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Therapeutic potential of sesamol and daidzein in letrozole-induced polycystic ovarian syndrome model in Wistar rats

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Current PCOS treatments may cause adverse effects and inadequately address endocrine and metabolic disturbances, warranting safer multitarget phytochemicals. This study evaluated the therapeutic potential of sesamol and daidzein in a letrozole-induced PCOS model in female Wistar rats. Acute oral toxicity studies were conducted in accordance with OECD guideline 425, based on which treatment doses were selected. PCOS was induced through a 21-day administration of letrozole (1 mg/kg body weight), followed by 14 days of treatment with sesamol (10 and 50 mg/kg) and daidzein (10 and 50 mg/kg), administered orally. Therapeutic potency was estimated through analysis of oestrous cycle regulation, fasting blood glucose, serum hormone concentrations, lipid profiles and histopathological examination of ovarian tissues. 1593 mg/kg was estimated as the LD_{50} of sesamol. Within the 14-day treatment period, sesamol and daidzein reduced elevated testosterone levels, improved oestrous cyclicity, and decreased ovarian cyst formation. At lower doses, oestrogen levels improved more noticeably, whereas changes in fasting blood glucose and lipid profile remained modest and inconsistent. Daidzein and sesamol especially revived hormonal balance and ovarian function, with limited impacts on metabolic parameters.

Keywords: Aromatase inhibitor model, Hyperandrogenism, Oestrous cyclicity, Ovarian histomorphology, Phytoestrogen, Wistar rat

Polycystic ovarian syndrome (PCOS) is an endocrine disease that affects 8% to 13% of women of reproductive age across the globe; however, there are variations, based on population demographics and diagnostic criteria[1,2]. PCOS is defined as a condition where there is hyperandrogenism, anovulation and PCO appearance, all of which have resulted in multiple health risks. It has become a major public health issue because of its link with the physical manifestations like hirsutism, acne and alopecia, along with the psychological impact like anxiety and depression.

The pathogenic process of PCOS is complicated and involves interaction among genetic susceptibility, environmental variables and lifestyle factors[2-4]. Insulin resistance is a prominent feature in the pathogenesis and progression of this disorder, and is present in 50–70% of women with PCOS[3]. The resulting hyperinsulinemia increases the ovarian androgen production, suppresses the follicular growth, and contributes to persistent anovulation and infertility. In addition, low-grade systemic inflammation with high levels of proinflammatory cytokines aggravates the endocrine and metabolic abnormalities of PCOS[4]. PCOS-like symptoms have also been described in animal species, especially in some dog and cat breeds, with irregular oestrous cycles and cystic ovarian abnormalities, which differs significantly from human PCOS.

The conventional management of PCOS has been mainly directed towards alleviating symptoms, with combination of oral contraceptives as the primary treatment for menstrual regulation and hyperandrogenism through the regulation of the menstrual cycle and suppression of androgen synthesis[5]. Anti-androgens like spironolactone and flutamide are often used for managing the other clinical manifestations such as hirsutism and acne. However, the adopted medical management are connected with significant adverse effects, including weight gain, mood alterations and an elevated risk of cardiovascular events. Furthermore, these drugs provide limited effectiveness

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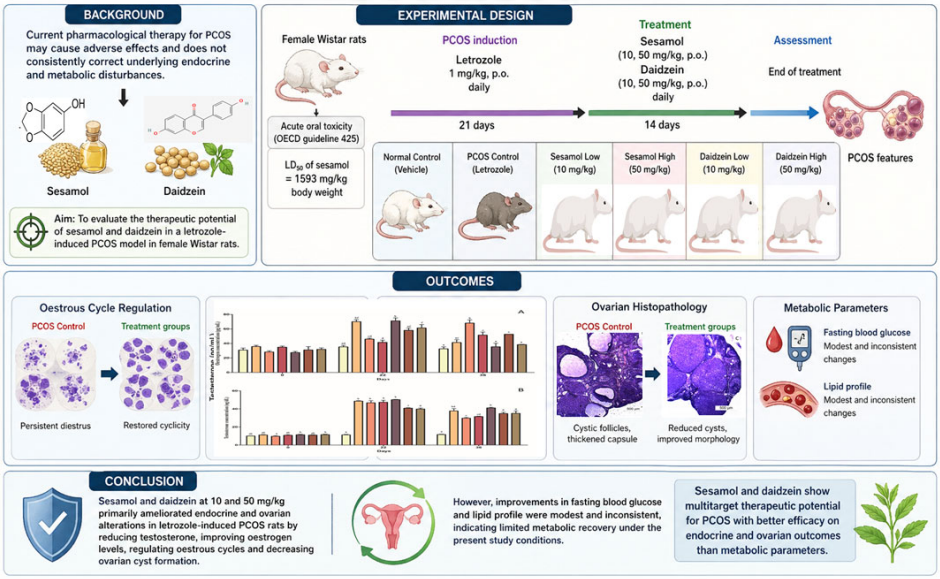

Graphical abstract

in correcting the fundamental metabolic abnormal functioning related with PCOS.

This gap has led to an increased interest in alternatives including the use of phytochemicals that have antioxidant, anti-inflammatory and hormone modulating capabilities. These plants derived bioactives target a number of molecular pathways involved in PCOS, and are being studied for their potential to treat PCOS with fewer side effects than the traditional therapies.

The diverse bioactivity and relevance to pathophysiology of PCOS makes sesamol and daidzein promising phytochemicals for management of PCOS. Sesamol is a phenolic lignan from sesame seeds with strong antioxidant and anti-inflammatory properties. It has been proven to attenuate oxidative stress and reduce chronic inflammatory cascades which are the major events involved in the pathogenesis and progression of metabolic and reproductive dysfunctions associated with PCOS.

Daidzein, which is abundantly present in soybeans, functions as a phytoestrogen through its binding capacity with oestrogen receptors and hormonal signalling. Daidzein increases the insulin sensitivity, lowers oxidative stress and modulates lipid metabolism, which helps to manage the metabolic and endocrine abnormalities caused by PCOS. These compounds work in different ways, are physiologically safe, and thus need to be studied further in animal models.

Hence, this study examined the efficacy of sesamol and daidzein in management of letrozole -induced PCOS in Wistar rats by investigating their influence on hormone levels, metabolic factors and ovarian histology.

Materials and Methods

Experimental animals

A total of fifty-one adult female Wistar rats, each weighing approximately 150–200 g, were procured from the Small Animal Breeding Station, College of Veterinary and Animal Sciences, Mannuthy, Thrissur, Kerala, India. All experimental procedures were conducted after obtaining approval from the Institutional Animal Ethics Committee (IAEC) of the institution with sanction number CVAS/MTY/IAEC/24/68. The animals were housed in well-ventilated polypropylene cages under standard laboratory conditions, which included a 12 h light/12 h dark cycle, ambient temperature of 25 ± 2°C, and relative humidity maintained between 50–70%. Animals had unrestricted access to standard pellet feed and filtered drinking water. A 14-day acclimatisation period was observed prior to the commencement of the study, and the experiment was carried out over a period of two months.

Test substances and chemicals

Letrozole (CAS No: 112809-51-5) was procured from M/s Chemland Industries, Gujarat, India. Sesamol (Cat. No: S3003-25G-A) and daidzein (Cat. No: HY-N0019) were purchased from M/s Sigma-Aldrich, India and M/s MedChemExpress, India,

respectively. Field's stain A (Cat. No: F30929) and Field's stain B (Cat. No: F31029) were obtained from M/s Nice Chemicals Pvt. Ltd., India.

Acute oral toxicity study of test compounds

As the no observed adverse effect level (NOAEL) of daidzein has been reported previously as 5000 mg/kg body weight in rats[6], no acute toxicity testing was conducted for daidzein in this study. A dose of 50 mg/kg body weight (1/100th of NOAEL) was selected as the upper treatment dose.

The acute oral toxicity of sesamol was evaluated following OECD Guideline 425 (Acute Oral Toxicity—Up-and-Down Procedure). Sesamol was suspended in a 0.5% carboxymethylcellulose (CMC) solution and administered orally using an 18-gauge oro-gastric feeding needle. Animals were fasted overnight prior to dosing, and the fasting body weight was recorded on the day of administration. Post-dosing, animals were observed continuously for the first 4 h and subsequently monitored twice daily for signs of toxicity and mortality, and once daily for clinical symptoms. Observations included behavioural changes and alterations in the skin, mucous membranes, respiratory, cardiovascular, central nervous, and somatomotor systems.

Sequential dosing was carried out in female Wistar rats at escalating dose levels of 175, 310, 550, 980, and 2000 mg/kg body weight at 24 h intervals. The test was terminated upon meeting the criteria for LD_{50} determination as per AOT425 guidelines.

***In vivo* efficacy of sesamol and daidzein in experimentally induced PCOS**

The dosing regimen for sesamol was established based on the determined LD_{50} value of 1593 mg/kg body weight. The highest dose selected was 50 mg/kg (approximately 1/30th of LD_{50}), while a lower dose of 10 mg/kg (approximately 1/150th of LD_{50}) was also tested. For daidzein, the highest dose chosen was 50 mg/kg body weight, corresponding to 1/100th of its reported NOAEL, with a lower dose set at 10 mg/kg. Both compounds were suspended in 0.5% CMC solution and administered orally via gavage.

Forty two female Wistar rats were randomly divided into seven groups, with six animals in each group. After a 14-day acclimatisation period, PCOS was induced in all groups except the normal control by administering letrozole (1 mg/kg body weight, orally) once daily for 21 consecutive days[7]. Treatment was initiated from day 22 and continued until day 36, as per the following groupings:

Group I: Normal control – received vehicle (0.5% CMC) only. Group II: PCOS model group – received letrozole without any post-treatment. Groups III: Standard treatment group - a 21-day letrozole cycle succeeded by clomiphene citrate (1 mg/kg body weight, orally). Groups IV and V: Low dose of sesamol treated group (10 mg/kg) and high dose of sesamol treated group (50 mg/kg), respectively. Groups VI and VII: daidzein low dose treated (10 mg/kg) and high dose treated (50 mg/kg) groups, respectively.

All treatments were given orally with freshly produced suspensions in 0.5% CMC. The animals were monitored for clinical symptoms, variation in body weight and other health indicators during the study.

On 0th, 22nd and 36th days, fasting blood glucose levels were evaluated by a calibrated glucometer (M/s Apollo Pharmacy Blood Monitoring System, APG01, India). Using an electronic precision balance, the body weights were documented at the same intervals and the mean weekly weight variation was evaluated[8].

The blood was collected on the 36th day under isoflurane anaesthesia, and the serum was segregated for biochemical analyses for the evaluation of oestrogen, testosterone, total cholesterol and triglycerides. For histopathological assessment, following euthanasia through ketamine overdose intraperitoneally, the ovaries were collected.

Vaginal cytology and analysis of oestrous cycle

Throughout the experimental duration, the vaginal smears were taken regularly from all animals to observe the progression and consistency of the oestrous cycle. Vaginal secretions were collected through lavage using 20 µL of sterile normal saline (0.9% NaCl), which was gradually inserted into the vaginal opening by a micropipette. The fluid was flushed in and out two to three times to dislodge the epithelial cells, which were then transferred onto clean glass slides.

Smears were air-dried, stained with Field's stain (Field's stain A and B; M/s Nice Chemicals Pvt. Ltd., India), and examined under a light microscope. The stage of the oestrous cycle was determined based on cytological characteristics: proestrus was characterised by nucleated epithelial cells, oestrus by anucleate cornified epithelial cells, metoestrus by a mix of leukocytes and epithelial cells, and dioestrus predominantly by leukocytes[9-11].

Serum hormone estimation

Blood samples were collected on days 0, 22 and 36 *via* tail vein under isoflurane anaesthesia. Serum was separated by centrifugation and stored at − 20°C until analysis. Serum 17β-estradiol levels were measured using a commercial ELISA kit (Rat 17β-Estradiol, GENLISATM ELISA, Ref: KLR1393; M/s KRISHGEN Biosystems, India) while serum testosterone concentrations were estimated using a rat-specific testosterone ELISA kit (Rat T, GENLISATM ELISA, Ref: KLR4465; M/s KRISHGEN Biosystems, India), following the manufacturer's instructions.

Lipid profile assessment

Serum total cholesterol and triglycerides were quantified using diagnostic kits procured from M/s Agappe Diagnostics Ltd., India. Analyses were conducted spectrophotometrically as per the manufacturer's protocol. The measurements were performed in triplicate to ensure accuracy and reproducibility.

Histopathological analysis

On the 36th day following euthanasia, both ovaries from each animal were cautiously extracted and instantly fixed in 10% neutral buffered formalin for a period of 48 h. By a standard paraffin-embedding protocol, the tissues were sliced serially at a thickness of 5 μm using a rotary microtome and mounted onto microscopic slides. The histological sections were stained using haematoxylin and eosin staining technique as per the procedure by Suvarna *et al*[12]. The prepared slides were examined under a compound light microscope for assessment of ovarian tissue organization, follicular development, presence of cystic follicles, corpora lutea and stromal integrity. Representative lesions were photographed.

Statistical analysis

The analysis of the collected data was done with the help of Statistical Software SPSS (IBMCorp.) version 24.0 (USA). Two-way repeated measures ANOVA was applied for analysis of variance of two independent variables with interaction. When significant differences were found, Duncan's multiple range test was used for pairwise comparisons between groups. Statistical significance was considered at the level of $P \leq 0.05$. Data were reported as means ± standard error (SE).

Results

Acute oral toxicity of sesamol

Oral administration of sesamol at the dose rate of 2000 mg/kg body weight was associated with intermittent tremors, lack of coordination, and respiratory distress within 5 min. Death occurred after 30 min of administration at the highest dose level. Administration of moderate doses of 310, 550, and 980 mg/kg in rats showed similar symptoms of toxicity. However, these symptoms were temporary, lasting only for three h. Administration of low dose of sesamol at 175 mg/kg, no signs of toxicity were observed.

LD_{50} determination

Based on the application of the AOT425StatPgm (version 1.0) software, the LD_{50} value of sesamol was found out to be 1593 mg/kg of body weight, thus affirming its acute toxicity level.

Histopathological evaluation following acute exposure

The viscera including liver, kidney, heart, brain, lung and uterus were analyzed by histopathology following the oral treatment of the rats with sesamol in doses of 310, 550, 980 and 2000 mg/kg body weight. The sections of the liver treated with the dose of 2000 mg/kg had minimal hepatocellular necrosis and vacuolation in cardiomyocytes while mild tubular epithelial degeneration was observed in kidney (Fig. 1a & b), while that from the dose of 980 mg/kg showed mild fatty change and minimal renal tubular epithelial degeneration (Fig. 2a & b). No

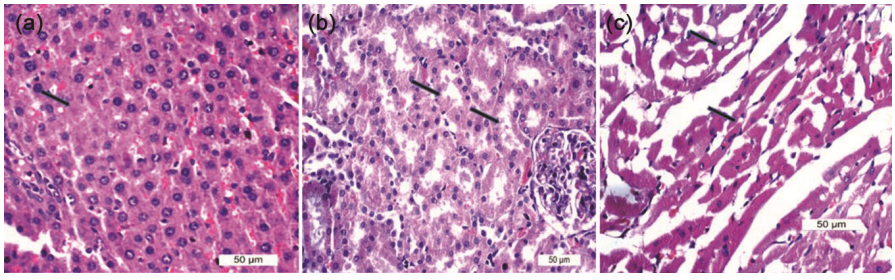

Fig. 1 — Histopathology of organs of animals treated with sesamol at 2000 mg/kg b. wt: (a) Liver, minimal necrosis (H&E, X400); (b) Kidney, mild tubular epithelial degeneration (H&E, X400); and (c) Heart, minimal vacuolation in cardiomyocytes (H&E, X400)

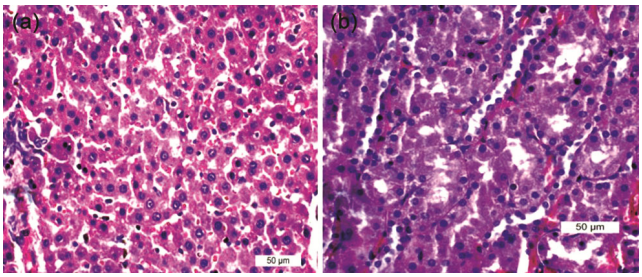

Fig. 2 — Histopathology of organs of animals treated with sesamol at 980 mg/kg b. wt: (a) Liver, mild fatty change (H&E, X400); and (b) Kidney, minimal tubular epithelial degeneration (H&E, X400)

observable histopathological lesions were identified in the heart, brain, lung, or uterus at any of the administered doses at 980, 550 and 310 mg/kg.

In vivo efficacy of sesamol and daidzein in experimentally induced PCOS

Exfoliative vaginal cytology

Microscopic examination of vaginal smears collected from rats in different experimental groups revealed characteristic patterns of the oestrous cycle. In the normal control group, the cycle followed the expected physiological sequence of proestrus, oestrus, dioestrus, and a return to proestrus. In the untreated PCOS group, a disruption in the cycle was observed, with animals showing a transition from dioestrus to proestrus, followed by oestrus, a return to dioestrus, and then proestrus again, indicating irregular cyclicity.

The standard treatment group, administered with clomiphene citrate, displayed a relatively regular sequence comprising proestrus, oestrus, metoestrus, and dioestrus. In the sesamol low-dose treated group (10 mg/kg), the cycle started with two days of proestrus, followed by oestrus and then dioestrus. The high-dose sesamol treated group (50 mg/kg) exhibited a pattern starting with proestrus, progressing to persistent oestrus over two consecutive days, and finally entering dioestrus on the fourth day.

The daidzein low-dose treated group (10 mg/kg) transitioned from proestrus to oestrus, followed by dioestrus and a subsequent return to proestrus. In the high-dose daidzein treated group (50 mg/kg), the animals followed a complete sequence of proestrus, oestrus, metoestrus and dioestrus. These observations indicated the partial restoration of cyclicity in the sesamol 10 mg/kg and daidzein 50 mg/kg groups, whereas persistent irregularity remained more evident in the untreated and high-dose sesamol treated groups.

During the third week of the study, corresponding to the late phase of letrozole administration, persistent dioestrus was observed in all groups except the normal control, which exhibited a complete oestrous cycle, confirming successful induction of a PCOS-like state in the affected groups.

Serum oestrogen concentration

Serum oestrogen levels were evaluated in all the seven experimental groups on days 0, 22, and 36. The data are depicted in (Fig. 3a). On day 0, oestrogen values were comparable across groups (27.46 ± 1.66 to 35.76 ± 1.70 pg/mL; $P > 0.05$). By day 22, the letrozole-induced groups showed increased oestrogen concentrations, ranging from 41.50 ± 3.10 to 71.17 ± 4.09 pg/mL, whereas the normal control remained at 35.34 ± 1.97 pg/mL. Groups 2, 5 and 7 showed a significant increase followed by a significant decline on day 36 ($P < 0.05$) whereas group 3 showed a progressive significant increase across time points ($P < 0.05$). Group 4 showed a significant increase only on day 36 ($P < 0.05$) while group 6 showed a significant increase followed by a non-significant decline ($P > 0.05$).

On day 36, the standard drug group showed the highest oestrogen concentration (68.08 ± 4.38 pg/mL), which was significantly higher than all the other groups ($P < 0.05$). The sesamol 10 mg/kg (51.54 ± 4.18 pg/mL) and daidzein 10 mg/kg (52.67 ± 0.18 pg/mL) treated groups showed significantly higher oestrogen concentration than the normal control (32.60 ± 2.69 pg/mL) at $P < 0.05$, whereas the oestrogen concentration in the untreated PCOS (41.27 ± 3.95 pg/mL), sesamol 50 mg/kg (35.41 ± 4.86 pg/mL), and daidzein 50 mg/kg (38.45 ± 0.31 pg/mL) treated groups remained comparable to the normal control ($P > 0.05$). Groups 2 and 5 showed comparable values on day 22 whereas groups 1, 2, 5 and 7 were comparable on day 36.

Thus, the lower doses of sesamol and daidzein produced better restoration of oestrogen than the corresponding higher doses, whereas clomiphene produced the greatest effect overall.

Testosterone concentration

Serum testosterone levels were measured on days 0, 22, and 36 across all the experimental groups, as shown in (Fig. 3b). On day 0, testosterone concentrations were comparable across groups (9.73 ± 0.64 to 11.50 ± 0.47 ng/dL; $P > 0.05$). On day 22, all letrozole-treated groups showed a significant increase ($P < 0.05$), reaching 39.93 ± 1.16 to 50.30 ± 0.36 ng/dL, whereas

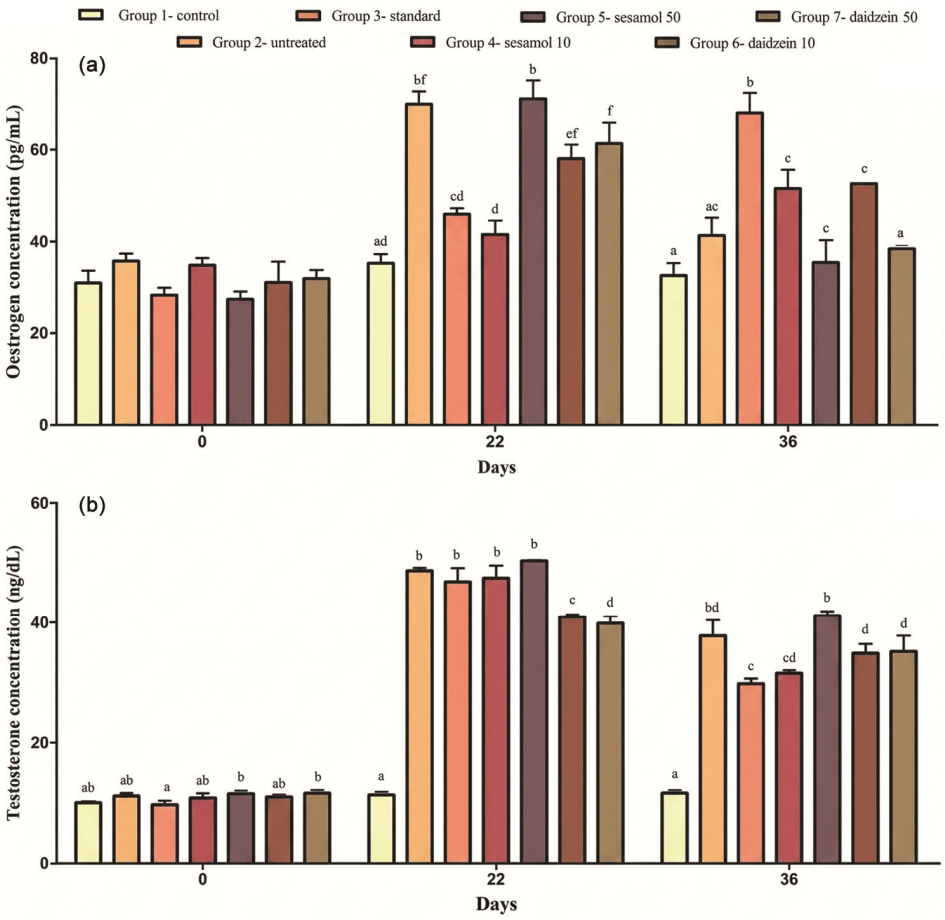

Fig. 3 — Effect of administration of various treatments on hormonal concentrations in Wistar albino rats: (a) oestrogen concentration, pg/mL; and (b) Testosterone concentration, ng/dL

the normal control remained at 11.27 ± 0.55 ng/dL. On day 22, groups 2, 3, 4 and 5 were comparable while groups 6 and 7 showed significantly lower testosterone than groups 2 to 5 ($P< 0.05$).

By day 36, even though the testosterone level declined significantly from day 22 in all the induced groups ($P< 0.05$), all the treated and untreated PCOS groups still remained significantly above the normal control value of 11.67 ± 0.35 ng/dL ($P< 0.05$). The standard drug group showed the lowest day 36 value (29.77 ± 0.88 ng/dL), followed by sesamol 10 mg/kg (31.57 ± 0.45 ng/dL), daidzein 10 mg/kg (34.90 ± 1.49 ng/dL), daidzein 50 mg/kg (35.10 ± 2.72 ng/dL), untreated PCOS (37.80 ± 2.66 ng/dL) and sesamol 50 mg/kg (37.87 ± 3.90 ng/dL) treated groups.

On day 36, the untreated PCOS and sesamol 50 mg/kg treated groups were comparable. Similarly, the standard drug and sesamol 10 mg/kg treated groups were also comparable on day 36. Likewise, daidzein 10 mg/kg and 50 mg/kg treated groups were comparable to each other. These data showed that sesamol at 10 mg/kg produced a clearer androgen lowering effect than sesamol at 50 mg/kg or daidzein at either tested dose.

These findings indicated that sesamol at the lower dose was more effective in ameliorating hyper-androgenism than the higher dose, and than that of daidzein at either tested dose.

Total cholesterol

Serum total cholesterol levels were assessed on days 0, 22, and 36, and the results are depicted in (Fig. 4a). On day 0, all groups were comparable, with values ranging from 39.23 ± 2.15 to 46.61 ± 1.53 mg/dL ($P> 0.05$). On day 22, group 2 showed a significant decrease relative to the normal mean of 40.61 ± 3.84 mg/dL (28.91 ± 1.47 mg/dL; $P< 0.05$), whereas groups 4 and 6 showed significant increases (59.49 ± 4.15 and 61.44 ± 3.80 mg/dL, respectively;

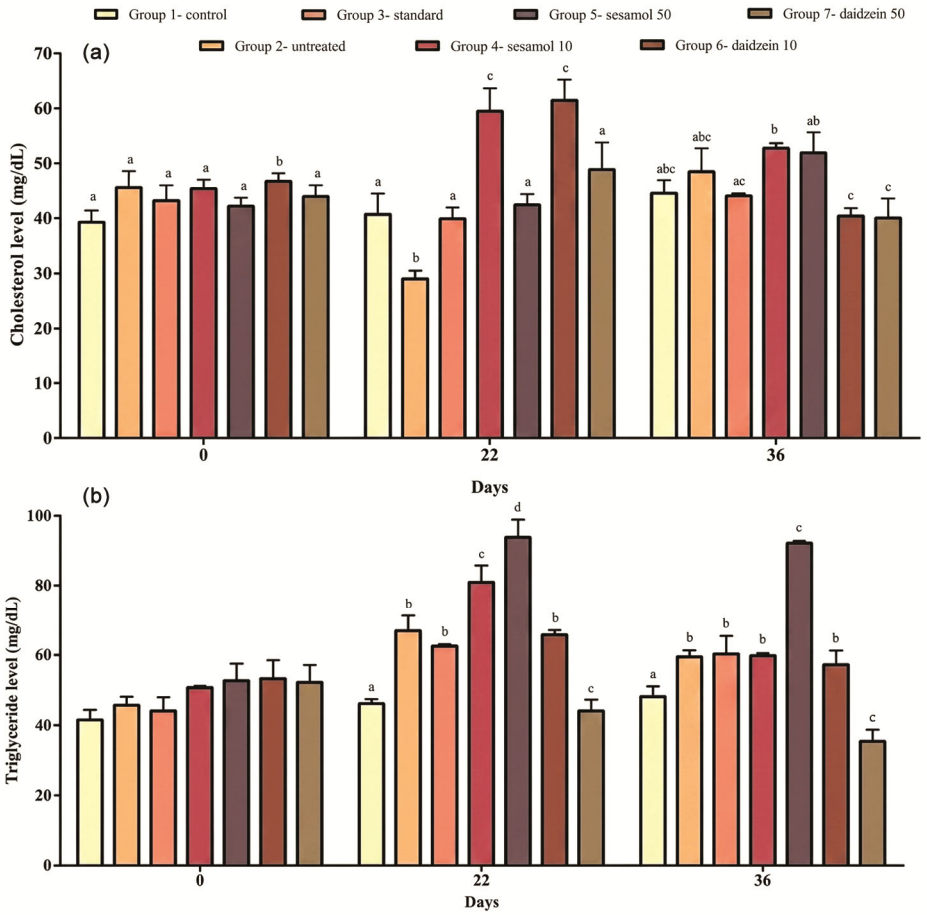

Fig. 4 — Effect of administration of various treatments on lipid profile in Wistar albino rats: (a) Total cholesterol level, mg/dL; and (b) Triglyceride level, mg/dL

$P< 0.05$). On day 22, groups 3, 5 and 7 did not differ significantly from the normal mean ($P> 0.05$).

By day 36, cholesterol values were 44.50 ± 2.42 mg/dL in the normal control, 48.37 ± 4.33 mg/dL in the untreated PCOS group, 43.99 ± 0.49 mg/dL in the standard group, 52.68 ± 0.89 mg/dL in the sesamol 10 mg/kg treated group, 51.85 ± 3.80 mg/dL in the sesamol 50 mg/kg treated group, 40.33 ± 1.46 mg/dL in the daidzein 10 mg/kg treated group, and 39.94 ± 3.63 mg/dL in the daidzein 50 mg/kg treated group. The total cholesterol values in the groups 4 and 5 were significantly higher than groups 6 and 7 ($P< 0.05$); the total cholesterol value in the group 4 was also significantly higher than the standard group ($P< 0.05$), while the total cholesterol values were comparable in groups 1, 2 and 5 ($P> 0.05$).

Thus, although the cholesterol graph showed some numerical variation, the pattern was not uniform and did not support a consistent treatment related metabolic improvement. The most notable day 36 deviation was the higher cholesterol value in the sesamol 10 mg/kg treated group relative to the standard drug treated group (52.68 ± 0.89 vs 43.99 ± 0.49 mg/dL; $P< 0.05$).

Overall, the cholesterol data suggested that effects on this metabolic parameter were modest and inconsistent under the present experimental conditions.

Serum triglycerides

Serum triglyceride concentrations were measured on days 0, 22, and 36, and the data are presented in (Fig. 4b). On day 0, values were comparable across all the experimental groups (41.41 ± 2.94 to 53.19 ± 5.13 mg/dL; $P> 0.05$). By day 22, groups 2, 3, 4, 5 and 6 showed significant increase ($P< 0.05$), with values of 66.79 ± 4.40, 62.39 ± 0.54, 80.89 ± 4.87, 93.73 ± 5.02 and 65.60 ± 1.53 mg/dL, respectively, whereas groups 1 and 7 remained comparatively lower at 46.01 ± 1.31 and 43.85 ± 3.33 mg/dL.

By day 36, triglyceride values were 47.94 ± 3.13 mg/dL in the normal control, 59.40 ± 1.73 mg/dL in the

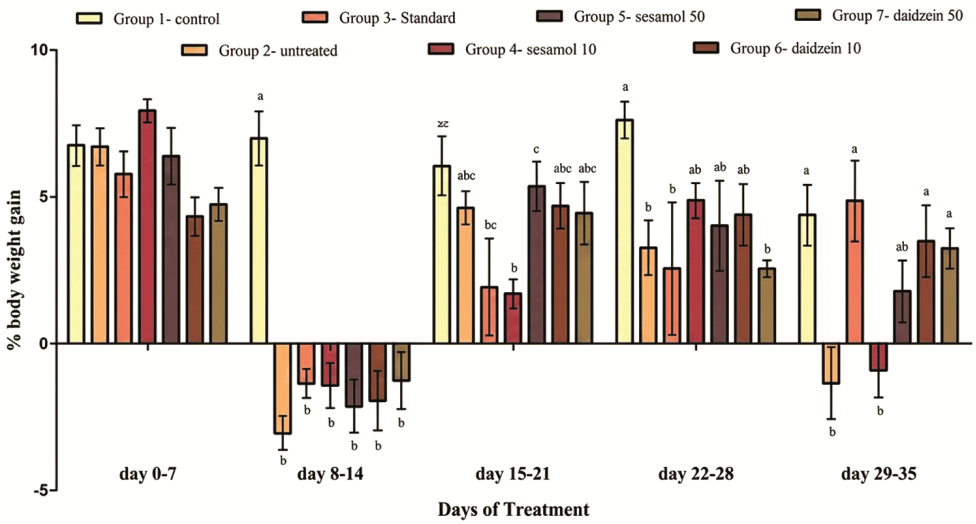

Fig. 5 — Mean weekly body weight gain of animals belonging to different treatment groups

untreated PCOS group, 60.05 ± 5.35 mg/dL in the standard drug treated group, 59.64 ± 0.61 mg/dL in the sesamol 10 mg/kg treated group, 91.99 ± 0.63 mg/dL in the sesamol 50 mg/kg treated group, 57.15 ± 3.84 mg/dL in the daidzein 10 mg/kg treated group, and 35.27 ± 3.39 mg/dL in the daidzein 50 mg/kg treated group. Groups 2, 3, 4 and 6 were significantly higher than the normal control group ($P< 0.05$), whereas group 7 was significantly lower than the normal control ($P< 0.05$). Group 5 showed the highest serum triglyceride value on day 36.

The serum triglyceride data showed considerable fluctuation and did not show a consistent therapeutic response. No consistent reduction in serum triglyceride levels were observed either in sesamol or daidzein treated groups over the 14-day treatment period despite the numerical increase observed in some groups.

Body weight changes

Body weight measurements were recorded at weekly intervals throughout the study, and the results are summarised in (Fig. 5). During the days 0 to 7, all the groups showed positive weight gain and intergroup differences were non-significant ($P> 0.05$), with values ranging from 4.32 ± 0.66% to 7.93 ± 0.39%; group 4 showed the highest body weight gain (7.93 ± 0.39%). During the days 8 to 14, all the groups except the normal control showed negative weight change, and all induced groups differed significantly from group 1 ($P< 0.05$). The untreated PCOS group showed the greatest reduction (-3.04 ± 0.58%), while the normal control maintained a positive gain of 6.99 ± 0.92%.

During the days 15 to 21, groups 1, 2, 5, 6 and 7 showed recovery with positive gains of 6.06 ± 1.00%, 4.62 ± 0.57%, 5.36 ± 0.85%, 4.69 ± 0.78% and 4.44 ± 1.06%, respectively, whereas groups 3 and 4 showed smaller body weight gains of 1.93 ± 1.65% and 1.70 ± 0.50% respectively. These intergroup differences were significant ($P< 0.05$). During days 22 to 28, most of the groups again showed positive body weight gain, ranging from 2.55 ± 0.29% to 7.61 ± 0.63%, but differences between groups were non-significant ($P> 0.05$).

During the final interval (days 29 to 36), groups 2, 4 and 5 differed significantly from the other groups ($P< 0.05$), with values of -1.34 ± 1.22%, -0.90 ± 0.93% and 1.78 ± 1.06%, respectively; however, these three groups did not differ significantly from one another ($P> 0.05$). Thus, the data of body weight does not show a clear or uniform treatment-dependent correction within the 14-day treatment period.

Fasting blood glucose level

The fasting blood glucose (FBG) level in all the experimental groups were recorded on days 0, 22 and 36, and represented graphically in (Fig. 6). The FBG on day 0 in all groups were similar, varying between 94.00 ± 2.78 to 105.17 ± 3.85 mg/dL ($p>0.05$). On day 22, the FBG was significantly high ($p<0.05$) in all the groups exposed to letrozole except the normal control group, varying between 131.50 ± 7.11 to 151.33 ± 1.86 mg/dL while in the normal control it was 99.83 ± 1.

Further, on day 22, the FBG levels dropped in all the induced groups and by day 36, the response was

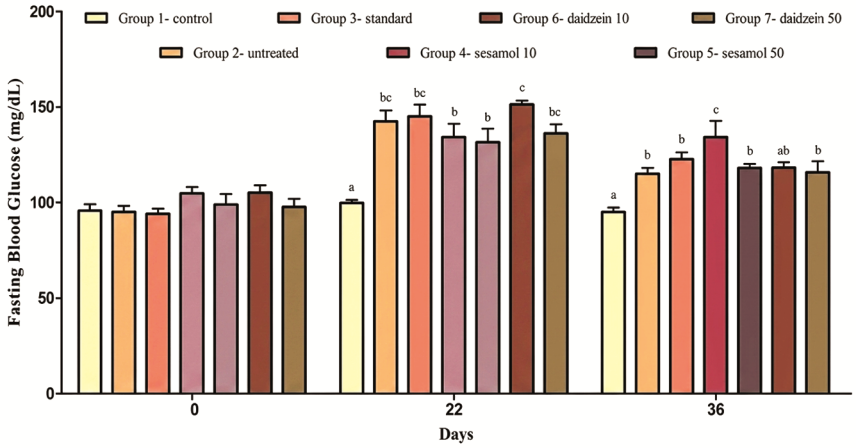

Fig. 6 — Effect of administration of various treatments on fasting blood glucose level in Wistar albino rats, mg/dL

therapy-dependent. The untreated PCOS group exhibited a FBG level of 115.00 ± 3.12 mg/dL whereas the standard group showed the FBG value of 122.50 ± 3.75 mg/dL. The sesamol 10 mg/kg, sesamol 50 mg/kg, daidzein 10 mg/kg and daidzein 50 mg/kg treated groups exhibited FBG levels of 134.00 ± 8.74 mg/dL, 118.00 ± 2.11 mg/dL, 118.17 ± 2.75 mg/dL, 115.67 ± 5.82 mg/dL respectively, whereas the normal control remained at 95.00 ± 2.22 mg/dL.

The FBG levels in groups 2, 3, 6 and 7 increased significantly at 22nd day and it decreased significantly at 36th day (P< 0.05). Groups 4 and 5 showed an increasing trend and then an insignificant change (P> 0.05). All the induced groups (treated and untreated) showed FBG levels which were significantly higher than normal control on day 36 (P< 0.05). FBG value was significantly higher in Group 4 as compared to the other induced groups (P< 0.05).

The data suggested that the effect of sesamol and daidzein on reducing the FBG was modest and inconsistent.

Histopathological evaluation

Histological examination of the ovarian samples showed considerable structural differences between the experimental groups. The ovarian sections of control group showed good preserved architecture including surface epithelium composed of a single layer of cuboidal cells. The cortex contained many follicles at different stages of development with distinct granulosa and theca layers. The medulla showed slight vascularization and good connective tissue integrity. Functional corpora lutea were present, represented by big luteinised cells with clear cytoplasm. The stroma appeared thick with a good network of vessels, without fibrosis, necrosis and inflammation (Fig. 7a).

In the untreated PCOS group, ovarian morphology was disrupted, showing multiple cystic follicles with thinning and degeneration of the granulosa cell layer, evidenced by pyknotic nuclei. The cyst walls exhibited thickened theca cell layers. Despite the presence of primordial, primary, and advanced secondary follicles, no mature Graafian follicles were observed. The ovarian stroma and surface epithelium, however, remained intact (Fig. 7b).

Sections from the standard drug treated group demonstrated the restoration of near-normal ovarian histoarchitecture. Different stages of follicular development, such as primordial, primary, secondary, and antral follicles, were present. There were distinct corpora lutea, which consisted of densely packed luteal cells containing large vacuoles. The stroma appeared to be normal, having well-developed vascular and surface epithelium systems (Fig. 7c).

Number of cysts in the sesamol 10 mg/kg treated group was relatively less when compared to the untreated group of PCOS. Different stages of follicular development, such as primordial, primary, and secondary follicles, were noted along with very slight degenerative changes in the granulosa cells. Formation of corpus luteum indicated recovery of ovulatory function and the stroma was cellular and vascularized (Fig. 7d).

In the sesamol 50 mg/kg treated group, cyst formation was reduced even more and there was improvement in the follicular development. There were follicles in all the stages ranging from primordial to mature antral type (Fig. 7e).

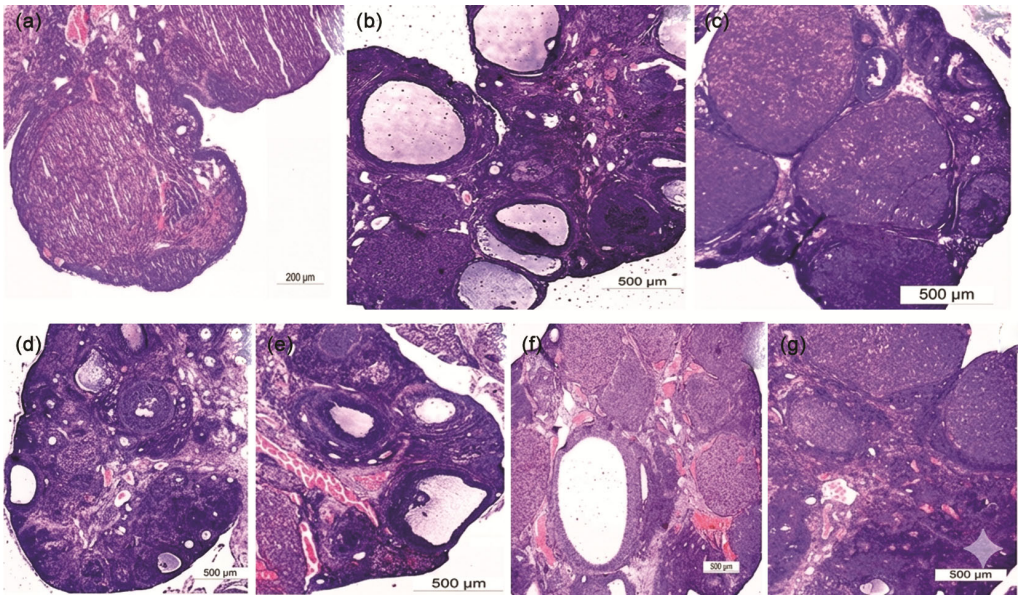

Fig. 7 — Histopathological images of the ovary of different treatment groups: (a) Normal control group- well preserved architecture with follicles at various stages of development (H&E, X100); (b) Untreated control group- multiple cysts of varying sizes lined by degenerating granulosa cells (H&E, X40); (c) Clomiphene group- apparently normal architecture (H&E, X40); (d) Sesamol 10 group- reduction in number of cysts as compared to group 2 (H&E, X40); (e) Sesamol 50 group- reduction in number of cysts and follicles at varying stages of development (H&E, X40); (f) Diadzein 10 group- luteal cysts lined by luteinised cells (H&E, X40); and (g) Diadzein 50 group- markedly reduced number of cysts and presence of multiple follicles and corpus lutea (H&E, X40)

The ovaries of the daidzein 10 mg/kg treated groups had cystic follicles and luteal cysts along with a number of corpora lutea and few follicles in their initial development phase. There was some degree of organization in the ovarian stroma (Fig. 7f). The ovaries of the daidzein 50 mg/kg treated group had many corpora lutea along with follicles in the initial and antral stage of development. Both the granulosa and theca cells were intact, which indicated normal follicular maturation (Fig. 7g).

These histological observations suggested that both sesamol and daidzein, especially at low concentrations, aid in the reversal of ovarian pathology caused by letrozole.

Discussion

PCOS can be defined as a syndrome exhibiting attributes such as hyperandrogenism, dysfunctional ovaries, and polyovulation. Studies are increasingly suggesting that there is a possibility of using phytochemicals for treating PCOS patients since they have multiple targets and exhibit minimal side effects compared to the conventional medicines. The efficacy of sesamol and daidzein, a phenolic lignan and soy derived isoflavone respectively, was studied in a widely used model of PCOS induced by letrozole in female Wistar rats.

The acute oral toxicity of sesamol was observed to be associated with severe signs of neurotoxicity at high doses (2000 mg/kg body weight), such as tremors and incoordination leading to immediate death of the animals. The LD_{50} value of sesamol was established to be 1593 mg/kg body weight. Khan *et al*. in C57BL/6 mice administered 2000 mg/kg of sesamol showed similar signs of acute toxicity with neurotoxic symptoms and death within 30 min[13]. At high concentrations, pro-oxidant properties of sesamol have been associated with mitochondrial death in HCT116 human colon cancer cells[14]. Thus, sesamol is considered safe at lower doses, but the potential neurotoxicity at supratherapeutic doses highlights the importance of established dose limits.

The histopathological analysis of the acute toxicity study also confirmed the same findings of toxicity as the rats administered higher doses of sesamol developed organ specific lesions mainly in liver, kidneys and heart. Necrosis and fatty degeneration were observed in the liver but tubular epithelial injury was observed in the kidney. Vacuolation of cardiomyocytes was observed in the cardiac tissues. The findings are in agreement with the findings of Khan *et al*. who reported similar degenerative changes in different organs after high dose of sesamol treatment[13]. The histopathological damage reported

appears to be mediated through sesamol-induced oxidative stress, lipid peroxidation and mitochondrial dysfunction. The present findings demonstrated the dualistic character of sesamol, where beneficial antioxidant effects at low doses might be nullified by cytotoxicity at higher exposures, thus warranting further chronic toxicity studies for determining the adequate therapeutic margins.

The PCOS-like features were successfully induced in rats by the 21-day letrozole injection, supporting the validity of this experimental paradigm. This model has been widely employed and is very well known for the capability of replicating important features of human PCOS including hyper-androgenemia, disruption in oestrous cycle and metabolic dysfunction. Letrozole is a non-steroid aromatase inhibitor which prevents androgens from converting into oestrogens; therefore, provides an environment which is very similar to pathophysiologic conditions in human PCOS.

Body weight dynamics provided additional evidence of letrozole-induced metabolic disruption in this study. All groups, except the normal control group, showed a transient loss of body weight during the 2nd week of letrozole therapy. Similar results have been reported in previous studies using letrozole-induced PCOS animals[18-20]. The transient weight loss might be due to initial hormonal imbalance and physiological stress. Importantly, weight gain was resumed in the last weeks especially in the untreated PCOS group confirming that the metabolic perturbations including obesity and insulin resistance occurred as the disease progressed[18-20].

Some of the variability in the body weight responses between humans and rodent models could be due to the inter species differences in basal metabolic rate and energy balance[21]. Moreover, it has been demonstrated that letrozole-induced PCOS without a high-fat diet may not reproduce the entire spectrum of metabolic alterations observed in human PCOS[22]. However, treatment with sesamol and daidzein attenuated the excessive weight gain during the recovery period, indicating that they might be involved in the stabilization of metabolic function.

Vaginal cytology confirmed the oestrous cycle disturbances induced by letrozole. All PCOS-induced groups developed persistent dioestrus from week 3 of letrozole therapy as also observed by Rajan *et al*.[19]. This cytological pattern is typical for anovulatory cycles and is one of the hallmark features of PCOS, and is remarkably similar to oligo- or amenorrhea in human patients[23,24]. It should be noted that the recovery of cyclical rhythms in the treated groups especially those treated with clomiphene and sesamol at 10 mg/kg reveals the potential of these treatments in normalizing reproductive hormonal communication.

Serum levels of oestrogens in the PCOS-induced groups changed significantly by day 22[25,26] revealing different sensitivity to the applied treatment. Even though clomiphene citrate had the best effect by day 36, it was only the low doses of sesamol and daidzein that resulted in increased oestrogen compared to the control. The effectiveness of both drugs decreased with increasing doses, implying a threshold dose for optimal hormonal regulation rather than a linear response of the effect to the dose. This contradicts the previous studies showing low concentrations of oestrogen in PCOS models[7,27]; this may indicate that compensating processes such as increased peripheral aromatisation or increased receptor sensitivity contributed to high concentrations of hormones found in this study.

Similarly, levels of serum testosterone were elevated significantly following letrozole injection and reduced after treatment in all groups. The effectiveness of clomiphene in reducing levels of androgens[28, 29] was greater than the effectiveness of sesamol at 10 mg/kg. In turn, sesamol at 50 mg/kg and daidzein at any of the doses, had no androgen reduction activity. This trend suggests that a hormetic effect is likely possible, where low levels would exert positive antioxidant or hormone regulation effects, whereas higher levels might reduce efficacy through receptor saturation or pro-oxidant effects[13,14]. However, these mechanistic explanations remain speculative and require targeted investigation.

Letrozole-induced PCOS was associated with significant elevations in FBG by day 22, consistent with previous reports indicating the development of glucose dysregulation in this model[30]. Although values declined after cessation of letrozole, no group returned to baseline by day 36, and treatment-related differences were small. Therefore, the present findings do not support a strong metabolic benefit of sesamol or daidzein on fasting glucose within the 14-day treatment period. Although studies have reported glucose lowering effects of sesamol and daidzein[31-33], the present findings suggest that endocrine and ovarian responses may occur earlier than measurable improvement in glucose, particularly in models not combined with a high-fat dietary challenge and under conditions of limited dose or treatment duration.

With regard to lipid metabolism, the present study showed only modest and inconsistent changes in serum total cholesterol. Apart from the higher cholesterol value in the sesamol 10 mg/kg treated group, most of the treatment groups did not differ significantly from the untreated PCOS group. These findings differ from studies reporting more pronounced dyslipidaemia in PCOS models, particularly when high-fat diets are combined with letrozole to intensify metabolic stress[34,35]. The absence of robust dyslipidaemic effects in the present study may be attributed to the use of a standard diet and the relatively short duration of PCOS induction. However, the elevated levels of cholesterol in the sesamol 10 mg/kg treated group can be viewed as the effects of the compound on lipids metabolic process, which may result from the modulation of HMG-CoA reductase or LDL receptor activities[36,37].

Similarly, serum concentrations of triglycerides did not show any consistent pattern of response to treatments. Although there were some numerical variations as well as variations between measurements done on days 22 and 36, concentrations of triglycerides remained within normal physiological values for rats (26-145 mg/dL) throughout the study[38]. Neither sesamol nor daidzein was able to enhance triglyceride levels better than the untreated PCOS group, suggesting that neither letrozole nor phytochemicals had any effect on lipids under the present experimental conditions. Studies have previously shown that combination of high fat diet with letrozole is more effective in reproducing hypertriglyceridemia of PCOS seen in humans[39-42]. Therefore, due to the lack of these diet-related stress factors, it is not possible to draw any definitive conclusions about the influence of sesamol and daidzein on lipids; however, the rather short period of 14 days of treatment may not have been enough for the effective normalization of lipids, despite the changes observed at hormonal levels and in histopathology.

Upon histological study of the ovaries, it was found that there were the usual PCOS lesions present in the untreated group, like multiple cysts filled with fluid, thin granulosa cell layer, and thickened theca cell layer[19,43]. The occurrence of these structural changes is usually caused by hyperandrogenemia, granulosa cell apoptosis, LH/FSH imbalance, and insulin resistance[44]. Administration of clomiphene citrate resulted in proper follicular structure, which includes well-formed antral follicles and corpora lutea. However, it is worth noting that sesamol and daidzein improved the morphology of ovaries, but their efficiency was non-linear in respect to doses in regard to all parameters. It was shown that the low dose treated group of sesamol experienced reduction in the number of cysts and the presence of follicles at different development stages, while the high dose treated group had a positive structural impact, but the hormonal results were worse. In turn, the daidzein treated group had a positive effect as well and experienced functioning corpora lutea and early antral follicles; however, this result was less noticeable compared to sesamol treatment at 10 mg/kg.

Conclusion

The present study observed the therapeutic potential of sesamol and daidzein in treating endocrine and ovarian disorders related to PCOS induced by letrozole in Wistar rats. The study revealed that the phytochemicals were able to improve oestrous cycles, prevent hyperandrogenism, partially regulate oestrogens, and improve ovarian histopathology. Significantly, sesamol treatment at 10 mg/kg proved to be more effective than the higher doses for hormone modulation. The histopathological analysis validated the biochemical observations, which revealed that there was reduced cyst formation but enhanced follicle formation with administration of both compounds. However, the changes brought about by these two compounds with respect to the level of fasting blood glucose and lipid profiles were moderate and inconsistent within the 14 days of treatment. This study revealed that sesamol and daidzein are potential phytotherapeutic agents that could be investigated in the preclinical studies of PCOS. Further investigations are required to determine the low dose benefit and to understand whether the long term treatment would lead to significant metabolic benefits.

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Conflict of interest

All authors declare no conflict of interest.

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