproductive age [8].

In women with PCOS insulin resistance is common and it causes lipoprotein disturbances that plays a role as predisposing factor for early development of diabetes, hyperlipidemia and cardiovascular disease [9].Two types of interventions: drug treatment and change in lifestyle with exercise and weight loss are mostly used as therapeutic approaches for PCOS. The available common drugs are effective for PCOS, but they have side effects such as amenorrhea, overweight, decreased bone mineral density and depression. Therefore, the use of non-pharmacological treatment methods is considered.

Using soy isoflavones as a non-pharmacological approach for PCOS patients is growing interest in how adding soy to the diet can help address metabolic syndrome and related health conditions. The effects of soy isoflavones, have been studied by several researchers and their biological activity related to estrogen receptor-mediated mechanisms have been observed [10].Soy isoflavones being phytoestrogens are adaptogens. Under increased or decreased levels of estrogen they can be beneficial. When isoflavones attached themselves to estrogen receptors, this hinders the effects of endogenous estrogen. Generally, phytoestrogens have about two percent of the strength of estrogens. Hence, when estrogen levels are high, switching a phytoestrogen for an estrogen means that there will be much less estrogenic activity at a given binding site. Conversely, if estrogen levels are low and estrogen-binding sites are free, phytoestrogens that have almost two percent estrogen activity will bind with them and result in a total increase in total estrogenic effect [11]. Considering the above facts, the objective of this study was to investigate the effect of different levels of soy isoflavones on blood insulin, lipid and reproductive hormones profile of the female rats with PCOS.

Materials And Methods:

This study was carried out in the Department of Food Science, Nutrition and Home Economics and Department of Pharmacy, Basic Sciences, Government College University, Faisalabad. The 36 Wistar albino female rats (38 days old), each with the weight 100 ± 10 gram, which had 2 consecutive estrus cycles were bought from National Institute of Health, Islamabad. They were kept at 28 ± 1 °C and 45 to 55% relative humidity under 12-h light: 12-h dark cycle. According to Principles of Laboratory Animal Care (NIH) all animals were kept and treated. The approved experimental procedure by the Animal Ethical Committee was considered. Ad *libitum* diet and water were offered to the rats. *Isocaloric* and *isonitrogenous*.diets were offered to the rats. The weekly intake of water and feed were monitored at least one week prior to treatments in order to regulate the amount of water drank per experimental animal. The soy isoflavones used for research were purchased from Xi'an Wharton Bio-Tech, Co, Ltd, China.

Different levels of soy isoflavones:

Thirty six PCOS induced Wistar female rats were used to observe the effect of different levels of isoflavones. The rats were divided into four groups (9 rats per group) named according to levels viz., PC (Positive control: without isoflavones), $SIF_{0.10}$ (soy isoflavone: 0.10 g/kg body weight), $SIF_{0.15}$ (soy isoflavones: 0.15 g/kg body weight) and $SIF_{0.20}$ (soy isoflavones: 0.20 g/kg body weight). These levels of soy isoflavones were given through oral gavage [12]. All animals included in PC, $SIF_{0.10,}$ $SIF_{0.15}$ and $SIF_{0.20}$ were induced PCOS through single intramuscular injection of estradiol-valerate (4 mg/rat/IM). In this in vivo experimental method, the rats were divided according to Completely Randomized Design. Each group consist of 9 rats and treatment w repeated 3 times to make 12 experimental units, involving 36 rats as a whole.

The trial was of 3 months. The first 2 months were correction period followed by one month collection period. The 30 days before the experimental protocol, 36 rats were given a single intramuscular injection of 4 mg/rat estradiol valerate (Riedeldehaen, Germany) firstly mixed in 0.2 ml oil to induce PCOS [13]. In the last week of study, all rats were bled to get blood samples to carry out biochemical and pathological analysis.

Feed and nutrient digestibility analysis:

Digestibility of nutrients were measured by total collection method. All modified diets/feeds and feces were analyzed to calculate ash, moisture, crude protein, crude fat, crude fiber, nitrogen free extract and dry matter content (AOAC, 2000).

Nutrient Digestibility (%) = Nutrient intake - Nutrient in feces × 100

Feces sample were collected via designing the cages in such a way that collect samples accurately [14]. Feces were stored at -20°C [15].

Biochemical Analysis:

In the end of trial 5 cc blood samples of all rats were drawn and prepared for biochemical analysis [13].

The plasma testosterone were estimated by method adopted by[16]. Plasma estrogen levels were checked according to reference method of [17]. The blood glucose were measured using ACCU Check Active® Machine and serum insulin were assessed by using Electrochemiluminescence immunoassay (Roche Diagnostics E170 insulin assay). The lipid profile were determined by the method described by Cholesterol [18], Triglycerides [19]. High density lipoproteins [20], Low density lipoproteins [21].

Statistical analysis:

The data obtained from all experimental sources were subjected to statistical analysis using completely randomized designed (CRD) according to the procedure described by [22]. Tukey's test was used to conclude statistically different groups.

Results

Nutrient Intake:

The result showing the effects of different level of soy isoflavones on weight of PCOS induced rats are shown in Table 1. Slightly significant decrease was observed in dry matter intake when compared with PC. Values of crude protein intake were found to be decreased in all rats treated with soy isoflavones as 7.94 ± 0.21 in $SIF_{(0.10)}$,8.31 ± 0.23 in $SIF_{(0.15)}$ and as 7.85 ± 0.24 in $SIF_{(0.20)}$. crude fiber intake was also significantly decreased in all groups with as lows as 0.97 ± 0.16 in $SIF_{(0.15)}$.Significant reduction in intake of ether extract and ash content of the feed was observed in all rats fed with different levels of soy isoflavones.

Table 1
Nutrient in take after administration of different levels of soy isoflavones in PCOS induced rats.

Parameters	PC	$SIF_{(0.10)}$	$SIF_{(0.15)}$	$SIF_{(0.20)}$
Dry matters intake (DMI) per day	26.61 ± 0.11^{a}	24.71 ± 0.11^{b}	24.65 ± 0.09^{b}	24.41 ± 1.12^{b}
Crude protein intake	9.17 ± 0.24^{a}	7.94 ± 0.21^{b}	8.31 ± 0.23^{b}	7.85 ± 0.24^{c}
Crude fiber intake CF	1.52 ± 0.16^{a}	0.87 ± 0.16^{bc}	0.97 ± 0.16^{b}	0.87 ± 0.16^{bc}
Ether extract intake EE	1.02 ± 0.59^{a}	0.99 ± 0.71^{b}	0.98 ± 0.71^{b}	0.87 ± 0.68^{c}
Ash	3.95 ± 0.33^{a}	2.53 ± 0.29^{b}	2.36 ± 0.31^{b}	2.34 ± 0.34^{c}

The values are means ± SD of three independent determinations. PC (positive control without isoflavones); $SIF_{0.10}$ (soy isoflavones:o.10 g/kg body weight); $SIF_{0.15}$ (soy isoflavones:o.15 g/kg body weight); $SIF_{0.20}$ (soy isoflavones:o.20 g/kg body weight)

Digestibility

The effects of different level of soy isoflavones on digestibility of PCOS induced rats were given in the Table 2. Dry matter digestibility was significantly decreased in all rats fed with various levels as 76.6 ± 0.64 in $SIF_{(o.15)}$, and as 70.2 ± 0.66 in $SIF_{(0.20)}$ .however ,crude fiber digestibility was significantly decreased with 18% decreased $SIF_{(0.20)}$.And significant reduction in values of ether extract was observed in all rats treated with different levels of soy isoflavones when compared with PC.

Table 2
Digestibility after administration of different levels of soy isoflavones in PCOS induced rats.

Parameters	PC	$SIF_{(0.10)}$	$SIF_{(0.15)}$	$SIF_{(0.20)}$
Dry matters digestibility (DMD)	75.89 ± 4.11^{a}	69.12 ± 2.12^{b}	71.21 ± 1.09^{b}	63.19 ± 1.12^{b}
Crude protein	82.7 ± 0.87^{a}	76.6 ± 0.64^{b}	77.1 ± 0.64^{b}	70.02 ± 0.66^{c}
Crude fiber	59.8 ± 0.68^{a}	54.1 ± 0.71^{b}	54.1 ± 0.71^{b}	48.5 ± 0.74^{c}
Ether extract	89.5 ± 0.78^{a}	83.6 ± 0.51^{b}	83.1 ± 0.48^{b}	77.0 ± 0.98^{c}

The values are means ± SD of three independent determinations. PC (positive control without isoflavones); $SIF_{0.10}$ (soy isoflavones:o.10 g/kg body weight); $SIF_{0.15}$ (soy isoflavones:o.15 g/kg body weight); $SIF_{0.20}$ (soy isoflavones:o.20 g/kg body weight)

Weight Changes

The result showing the effects of different level of soy isoflavones on weight PCOS induced rats are shown in the Table 3. Statistically significant reduction in final weight was observed in SIF $_{(0.20)}$ as 72.5 ± 8.53 when compared with PC, after administration of soy isoflavones in PCOS induced female rats.In all rats treated with various levels of soy isoflavones significant increase was observed in feed conversion ratio (FCR) with the maximum increase noted in SIF $_{(0.20)}$of about 20% as compared to PC.

Table 3
Weight/ht changes after administration of different levels of soy isoflavones in PCOS induced rats.

Parameters	PC	SIF$_{(0.10)}$	SIF$_{(0.15)}$	SIF$_{(0.20)}$
Initial weight	95.6 ± 1.52	97.2 ± 1.52	96.3 ± 1.52	97.7 ± 1.52
Weight after PCOS	124.6 ± 1.73	124.2 ± 1.73	127.2 ± 1.73	126.6 ± 1.73
Weight Gain First Month	30.0 ± 1.20	27.0 ± 1.20	30.0 ± 1.20	28.0 ± 1.20
Final Weight gain	93.4 ± 4.75 ^{b}	79.4 ± 4.75^{a}	77.2 ± 4.75^{a}	72.5 ± 4.75^{a}
Feed Conversion Ratio	15.4 ± 0.45^{b}	17.7 ± 0.65^{b}	18.2 ± 0.57^{ab}	19.3 ± 0.69^{a}

The values are means ± SD of three independent determinations. PC (positive control without isoflavones); SIF $_{0.10}$ (soy isoflavones:o.10 g/kg body weight); SIF $_{0.15}$ (soy isoflavones:o.15 g/kg body weight); SIF $_{0.20}$ (soy isoflavones:o.20 g/kg body weight)

Reproductive Hormones:

The results showing the effects of different level of soy isoflavones on reproductive hormones of PCOS induced rats are shown in Table 4. In all rats treated with SIF$_{0.10}$, SIF$_{0.15}$ and SIF$_{0.20}$ almost significant results were observed. Progesterone values were observed to be increased in all levels with maximum increase in SIF$_{0.20}$ that is 13.21 ± 0.45 showing 30% increase from PC. Decrease in testosterone values was noted in all treatment levels. Notable change was seen in FSH/LH ratio, with increase in FSH values as noted maximum in SIF$_{0.10}$ and SIF$_{0.20}$, but is comparatively low in SIF$_{0.15}$. Whereas, remarkable decrease was observed in LH values with lowest most observed in SIF$_{0.20}$ as (13.91 ± 0.67 mIU/ml). Increase was observed in prolactin values from PC in both SIF$_{0.10}$ and SIF$_{0.20}$ levels, however, reduction was noted in SIF$_{0.15}$ (31.93 ± 1.04 ng/ml) from PC. In estrogen concentration significant increase was observed in SIF$_{0.20}$ but, no statistically significant change was noted in SIF$_{0.10}$ and SIF$_{0.15}$ from PC.

Lipid profile:

Effects of different levels of soy isoflavones on lipid profile of PCOS induced rats are given in the Table 5. Significant reduction in cholesterol was observed in SIF$_{0.15}$ and SIF$_{0.20}$ as 141.60±2.56 (mg/dl) and 127.56±2.69 (mg/dl) while insignificant results were obtained in SIF$_{0.10}$ 154.63±2.86 (mg/dl) as

compared to PC. Level of serum HDL was significantly improved in all $SIF_{0.10}$, $SIF_{0.15}$ and $SIF_{0.20}$ as 26.88±1.59 (mg/dl), 32.40±1.42 (mg/dl) and 53.44±1.50 (mg/dl) in comparison to PC. Serum LDL was significantly reduced to 68.89±4.36 (mg/dl) in $SIF_{0.20}$, and to 108.20±4.14 (mg/dl) in $SIF_{0.15}$ whereas, insignificant reduction was observed in $SIF_{0.10}$ (127.75±4.62 mg/dl) as compared to PC. Highly significant reduction was noted in triglycerides level in $SIF_{0.20}$ as 115.00±2.04 (mg/dl), however, significant reduction was observed in both $SIF_{0.10}$ and $SIF_{0.15}$ as compared to PC after three months of treatment with soy isoflavones

Table 4
Reproductive hormones after administration of different levels of soy isoflavones in PCOS induced rats.

Parameters	PC	$SIF_{(0.10)}$	$SIF_{(0.15)}$	$SIF_{(0.20)}$
Progesterone mg/mL	9.16 ± 0.45^{b}	11.84 ± 0.48^{a}	12.90 ± 0.43^{a}	13.21 ± 0.45^{a}
Testosterone ng/dL	112.70 ± 2.85^{a}	97.34 ± 3.03^{b}	89.29 ± 2.71^{bc}	79.61 ± 2.85^{c}
FSH mIU/mL	4.37 ± 0.32^{a}	4.57 ± 0.34^{a}	4.55 ± 0.30^{a}	4.67 ± 0.32^{b}
LH mIU/mL	18.18 ± 0.67^{a}	16.50 ± 0.71^{ab}	15.31 ± 0.64^{bc}	13.91 ± 0.67^{c}
Prolactin ng/mL	35.16 ± 1.10^{bc}	37.55 ± 1.17^{b}	31.93 ± 1.04^{c}	44.29 ± 1.10^{a}
Estrogen pg/mL	63.40 ± 2.88^{b}	63.24 ± 3.05^{b}	60.28 ± 2.73^{b}	92.90 ± 2.88^{a}

The values are means ± SD of three independent determinations. PC (positive control without isoflavones); $SIF_{0.10}$ (soy isoflavones:o.10 g/kg body weight); $SIF_{0.15}$ (soy isoflavones:o.15 g/kg body weight); $SIF_{0.20}$ (soy isoflavones:o.20 g/kg body weight)

Insulin Level:

Effects of different levels of soy isoflavones on insulin level of PCOS induced rats are given in the Table 5. Significant reduction was observed in serum insulin level of all rats treated with $SIF_{0.10}$, $SIF_{0.15}$ and $SIF_{0.20}$, with the maximum reduction in $SIF_{0.20}$ as 5.94 ± 0.37 (mg/dl) as compared to PC.

Discussions

The purpose of this study was to investigate the effects of different levels of soy isoflavones on reproductive hormones, lipid profile, serum insulin, digestibility and weight changes in polycystic ovarian syndrome (PCOS) induced female rats.

In this study significant changes were observed in the levels of reproductive hormones after administration of soy isoflavones on different levels say $SIF_{0.10}$, $SIF_{0.15}$ and $SIF_{0.20}$ for three months. Significant increase in progesterone hormone was observed in all levels of soy isoflavones supplementation with maximum increase in $SIF_{0.20}$ that is 13.21 ± 0.45 showing 30% increase from PC, whereas, $SIF_{0.15}$ and $SIF_{0.10}$ showing 13% and 2% change only [23] found similar result with 120 mg/day dosage of soy isoflavones after 8 weeks of supplementation. In PCOS testosterone level become increased because of the high levels of LH or high levels of insulin [24]. This excessive testosterone cause other problematic issues like acne, hirsutism and hair loss and also inhibits normal ovulation. So, reducing testosterone to the normal healthy level is the prime goal of PCOS treatment. And our study found statistically significant decrease in testosterone values in all treatment levels as 97.34 ± 3.03 (ng/dl) in $SIF_{0.10}$, 89.29 ± 2.71 (ng/dl) in $SIF_{0.15}$ and 79.61 ± 2.85 (ng/dl) in $SIF_{0.20}$. [25, 26] found similar results on decrease in testosterone levels by using different dosage levels of soy isoflavones for different time periods.

Moreover, in FSH/LH ratio, no statistically significant increase in FSH values were noted in $SIF_{0.10}$ and $SIF_{0.15}$, but a significant increase of 6.8% was observed in $SIF_{0.20}$. Similar results were found by [10] where no statistically significant change in FSH level was observed after 2 months of supplementation. But increase in FSH concentration in $SIF_{0.20}$ was contradictory with all studies, might be this increase was a result of high dosage of soy isoflavone. Whereas, remarkable decrease was observed in LH values with lowest most observed in $SIF_{0.20}$ as (13.91 ± 0.67 mIU/ml) [26] suggested similar benefits by using 36 mg/day of soy isoflavones supplementation for 3 months in women having PCOS. LH lowering effects of soy isoflavones was also suggested in other studies [27, 28, 29, 23]. Increase was observed in prolactin values from PC in both $SIF_{0.10}$ and $SIF_{0.20}$ levels, however, reduction was noted in $SIF_{0.15}$ (31.93 ± 1.04 ng/ml) from PC. Similar findings were suggested by [23] after using 120 mg of soy isoflavones per day for 8 weeks. In estrogen concentration significant increase of 46% was observed in $SIF_{0.20}$ but, no statistically significant change was noted in $SIF_{0.10}$ and $SIF_{0.15}$ as compaired to PC. Increase in estrogen levels were noted in a meta-analysis of 47 studies on both pre and post-menopausal women by [29]. Whereas, many studies suggested significant decrease in estrogen level after different level of soy isoflavones administration [30, 31, 32].

Dyslipidemia is a common problem in women with PCOS [33]. Combating with abnormal lipid profile is an important consideration in PCOS treatment. Our study showed significant reduction in cholesterol, 14% in $SIF_{0.15}$ and 22% in $SIF_{0.20}$ as 141.60 ± 2.56 (mg/dl) and 127.56 ± 2.69 (mg/dl) while statistically insignificant results were obtained in $SIF_{0.10}$ with 5% change as compared to PC [34, 35, 36] found similar results in total cholesterol reduction with soy isoflavones supplementation. Serum HDL concentration was significantly improved in all levels ($SIF_{0.10}$, $SIF_{0.15}$ and $SIF_{0.20)}$ as 26.88 ± 1.59 (mg/dl), 32.40 ± 1.42

(mg/dl) and 53.44 ± 1.50 (mg/dl) in comparison to PC. Similarly significant decrease in LDL after 3 months of 36 mg/day genistein supplementation was observed by [26]. Significant reduction in serum LDL was noted as 68.89 ± 4.36 (mg/dl) in $SIF_{0.20}$, and to 108.20 ± 4.14 (mg/dl) in $SIF_{0.15}$ whereas, insignificant reduction was observed in $SIF_{0.10}$ (127.75 ± 4.62 mg/dl) as compared to PC. The results of meta-analysis by [35, 36] showed similar reduction in LDL concentration after soy isoflavones supplementation. Overall, significant decrease was noted in triglycerides concentration, with highly significant in $SIF_{0.20}$ as 115.00 ± 2.04 (mg/dl). However, significant reduction was observed in both $SIF_{0.10}$ and $SIF_{0.15}$ as compared to PC after three months soy isoflavone's supplementation. A significant reduction in triglycerides concentration after 50 mg/day of soy isoflavones intake for 12 weeks in 70 women with PCOS was suggested by [8]. Other studies by [37, 38, 35] also found triglyceride improving effects of soy isoflavones supplementation.

Overall, significant reduction was noted in serum insulin level of all rats treated with $SIF_{0.10}$, $SIF_{0.15}$ and $SIF_{0.20}$, with the maximum reduction in $SIF_{0.20}$ as 5.94 ± 0.37 (mg/dl) as compared to PC. Similar trial of 70 women with PCOS given 50 mg/day soy isoflavones for 12 weeks was conducted by [8] found significant decrease in circulating serum levels of insulin homeostasis model of assessment estimated insulin resistance and increased quantitative insulin sensitivity check index. But soy isoflavones were found to have more potent effects on fasting insulin level [39, 40] .

Conclusion

Our study revealed that soy isoflavones have potential role on reproductive hormones, insulin levels, weight, lipid profile and nutrient digestibility in PCOS induced rats. Significant effects were observed on testosterone, LH, prolactin, estrogen, cholesterol, HDL, LDL, triglyceride and insulin, whereas progesterone and FSH showed statistically significant results only in level $SIF_{0.20}$, however no effects were observed in lower dosage levels, might be due short duration of the study. Although significant results were found in high dosage level of soy isoflavones, but long term administration of this level may cause toxicity. So, long term study with high dosage level of soy isoflavones is required for further investigation.

Declarations

Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable

Availability of data and material

The data generated for this study available in the manuscript

Competing interests

The authors declare that they have no competing interests.

Funding

The funding for this manuscript was provided by Higher Education Commission, Islamabad, Pakistan

Acknowledgements

The authors are highly acknowledged Institute of Home and Food Sciences for providing the facilities for conducting the research work.

Authors' contributions

The contribution of the each author for this manuscript was as follows, MN designed the experimental plan. NM conducted the analysis and drafted the manuscript, MSA reviewed the manuscript. It is also confirmed that all the authors read and approved the final manuscript.

References

1. Setchell KD, Brown NM, Desai P, Zimmer-Nechemias L, Wolfe BE, Brashear WT, Heubi JE. Bioavailability of pure isoflavones in healthy humans and analysis of commercial soy isoflavone supplements. J nutr. 2001;131(4):1362S–1375S.
2. Ricci E, Cipriani S, Chiaffarino F, Malvezzi M, Parazzini F. Soy isoflavones and bone mineral density in perimenopausal and postmenopausal Western women: a systematic review and meta-analysis of randomized controlled trials. J Women's Health. 2010;19(9):1609–17.
3. Knight DC, Eden JA. A review of the clinical effects of phytoestrogens. Obstet Gynecol. 1996;87(5):897–904.
4. Helmy S, Leibfarth FA, Oh S, Poelma JE, Hawker CJ. & Read de Alaniz, J. Photoswitching using visible light: A new class of organic photochromic molecules. J AM Chem Soc. 2014;136(23):8169–72.
5. Lephart ED, Rhees RW, Setchell KDR, BuL H, Lund TD. Estrogens and phytoestrogens: brain plasticity of sexually dimorphic brain volumes. J Steroid Biochem Mol Biol. 2003;85(2):299–309.
6. Kuiper GG, Carlsson BO, Grandien KA, Enmark E, Häggblad J, Nilsson S, Gustafsson JA. Comparison of the ligand binding specificity and transcript tissue distribution of estrogen receptors α β. Endocrinol. 1997;138(3):863–70.
7. Davis SR, Murkies AL, Wilcox G. Phytoestrogens in clinical practice. Integrative Medicine. 1999;1(1):27–34.
8. Jamilian M, Asemi Z. The effects of soy isoflavones on metabolic status of patients with polycystic ovary syndrome. J Clin Endocrinal Metab. 2016;101(9):3386–94.

9. Rizk MA, Sallam GM, Abdel-Azim NA, Ghallab MM. Spider occurrence in fields of some medical and ornamental plants in Fayoum-Egypt. Acari. 2012;6:41–7.
10. Forouhari SE, Heidari Z, Tavana Z, Salehi M, Sayadi M. The effect of soya on some hormone levels in women with polycystic ovary syndrome (balance diet): a cross over randomized clinical trial. Bull Env Pharmacol Life Sci. 2013;3(1):246–50.
11. Barnes S, Boersma B, Patel R, Kirk M, Darley-Usmar VM, Kim H, Xu J. Isoflavonoids and chronic disease: mechanisms action. Biofactors. 2000;12(1–4):209–15.
12. Turner M, Yu O, Subramanian S. (2012). Genome organization and characteristics of soybean microRNAs. BMC Genom.2012; 13(1), 169.
13. Ghasemzadeh A, Farzadi, Laya, Khaki A. & Khan Ahmadi, SH.Effect of Allium cepa seeds ethanolic extract on experimental polycystic ovary syndrome (PCOS) apoptosis induced by estradiol-valerate. Life Sci J. 2013;10(4 s):170–5.
14. Perline. Irvin H.An inexpensive mouse urine collection system. Physiol Behav. 1971;6(5):597.
15. Janczyk P, Pieper R, Souffrant WB, Bimczok D, Rothkötter HJ, Smidt H. Parenteral long-acting amoxicillin reduces intestinal bacterial community diversity in piglets even 5 weeks after the administration. ISME J. 2007;1(2):180–3.
16. Yilmaz M, Bi˙ri˙ A, Bukan N, Karakoç A, Sancak B, Törüner F, Paşaoğlu H. Levels of lipoprotein and homocysteine in non-obese and obese patients with polycystic ovary syndrome. Gynecol endocrinol. 2005;20(5):258–63.
17. Wu AH, Yu MC, Tseng CC, Stanczyk FZ, Pike MC. Diabetes and risk of breast cancer in Asian-American women. Carcinog. 2007;28(7):1561–6.
18. Stockbridge, Hal. Hardy Roger Ian & Glueck Charles J.Public cholesterol screening: motivation for participation, follow-up outcome, self-knowledge, and coronary heart disease risk factor intervention. J Lab Clin Med. 1989;114(2):142–51.
19. Annoni GG, Bottasso RM, Ciaci D, Donato MF. & Tripoli A.Clinical Investigation of triglycerides. J Res Lab Med. 1982;9:115.
20. Assmann G. Tangier disease and the possible role of high density lipoproteins in atherosclerosis. Atherosclerosis Rev.1979; 6(1).
21. McNamara JR, Cohn JS, Wilson PW, Schaefer EJ. Calculated values for low densitylipoprotein cholesterol in the assessment of lipid abnormalities and coronary disease risk. Clin Chem. 1990;36(1):36–42.
22. Steel R, Torrie J. Principles and procedures of statistics; a Biomedical approach M C Graw-Hill Book Company. Toronto Rev Vet. 1997;13:6, 481.
23. Li SH, Liu XX, Bai YY, Wang XJ, Sun K, Chen JZ, Hui RT. Effect of oral isoflavone supplementation on vascular endothelial function in postmenopausal women: a meta-analysis of randomized placebo-controlled trials. AM J Clin Nutr. 2010;91(2):480–6.

24. Allahbadia GN, Merchant R. Polycystic ovary syndrome and impact on health. Middle East Fertil Soc J. 2011;16(1):19–37.
25. Rajan RK, Balaji B. Soy isoflavones exert beneficial effects on letrozole-induced rat polycystic ovary syndrome (PCOS) model through anti-androgenic mechanism. Pharm boil. 2017;55(1):242–51.
26. Khani B, Mehrabian F, Khalesi E, Eshraghi A. Effect of soy phytoestrogen on metabolic and hormonal disturbance of women with polycystic ovary syndrome. Journal of research in medical sciences: J Res Med Sci. 2011;16(3):297.
27. Cassidy A, Bingham S, Setchell KD. Biological effects of a diet of soy protein rich in isoflavones on the menstrual cycle of premenopausal women. Am J Clin Nutr. 1994;60(3):333–40.
28. Yuan XX, Zhang B, Li LL, Xiao CW, Fan JX, Geng MM, Yin YL. Effects of soybean isoflavones on reproductive parameters in Chinese mini-pig boars. J Anim Sci Biotechnol. 2012;3(1):31.
29. Hooper L, Ryder JJ, Kurzer MS, Lampe JW, Messina MJ, Phipps WR, Cassidy A. Effects of soy protein and isoflavones on circulating hormone concentrations in pre-and post-menopausal women: a systematic review and meta-analysis. Hum Reprod Update. 2009;15(4):423–40.
30. Liu K. Chemistry and nutritional value of soybean components. In: Soybeans. Boston: Springer; 1997. pp. 25–113.
31. Kurzer MS. Hormonal effects of soy in premenopausal women and men. J Nutr. 2002;132(3):570S–573S.
32. Foth D, Nawroth. Effect of soy supplementation on endogenous hormones in postmenopausal women. Gynecol Obstet. 2003;55(3):135–8.
33. Kim JJ, Choi YM. Dyslipidemia in women with polycystic ovary syndrome. Obstet Gynecol Sci. 2O13; 56(3), 137–142.
34. ROMÂNĂ A. Ultra-performance liquid chromatography method for the quantification of some phytoestrogens in plant material. Rev Roum Chim. 2010;55(8):459–65.
35. Zhan S, Ho SC. Meta-analysis of the effects of soy protein containing isoflavones on the lipid profile. Am J Clin Nutr. 2005;81(2):397–408.
36. Taku K, Umegaki K, Sato Y, Taki Y, Endoh K, Watanabe S. Soy isoflavones lower serum total and LDL cholesterol in humans: a meta-analysis of 11 randomized controlled trials. Am J Clin Nutr. 2007;85(4):1148–56.
37. Jassi HK, Jain A, Arora S, Chitra R. Effect of soy proteins vs soy isoflavones on lipid profile in postmenopausal women. *Indian* J Clin Biochem. 2010;25(2):201–7.
38. Romualdi D, Costantini B, Campagna G, Lanzone A, Guido M. Is there a role for soy isoflavones in the therapeutic approach to polycystic ovary syndrome? Results from a pilot study. FERTIL STERIL. 2008;90(5):1826–33.
39. Aubertin-Leheudre M, Lord C, Khalil A, Dionne IJ. Isoflavones and clinical cardiovascular risk factors in obese postmenopausal women: a randomized double-blind placebo-controlled trial. J Women's Health. 2008;17(8):1363–9.

40. Villa P, Costantini B, Suriano R, Perri C, Macrì F, Ricciardi L, Lanzone A. The differential effect of the phytoestrogen genistein on cardiovascular risk factors in postmenopausal women: relationship with the metabolic status. J Clin Endocrinol Metab. 2009;94(2):552–8.