doi.org/10.36721/PJPS/2026.40.16193.1

Bergapten ameliorates metabolic, inflammatory and reproductive abnormalities in a high-fat diet- and letrozole-induced rat model of polycystic ovary syndrome

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Abstract: **Background**: Polycystic ovary syndrome (PCOS) is a multifactorial endocrine and metabolic disorder commonly associated with obesity, insulin resistance, oxidative stress and chronic inflammation. Despite current therapeutic approaches, effective agents targeting both metabolic and reproductive abnormalities remain limited. **Objectives**: The present study investigated the effects of bergapten on metabolic, hormonal, inflammatory and oxidative stress–related alterations in a high-fat diet (HFD) and letrozole-induced rat model of PCOS. **Methods**: In a randomized experimental design, female *Wistar* rats ($N = 42$) were allocated into seven distinct groups (n = 6/group): Normal Control, Letrozole Control, HFD + Letrozole Control, Metformin (300 mg/kg) and bergapten (5, 10 and 20 mg/kg). Obesity-associated PCOS was induced by administering a high-fat diet for 12 weeks combined with letrozole for 28 days. Afterwards, respective treatments were administered orally for 28 consecutive days. Body weight, oral glucose tolerance, serum lipid profiles, reproductive hormones, adipokines, oxidative stress biomarkers, inflammatory mediators, ovarian histopathology and gene expression changes were evaluated. **Results**: Bergapten produced dose-dependent improvements in metabolic parameters, with the 20 mg/kg dose exhibiting the greatest restoration of body weight, glucose tolerance and lipid profile abnormalities. The effects of the highest dose were comparable to those observed with metformin (300 mg/kg), whereas the 5 and 10 mg/kg doses demonstrated partial improvements. Hormonal disturbances, oxidative stress and inflammation were significantly ameliorated following bergapten administration. Bergapten demonstrated a favorable safety profile, with an oral LD_{50} > 2000 mg/kg and no clinical signs of toxicity, establishing a substantial safety margin (100- to 400-fold) above the highest therapeutic dose used in this study. **Conclusion**: Bergapten improved metabolic and reproductive parameters in a PCOS model by reducing inflammatory cytokines and regulating key gene pathways (*Nrf2/Keap1*, *PPAR-γ* and steroidogenic enzymes). Further studies are needed to confirm causation and clinical applicability.

Keywords: Bergapten; Nuclear factor erythroid 2-related factor-2; Oxidative stress; Obesity; Polycystic ovarian syndrome

Submitted 30-01-2026 – Revised 26-04-2026 – Accepted 25-05-2026

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INTRODUCTION

Polycystic ovarian syndrome (PCOS), also referred to as hyperandrogenic anovulation or Stein–Leventhal syndrome, is one of the most prevalent endocrine disorders affecting women of reproductive age. It is a chronic and heterogeneous condition clinically characterized by hyperandrogenism, menstrual irregularities, infertility, obesity and hirsutism (Singh *et al*., 2023). The global burden of PCOS is substantial, with an estimated 116 million women, representing approximately 3.4% of the female population, affected worldwide (Deswal *et al*., 2020). Due to its association with reproductive dysfunction, metabolic abnormalities and long-term cardiometabolic complications, PCOS poses a considerable health and socioeconomic burden. Although the disorder may develop from adolescence onward, diagnosis most commonly occurs between 20 and 30 years of age (Salari *et al*., 2024).

Obesity is a major metabolic comorbidity and an important aggravating factor in PCOS. Epidemiological evidence indicates that a substantial proportion of women with PCOS are overweight or obese, conditions strongly associated with infertility and endocrine dysfunction (Abinaya *et al*., 2019). Excess adiposity contributes to insulin resistance and hyperinsulinemia, which stimulate ovarian androgen production and disrupt normal follicular maturation. Moreover, obesity exacerbates reproductive abnormalities and increases the risk of associated complications, including menstrual dysfunction, infertility and cardiometabolic disorders (El Hayek *et al*., 2016; Shoaib *et al*., 2023). Clinical evidence further suggests that weight reduction can improve insulin sensitivity, restore ovulatory function and normalize endocrine balance in women with PCOS (Shaharyar *et al*., 2026).

The pathophysiology of PCOS is multifactorial and involves complex interactions among endocrine dysregulation, insulin resistance, chronic inflammation, oxidative stress and hyperandrogenism, ultimately

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disrupting the hypothalamic–pituitary–ovarian axis and impairing folliculogenesis (Ding *et al*., 2021). In obesity-associated PCOS, dysregulated adipokine signaling, characterized by reduced adiponectin and elevated leptin, tumor necrosis factor-α (TNF-α) and interleukin-6 (IL-6), further contributes to metabolic and inflammatory imbalance (Kim, 2024). Oxidative stress has also emerged as a key contributor to ovarian dysfunction by disrupting cellular homeostasis and worsening both metabolic and reproductive abnormalities (Amer *et al*., 2026).

At the molecular level, several signaling pathways are implicated in the progression of PCOS. The nuclear factor erythroid 2-related factor 2 (*Nrf2*)/Kelch-like ECH-associated protein 1 *(Keap1*) pathway is a major regulator of antioxidant defense and its dysregulation has been associated with increased oxidative stress and tissue injury. Similarly, peroxisome proliferator-activated receptor gamma (*PPAR-γ*) plays an important role in lipid metabolism and insulin sensitivity, whereas steroidogenic enzymes such as *CYP19A1* (aromatase) and *CYP11A1* regulate estrogen biosynthesis and ovarian steroidogenesis. Alterations in these pathways may contribute to the metabolic and hormonal disturbances observed in PCOS (Heidarzadehpilehrood *et al*., 2022; Goodarzi *et al*., 2011).

Bergapten (5-methoxypsoralen) is a naturally occurring furocoumarin present in medicinal plants of the Umbelliferae family, including *Cnidii Fructus*, *Angelica dahurica* and *Peucedanum ostruthium*, as well as in grapefruit juice and citrus essential oils. Previous studies have demonstrated the therapeutic potential of bergapten in metabolic and inflammatory disorders through insulin-sensitizing, lipid-regulating, antioxidant and anti-inflammatory effects (Liang *et al*., 2021; Aslam *et al*., 2022; Malik *et al*., 2024). Bergapten has also shown pharmacological activity in experimental inflammatory conditions, including anti-arthritic effects at doses of 5, 10 and 20 mg/kg in rats (Ahmad *et al*., 2025). Furthermore, it has been reported to modulate inflammatory mediators, including inducible nitric oxide synthase (iNOS), cyclooxygenase-2 (COX-2) and poly-ADP ribose polymerase (PARP), suggesting possible relevance in disorders characterized by oxidative stress and chronic inflammation. Recent reviews further support the therapeutic relevance of plant-derived bioactive compounds in PCOS through modulation of oxidative stress, inflammatory mediators and metabolic dysfunction, suggesting that phytochemicals may represent promising adjunctive approaches in PCOS management (Dashti *et al*., 2026; Shen and Liu, 2026).

Despite these promising pharmacological properties, the therapeutic potential and mechanistic role of bergapten in obesity-associated PCOS remain insufficiently investigated. In particular, its influence on oxidative stress–related signaling, inflammatory mediators and steroidogenic pathways in PCOS has not been comprehensively evaluated. Therefore, the present study aimed to investigate the effects of bergapten in a high-fat diet and letrozole-induced rat model of PCOS, with specific emphasis on gene expression changes associated with oxidative stress regulation, inflammation and steroidogenesis, including *Nrf2/Keap1*, *PPAR-γ, CYP19A1* and *CYP11A1* pathways.

MATERIALS AND METHODS

Chemicals and reagents

Analytical-grade chemicals and reagents were used throughout the study. Metformin was purchased from Martin Dow Pharmaceuticals, Pakistan. Letrozole (5 g) was obtained from Saffron Pharmaceuticals, Faisalabad, Pakistan. Bergapten (purity ≥98%) was procured from Biosynth s.r.o., Bratislava, Slovakia. Dimethyl sulfoxide (DMSO) and carboxymethyl cellulose (CMC) were purchased from Sigma-Aldrich, Dorset, UK. Formalin and chloroform were obtained from Merck, Darmstadt, Germany. Fresh solutions were prepared daily before administration.

Experimental animals

Forty-two healthy nulliparous female *Wistar* rats (100-150 g of weight and 4-6 weeks old) were acquired from the animal house of Government College University Faisalabad (GCUF) and kept in the animal house of the Department of Pharmacology, GCUF. Animals were maintained under standard laboratory conditions (25 ± 2 ^{o}C, 55 ± 5% relative humidity and a 12 h light/dark cycles) with free access to water and standard pellet diet. An acclimatization period of seven days was provided to adapt rats before start of experiment. All animal procedures were approved by the Research Ethical Committee of Government College University Faisalabad (Approval No. GCUF/ERC/633) and were conducted in accordance with institutional and National Research Council guidelines.

A formal and prior sample size calculation was not performed. Group sizes were selected based on previously published preclinical studies using comparable letrozole and high-fat diet-induced PCOS rat models and in accordance with institutional ethical recommendations to minimize animal use while maintaining experimental validity (Xu *et al*., 2020; Huang *et al*., 2024).

Acute toxicity study

Acute oral toxicity of bergapten was evaluated according to OECD guideline 423. Female Wistar rat (n=6) were administered a single oral dose of bergapten (2000 mg/kg, p.o.) suspended in 0.5% CMC. Following administration, animals were continuously observed during the first 4 h and periodically during the first 24 h for any signs of

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toxicity, including changes in skin and fur appearance, eyes and mucous membranes, behavioral patterns, tremors, convulsions, salivation, lethargy, sleep, coma, food and water intake and mortality. Animals were further monitored daily for 14 days for delayed toxic effects and mortality. Body weight changes and general health status were also recorded throughout the observation period (Andleeb *et al*., 2022; Raza *et al*., 2025).

Obesity and PCOS induction

Animals were randomly allocated into two experimental groups using a simple randomization method.

Inclusion and exclusion criteria

Healthy nulliparous female Wistar rats weighing 100–150 g with regular estrous cycles were included at Day 0. After induction, obesity/PCOS was confirmed by a Lee index >320 and persistent estrus on vaginal cytology. Animals we excluded in case of illness, abnormal weight, death, excessive weight loss, dosing problems, or unsuitable samples. No animals met these criteria, so all animals were included in the final analysis. Before starting experiment, body weight of animals were measured in grams. The Group A (n = 12) were maintained on a standard pellet diet. Group B (n = 30) were administered high fat diet (HFD) for 69 days to induce obesity. The high-fat diet consisted of 60% kcal from fat, 20% kcal from carbohydrates and 20% kcal from protein (Reddy *et al*., 2016, Begum *et al*., 2022). A high-fat diet (HFD) was prepared in the laboratory to induce metabolic disturbances associated with the experimental PCOS model. The diet was formulated using casein (200 g), corn starch (3.0 g), sucrose (72.5 g), tallow (177.5 g), soybean oil (25 g), wheat bran (300 g) and choker bran (222 g) per 1 kg of diet. Thus, the formulation consisted of approximately 20.0% casein, 0.3% corn starch, 7.25% sucrose, 17.75% tallow, 2.5% soybean oil, 30.0% wheat bran and 22.2% choker bran (w/w).

Preparation of high-fat diet

All dietary ingredients were weighed accurately according to the specified formulation. The dry ingredients, including casein, corn starch, sucrose, wheat bran and choker bran, were thoroughly mixed to obtain a homogeneous dry mixture. Tallow was gently warmed until sufficiently softened/melted and was then combined with soybean oil. The lipid mixture was gradually incorporated into the dry ingredients with continuous mixing to ensure uniform distribution of the fat throughout the diet. An appropriate amount of water was subsequently added to obtain a cohesive dough-like mixture. The prepared diet was thoroughly kneaded and shaped into small pellets. The pellets were allowed to dry at room temperature until they attained a suitable consistency and were then stored in clean, airtight containers until use. The HFD was provided ad libitum to the designated animals throughout the dietary induction period.

Following HFD regimen, all animals were weighed weekly and obesity status was determined using Lee obesity index with following formula.

$$\text{Lee Index} = (\sqrt[3]{[\text{Body weight (g)}]} / \text{Naso-anal length [cm]}) \times 1000$$

Animals with a Lee index > 320 were classified as obese and selected for further study (Shoaib *et al*., 2023).

After 69 days Group A was divided in normal control and letrozole control. Normal control was given routine di*et al*ong with oral dose of 5 ml/Kg of 0.5 % carboxymethyl cellulose as vehicle. Letrozole control was given 1 mg/kg letrozole treatment for 28 days. While group B was also administered with 1 mg/kg letrozole along with high fat diet for further 28 days and named as HFD + letrozole group (Hussain *et al*., 2023, Begum *et al*., 2022). After 21 days of giving letrozole to both groups (letrozole control and HFD + letrozole) vaginal smears were taken to observe estrous cycle. Persistent diestrus/metestrus, defined as the presence of leukocyte-predominant vaginal smears for at least 7 consecutive days with absence of normal cyclic progression, was confirmed by vaginal cytology, indicating successful disruption of estrous cyclicity. Vaginal cytology performed and the estrous cycle was confirmed after 28 days. The weight deviation record was also being taken on a weekly basis. Moreover, blinding was not performed during treatment administration, outcome assessment or data analysis.

Experimental design

After successful induction of PCOS and obesity, normal control was continued with routine diet and letrozole control was given letrozole for another 28 days. Whereas, HFD+ letrozole group was further subdivided into 5 groups (n=6). HFD+ letrozole group was treated with high fat diet (HFD) and letrozole (LT). Metformin 300 mg/kg was given metformin (Standard control), BA 5 mg/kg was treated with bergapten 5mg/kg, BA 10mg/kg with bergapten 10 mg/kg and BA 20mg/kg with bergapten 20 mg/kg for 28 days. The doses of bergapten (5, 10 and 20 mg/kg) were selected on the basis of previously published studies demonstrating pharmacological efficacy at multiple dose levels within this range in rodent models of metabolic and inflammatory disorders. These doses were chosen to assess the relationship between dose and therapeutic response (Aslam *et al*., 2022; Liang *et al*., 2021; Malik *et al*., 2024). This dose range was chosen to evaluate a dose-response relationship within pharmacologically relevant and safe boundaries as confirmed by acute toxicity testing. Flow diagram of experimental design is shown in figure. 1.

Oral glucose tolerance test (OGTT)

After 28 days of drug treatment, rats were fasted overnight (12h). Thirty minutes after administration of ongoing treatment, 2 g/kg glucose solution was administered orally. Blood samples were collected from tail tip using

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EVOCHECK GM700S electronic glucometer and readings were recorded at 0, 60, 120 and 180 minutes respectively.

Biochemical testing

After completing treatment period, animals were anesthetized by pentobarbital sodium (40 mg/kg, i.p) and blood samples were collected by cardiac puncture. Serum was obtained by centrifugation at 4000 rpm for 10 min. Serum samples were tested for lipid profile including total cholesterol (TC), triglycerides (TG) and high-density lipoproteins (HDL) according to manufacturer's guidelines (DiaSys Diagnostic Systems, Germany). LDL was calculated by deducting HDL from TC (Iqbal *et al*., 2025).

Hormone level testing

Moreover, levels of hormones including follicle-stimulating hormone (FSH), serum insulin, testosterone, luteinizing hormone (LH), progesterone (Boditech Med Inc, Korea) and estradiol (Shanghai Coon Koon Biotech Co. Ltd, China) were determined by using commercially available ELISA kits (Elabscience®, China), according to manufacturer's instructions.

Estimation of oxidative stress biomarkers

Oxidative stress status was evaluated in serum by determining the activity of catalase (CAT; Catalog No. E-BC-K106, Elabscience®, China) and superoxide dismutase (SOD; Catalog No. E-BC-K020, Elabscience®, China), as well as the levels of glutathione (GSH; Catalog No. E-EL-R2491, Elabscience®, China) and malondialdehyde (MDA; Catalog No. E-EL-0060, Elabscience®, China), according to the manufacturer's protocols. Absorbance was measured using a microplate ELISA reader (BioTek Instruments Inc., USA).

Estimation of ovary weight index (OWI)

The anesthetized animals were euthanized through cervical dislocation; ovaries were carefully removed. Ovaries were cleaned and weighed properly by using weighing balance (Begum *et al*., 2022). Afterwards, OWI was calculated by following formula:

OWI= Ovary weight (mg)/ Body weight (g)×100

Histopathological analysis

Following sacrifice, ovaries were excised, rinsed with ice-cold normal saline and fixed in 10% formalin for 24 h. Fixed tissues were processed routinely, embedded in paraffin wax and sectioned at 5 µm thickness using a rotary microtome. Tissue sections were mounted on glass slides, deparaffinized in xylene, rehydrated through graded ethanol and stained with hematoxylin and eosin (H&E). After dehydration and mounting, sections were examined under a photomicroscope (Meiji Techno®, USA) at 40× magnification. Histopathological evaluation was qualitative and included assessment of ovarian architecture, follicular development, cystic follicles and corpus luteum formation as previously described (Younas *et al*., 2022).

Quantification of genetic biomarkers

Gene expression of the target genes was quantified using qRT-PCR in blood.

Total RNA was extracted from blood samples with TRIzol reagent and its concentration and purity were evaluated spectrophotometrically using a NanoDrop system. Reverse transcription into cDNA was carried out using the WizScript® cDNA Synthesis Kit (W2202). Amplification was performed on a Bio-Rad® real-time PCR system using 2-Sybr qPCR reagents (GeneAll®, Korea). Each reaction mixture consisted of 1 µL each of forward and reverse primers, 1 µL cDNA template, 10 µL SYBR Green master mix and 7 µL nuclease-free water. Thermal cycling conditions comprised 40 cycles of denaturation at 95°C, annealing at 60°C and extension at 72°C. Relative expression of target genes were determined by applying the 2−ΔΔCt method and given as below (Iqbal *et al*., 2025).

ΔCt = Ct target- Ct reference

$\Delta\Delta Ct = \Delta Ct$ sample-ΔCt control

Relative expression = $2^{-\Delta\Delta Ct}$

GAPDH was used as housekeeping gene and normal control group used as calibrator. Primer sequences were designed using Primer BLAST. In addition, primer specificity was assessed using *in-silico* validation and PCR for *in-vitro* validation, which confirmed single product amplification and validated using standard PCR conditions. The sequence of primers was given in table 1.

Statistical analysis

Data were analyzed using one-way ANOVA for parameters measured at a single time point across groups, followed by Tukey's post-hoc test. Two-way ANOVA was applied for body weight and OGTT data, where both treatment group and time were independent variables. OGTT data represent repeated measurements over time (0, 60, 120, 180 min) and were analyzed using two-way repeated-measures ANOVA to account for within-subject temporal correlation, followed by Tukey's post-hoc test. All analyses were performed using GraphPad Prism version 8.01 (GraphPad Software, Inc., San Diego, California, USA). Statistical significance was set at $p < 0.05$.

RESULTS

Effect on acute toxicity

No mortality or clinical signs of toxicity were observed at the limit dose of 2000 mg/kg, indicating that the LD_{50} of bergapten more than 2000 mg/kg.

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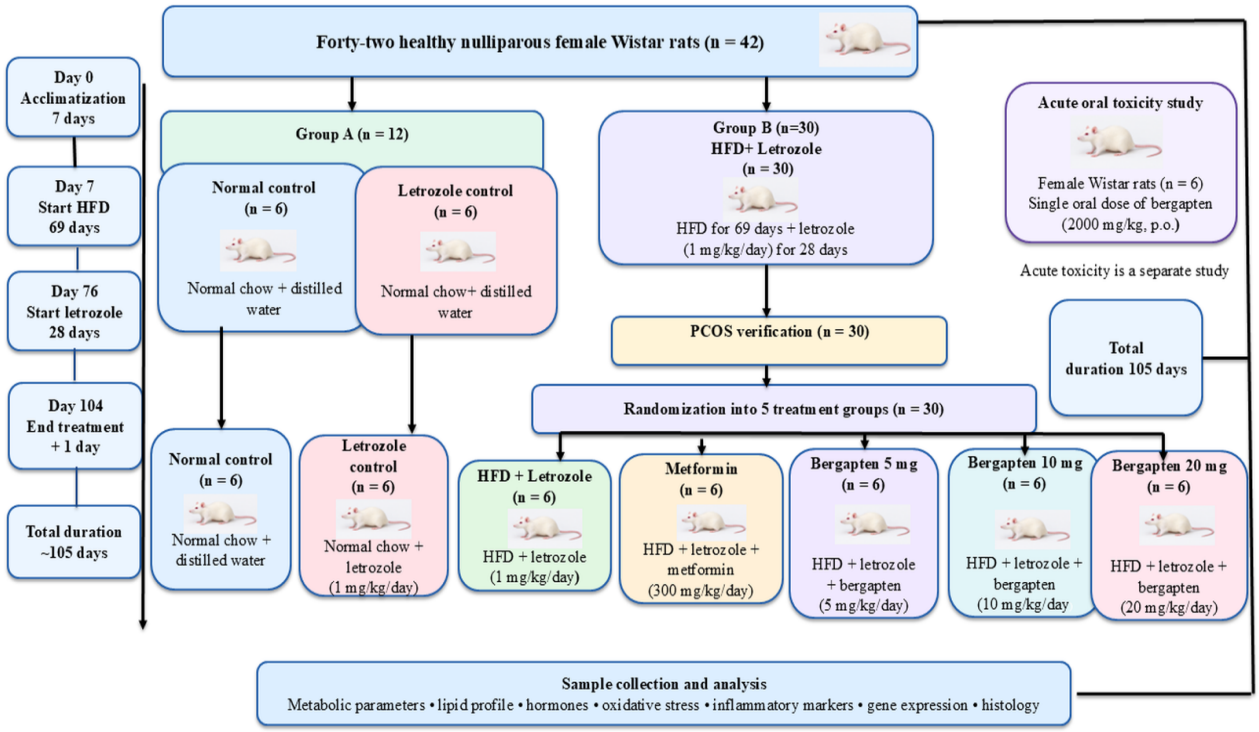

Fig. 1: Schematic representation of the experimental design used to establish the polycystic ovary syndrome (PCOS) model and evaluate the effects of bergapten (BA).

Table 1: Forward/reverse primers used for genetic biomarkers analysis by RT-PCR.

Marker	Sequence (5'->3')	Reverse/Forward	Product length (bp)	Gene accession number
Cyp11A1	TCAGCGATGACCTATTCCGC	Forward	168	NM_017286.3
	AGTCTGGAGGCATGTTGAGC	Reverse		
Cyp19A1	GGAGCCACAGCCAAACTACT	Forward	160	NM_017085.3
	CACAGTTTCCGCGACCATGA	Reverse		
Keap1	TTCGCCTACACGGCCTC	Forward	156	NM_057152.2
	GAAGTTGGCGATGCCGATG	Reverse		
IL-6	GCCCTTCAGGAACAGCTATGA	Forward	80	NM_012589.2
	TGTCAACAACATCAGTCCCAAGA	Reverse		
TNF-α	ATGGGCTCCCTCTCATCAGT	Forward	106	NM_013693.3
	GCTTGGTGGTTTGCTACGAC	Reverse		
PPAR- γ	AGCATGGTGCCTTCGCTGAT	Forward	236	NM_013124.3
	AGCAACCATTGGGTCAGCTC	Reverse		
GAPDH	TGCCACTCAGAAGACTGTGG	Forward	85	NM_017008.4
	GGATGCAGGGATGATGTTCT	Reverse		
Nrf-2	GCACATCCAGACAGACACCA	Forward	168	NM_031789.3
	CTCTCAACGTGGCTGGGAAT	Reverse		
IL-8	TCGCCAATGAGCTGCGCTGT	Forward	167	NM_030845.2
	GGGACACCCTTTAGCATCTT	Reverse		

Effect on body weight, ovary weight index

HFD + letrozole administration significantly increased body weight compared with the normal control, confirming successful induction of obesity-associated PCOS (Fig. 2A). Increased body weight was also observed in the letrozole control group. Treatment with bergapten and metformin significantly reduced body weight relative to the HFD + letrozole control, with bergapten demonstrating a dose-dependent improvement and greater efficacy at higher doses.

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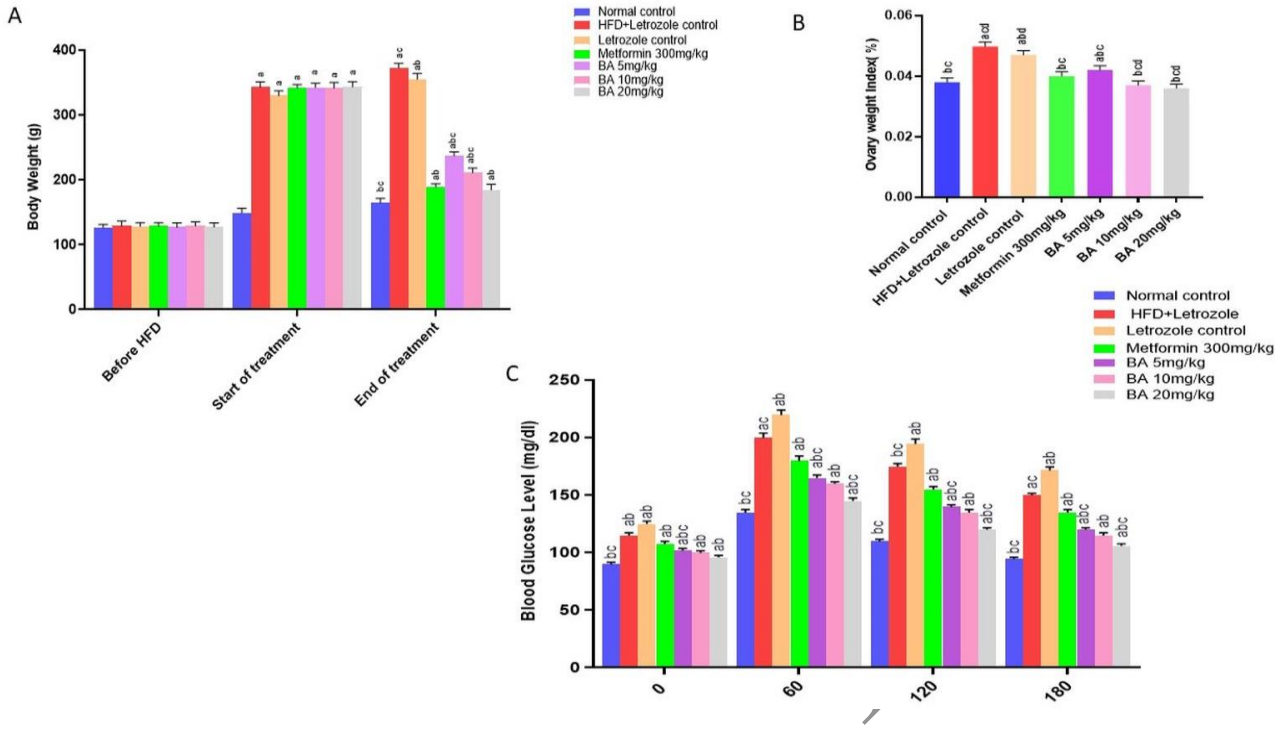

Fig. 2: Effect of bergapten on (A) body weight, (B) ovary weight index and (C) oral glucose tolerance test in obese-PCOS rats. Results as mean ± SD (n = 6); two-way ANOVA for body weight and OGTT; one-way ANOVA for ovary weight index followed by Tukey's test. (a) significant difference compared with normal control group; (b) compared with HFD + letrozole group; and (c) standard control group (metformin 300 mg/kg).

Similarly, the ovary weight index (OWI) was significantly elevated in the HFD + letrozole control group compared with normal animals, indicating ovarian alterations associated with PCOS induction (Fig. 2B). Metformin and bergapten treatment significantly attenuated these changes, with bergapten-treated groups showing improved ovarian weight indices.

Effect on OGTT

Data of OGTT revealed impaired glucose regulation in the HFD + letrozole group, as reflected by significantly elevated blood glucose levels at 60, 120 and 180 min compared with the normal control (Fig. 2C). Bergapten and metformin treatment significantly improved glucose tolerance, with reduced glucose concentrations observed at all measured time points. Elevated glucose levels were also observed in the letrozole control group.

Effect of bergapten on lipid profile

HFD + letrozole administration significantly disrupted lipid homeostasis, as evidenced by increased LDL, VLDL, total cholesterol, triglycerides, total lipids and cholesterol/HDL ratio, along with reduced HDL levels compared with the normal control group, confirming the development of dyslipidemia (Figs. 3A–G). Similar but less pronounced alterations were observed in the letrozole control group. Metformin treatment significantly improved lipid abnormalities relative to the HFD + letrozole control. Likewise, bergapten treatment dose-dependently ameliorated dyslipidemia, evidenced by significant reductions in LDL, VLDL, total cholesterol, triglycerides, total lipids and cholesterol/HDL ratio, accompanied by increased HDL concentrations (Fig. 3A–G). Higher bergapten doses (10 and 20 mg/kg) demonstrated greater efficacy and showed responses comparable to the standard control for several lipid parameters.

Effect of bergapten on hormones and adipocytokines

The effects of bergapten on serum reproductive hormones are presented in Figure. 4A–E. HFD + letrozole administration significantly disrupted hormonal balance, characterized by reduced follicle-stimulating hormone (FSH), progesterone and estrogen levels, alongside elevated luteinizing hormone (LH) and testosterone concentrations compared with the normal control group. Similar hormonal disturbances were observed in the letrozole control group.

Metformin treatment significantly improved hormonal alterations relative to the HFD + letrozole control. Likewise, bergapten administration dose-dependently restored hormonal balance, evidenced by increased FSH, progesterone and estrogen levels and reduced LH and testosterone concentrations (Fig. 4A–E). Higher bergapten doses (10 and 20 mg/kg) demonstrated greater efficacy, with responses approaching those of the metformin-treated group for several hormonal parameters. The effects of bergapten on adipocytokines are shown in figures. 4F–G.

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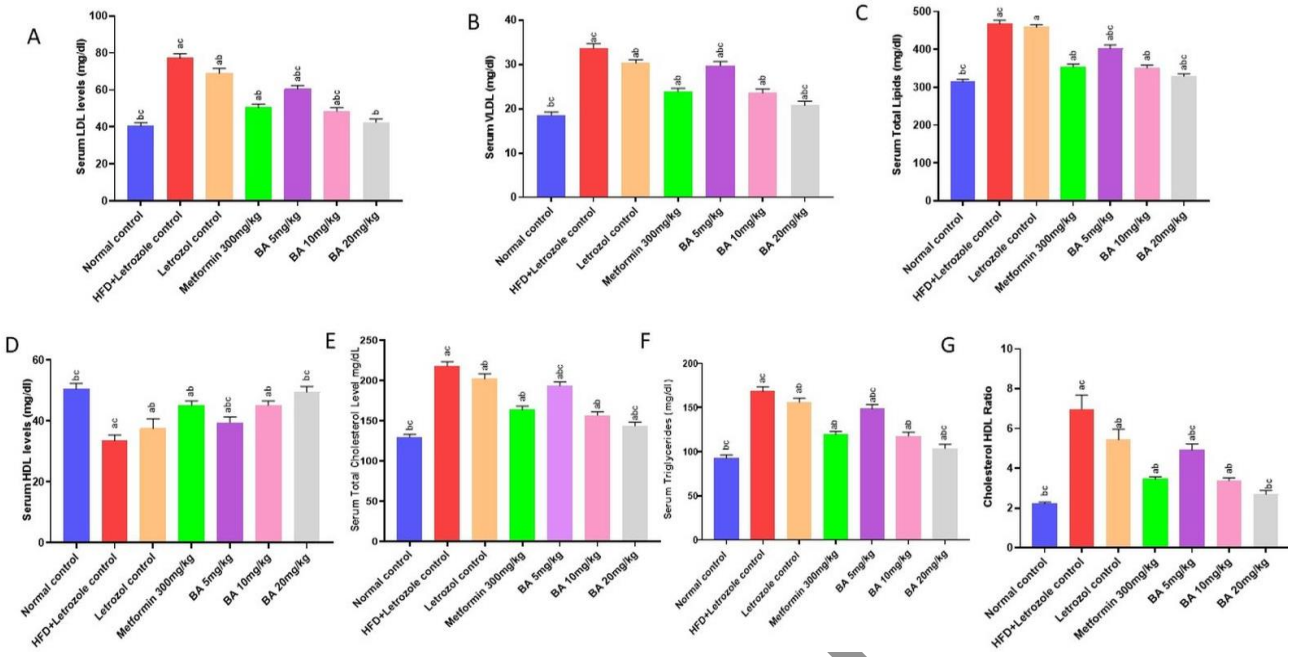

Fig. 3: Effect of bergapten treatment on level of (A) LDL; (B) VLDL; (C) total lipids; (D) HDL; (E) cholesterol; (F) TG; and (G) cholesterol/HDL ratio in obese-PCOS rats. Results as mean ± SD (n = 6); one-way ANOVA; Tukey's test. (a) significant difference compared with normal control group; (b) compared with HFD + letrozole group; and (c) standard control group (metformin 300 mg/kg).

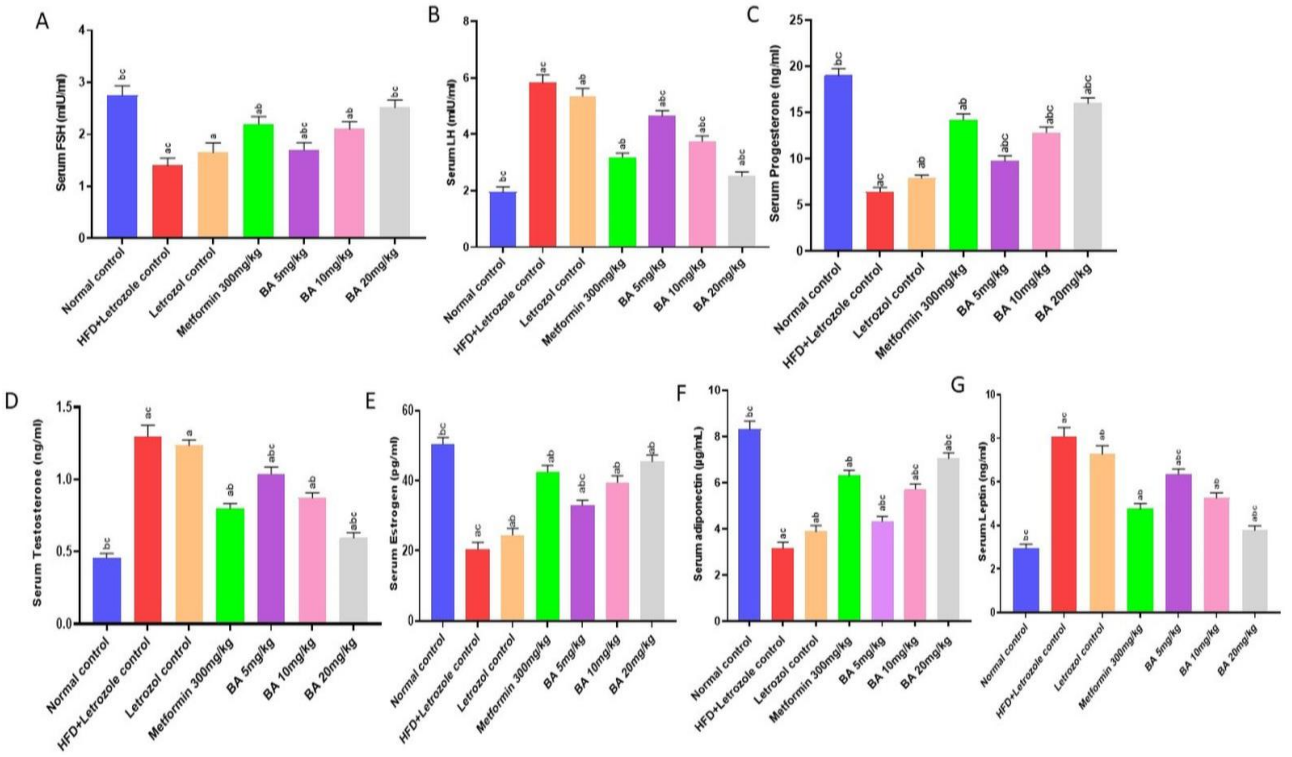

Fig. 4: Effect of bergapten treatment on level of (A) FSH; (B) LH; (C) progesterone; (D) testosterone; and (E) estrogen; (F) Adiponectin; (G) Leptin in obese-PCOS rats. Results as mean ± SD (n = 6); one-way ANOVA; Tukey's test. (a) significant difference compared with normal control group; (b) compared with HFD + letrozole group; and (c) standard control group (metformin 300 mg/kg).

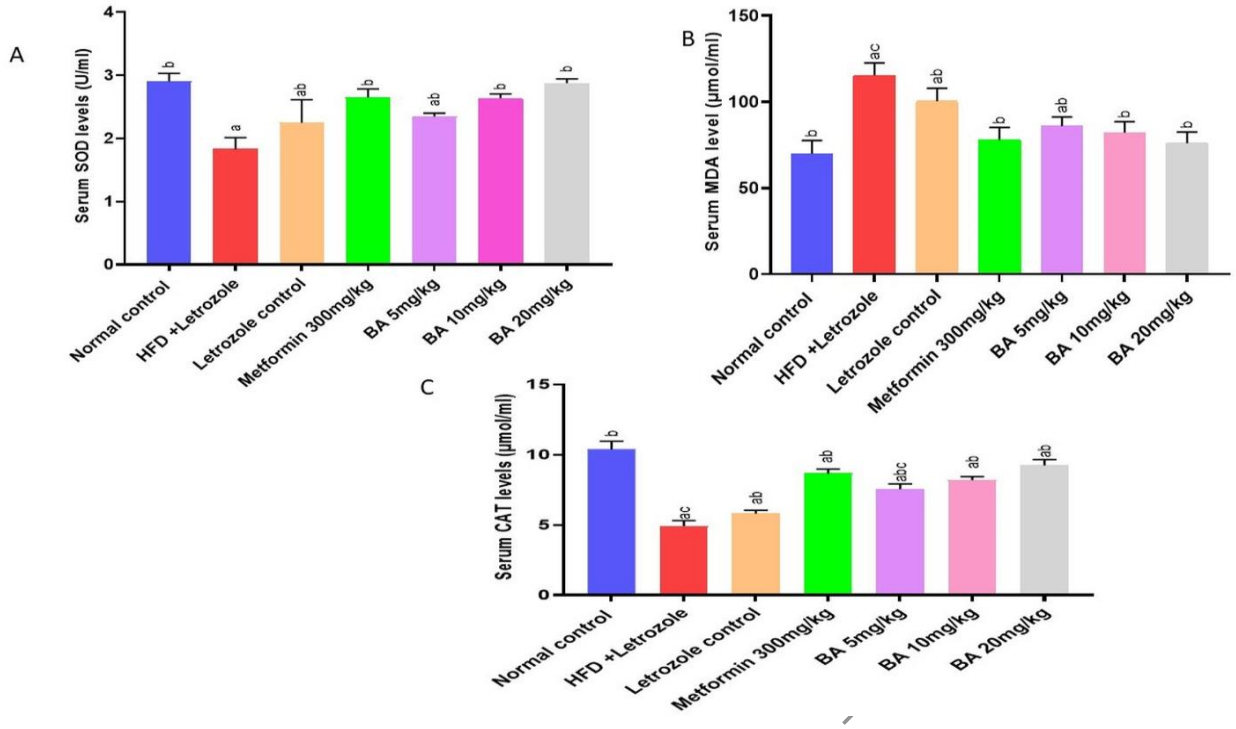

Fig. 5: Effect of bergapten treatment on (A) SOD activity; (B) MDA level; and (C) CAT activity in obese-PCOS rats. Results are expressed as mean ± SD (n = 6); one-way ANOVA; Tukey's test. (a) significant difference compared with normal control group; (b) compared with HFD + letrozole group; and (c) standard control group (metformin 300 mg/kg).

HFD + letrozole-induced PCOS significantly increased leptin concentrations and reduced adiponectin levels compared with normal controls, indicating adipokine dysregulation. Metformin and bergapten treatment significantly ameliorated these alterations, as reflected by decreased leptin and increased adiponectin concentrations relative to the HFD + letrozole control. Bergapten exhibited dose-dependent effects, with higher doses showing greater restoration of adipocytokine balance. Similar dysregulation was also observed in the letrozole control group.

Effect of bergapten on oxidative stress biomarkers

The effects of bergapten on oxidative stress biomarkers are shown in figure 5. HFD + letrozole-induced PCOS significantly impaired antioxidant status, as evidenced by reduced superoxide dismutase (SOD) and catalase (CAT) activities, along with elevated malondialdehyde (MDA) levels compared with the normal control group. Similar alterations were observed in the letrozole control group. Metformin and bergapten treatment significantly ameliorated oxidative stress–related alterations relative to the HFD + letrozole control. Bergapten administration dose-dependently increased SOD and CAT activities while reducing MDA levels, with higher doses demonstrating greater efficacy (Fig. 5A–C).

Effect of bergapten on gene expression and inflammatory biomarkers

The effects of bergapten on oxidative stress, steroidogenesis and inflammation-related gene expression are presented in figures 6A–H. HFD + letrozole-induced PCOS significantly altered the expression of genes associated with oxidative stress regulation, characterized by reduced *Nrf2* and *PPAR-γ* expression and increased Keap1 expression compared with the normal control group. Similar alterations were observed in the letrozole control group. Metformin and bergapten treatment significantly modulated these expression changes, with bergapten dose-dependently increasing *Nrf2* and *PPAR-γ* expression while reducing *Keap1* levels relative to the HFD + letrozole control (Fig. 6A–C).

Similarly, HFD + letrozole administration significantly dysregulated steroidogenic gene expression, as reflected by reduced *CYP19A1* and elevated *CYP11A1* expression compared with normal controls (Figs. 6D–E). Bergapten and metformin treatment significantly improved these alterations, evidenced by increased *CYP19A1* and reduced *CYP11A1* expression, with higher bergapten doses showing greater effects.

Inflammatory biomarkers were also markedly altered in the HFD + letrozole group, as indicated by increased mRNA expression of *TNF-α*, *IL-6* and *IL-8* relative to normal controls (Fig. 6F–H). Metformin and bergapten treatment significantly attenuated these inflammatory changes, with bergapten producing dose-dependent reductions in pro-inflammatory cytokine expression.

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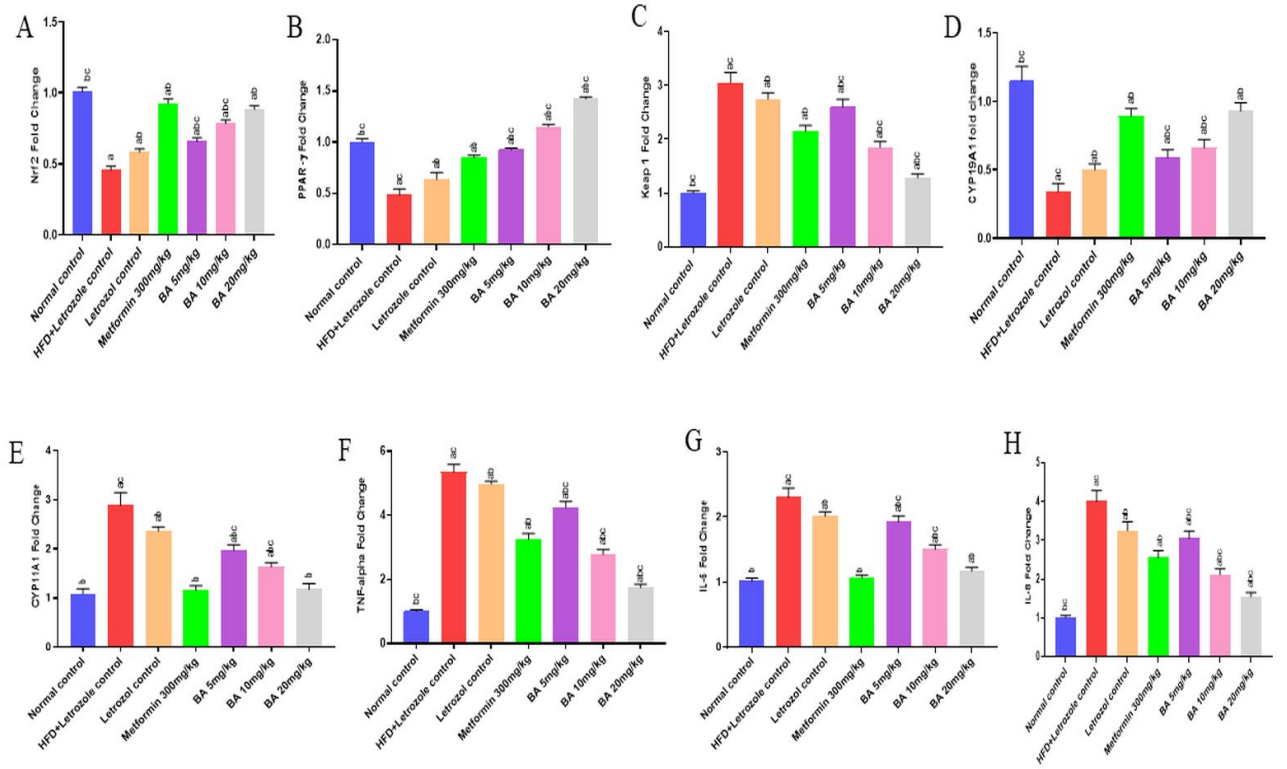

Fig. 6: Effect of bergapten treatment on (A) *Nrf2* level; (B) PPAR-γ; (C) *Keap1*; (D) *CYP19A1*; (E) *CYP11A1*; (F) *TNF-α*; (G) *IL-6*; (H) *IL-8* in obese PCOS rats. Results are expressed as mean ± SD (n = 6); one-way ANOVA; Tukey's test. (a) significant difference compared with normal control group; (b) compared with HFD + letrozole group; and (c) standard control group (metformin 300 mg/kg).

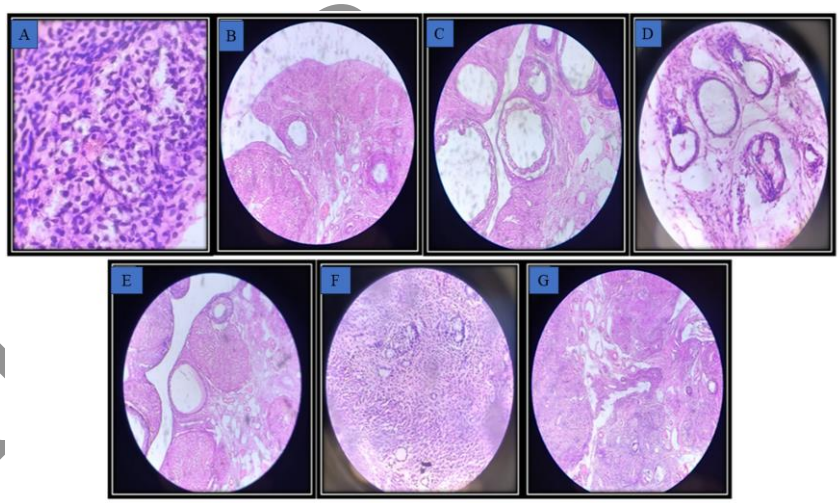

Fig. 7: Effect of bergapten on histopathology of ovary in obesity-induced infertile rats at 40X (A) normal control group; (B) HFD-disease control; (C) Letrozole control; (D) Standard control (metformin 300 mg/kg); (E) 5 mg/kg BA treatments; (F) 10 mg/kg BA treatment; (G) 20 mg/kg BA treatment.

Histopathological evaluation

Normal control group Fig. 7(A) exhibited healthy ovarian cortex with multiple healthy follicles, defined corpora luteum, compact vascularized stroma with no cystic formations. Disease control group Fig. 7(B) subjected to a high-fat diet exhibited multiple large, thin-walled cystic follicles with irregular granulosa cell layers and mild stromal edema. Letrozole treated animals Fig. 7(C) produced enlarged fluid-filled follicles with thin granulosa layers and absent oocytes, thus surrounding stroma was loose and edematous. Metformin (300 mg/kg) treatment group Fig. 7(D) displayed partial reversal of cystic changes, dense and hypercellular ovarian stroma with increased fibroblast-like cells. Treatment with bergapten

demonstrated dose-dependent histological restoration. The group treated with 5 mg/kg Fig. 7(E) showed moderate preservation of follicular architecture with a reduced number of cystic follicles. While high doses of BA 10 mg/kg Fig. 7(F) and 20 mg/kg Fig. 7(G) showed near-normal ovarian histology, characterized by regression of cystic follicles, reappearance of corpora luteum and restoration of granulosa as well as thecal cells organization with undamaged architecture.

DISCUSSION

Obesity is an important aggravating factor in PCOS, contributing to insulin resistance, oxidative stress, chronic inflammation and reproductive dysfunction (Li *et al*., 2019). In the present study, we used a high-fat diet and letrozole-induced rat model to mimic obesity-associated PCOS and evaluate the therapeutic effects of bergapten on metabolic, hormonal, inflammatory and molecular alterations. Consistent with previous reports, HFD combined with letrozole successfully reproduced key features of obesity-associated PCOS, including metabolic dysregulation and reproductive disturbances (Kafali *et al*., 2004). Letrozole-induced aromatase inhibition has been widely reported to disrupt ovarian steroidogenesis and induce hyperandrogenic and cystic ovarian changes resembling human PCOS. Concurrently, HFD exposure contributes to excessive weight gain, adiposity and impaired glucose regulation, thereby aggravating the metabolic disturbances associated with PCOS (Reddy *et al*., 2016). In agreement with previous findings, the HFD + letrozole model in the present study reproduced key metabolic and reproductive abnormalities of obesity-associated PCOS, including weight gain, impaired glucose tolerance and dyslipidemia. Weight reduction is considered an important therapeutic strategy in PCOS, as even modest decreases in body weight have been associated with improved ovulatory and endocrine function (Moran *et al*., 2010). In the present study, bergapten and metformin treatment were associated with reduced body weight relative to disease controls, suggesting improvement in obesity-related metabolic disturbances. Impaired glucose tolerance is a hallmark of obesity-associated PCOS and is closely linked to insulin resistance (Bednarz *et al*., 2022). Bergapten-treated animals demonstrated improved glucose tolerance, comparable to metformin-treated rats, indicating a potential beneficial effect on metabolic homeostasis. Although the underlying mechanism was not directly investigated, these findings may be associated with improved insulin sensitivity or peripheral glucose utilization. However, further mechanistic studies are required to confirm these effects. In contrast, persistent hyperglycemia observed in HFD + letrozole-treated rats was consistent with previous reports of diet-induced metabolic dysfunction (Tao *et al*., 2019).

The present study also demonstrated marked dyslipidemia in the disease control group, characterized by elevated LDL, VLDL, total cholesterol and triglycerides, together with reduced HDL levels, findings consistent with obesity-associated PCOS. Bergapten treatment dose-dependently improved these lipid abnormalities, with effects comparable to metformin at higher doses. Similar lipid-lowering effects have been reported for bioactive compounds in experimental PCOS models, including short-chain fatty acids that improve lipid homeostasis in letrozole-induced PCOS (Acharya *et al*., 2024). These findings suggest that bergapten may contribute to improved metabolic regulation in obesity-associated PCOS. Vaginal smear cytology of healthy animals demonstrated a normal estrous cycle pattern, whereas PCOS-induced rats exhibited irregular cyclicity characterized by prolonged metestrus, consistent with previous findings in letrozole-induced PCOS models (Thakor and Patel, 2014). These observations support successful induction of reproductive dysfunction in the disease control group. Hormonal imbalance is a defining feature of PCOS, with disruption of gonadotropin secretion contributing to impaired follicular development, anovulation and reproductive dysfunction (Murray and Orr, 2020). In the present study, HFD + letrozole administration altered reproductive hormone levels, characterized by increased LH and testosterone concentrations together with reduced progesterone and estrogen levels, findings consistent with endocrine disturbances observed in PCOS (Sharma *et al*., 2023; Yang *et al*., 2024). Bergapten treatment dose-dependently improved these hormonal alterations, with higher doses producing responses comparable to metformin for several parameters. These findings suggest a potential role of bergapten in attenuating endocrine dysregulation associated with obesity-related PCOS. Adipokine imbalance is increasingly recognized as an important contributor to metabolic dysfunction in PCOS, particularly under obese conditions. The elevated leptin and reduced adiponectin concentrations observed in disease control animals are consistent with obesity-associated metabolic dysregulation. Bergapten treatment significantly reduced leptin levels and increased adiponectin concentrations, indicating possible improvement in adipokine homeostasis. However, the precise mechanisms underlying these effects require further investigation. Histopathological examination demonstrated ovarian alterations consistent with PCOS induction, including cystic follicles, attenuated granulosa cell layers and absence of corpora lutea in the HFD + letrozole control group. Bergapten treatment improved ovarian morphology, particularly at 20 mg/kg, as reflected by reduced cystic changes and improved follicular organization. Similar ovarian protective effects have been reported for bioactive compounds in experimental PCOS models, including carvacrol, which has been associated with antioxidant and anti-inflammatory properties (Gao and Li, 2023). Although

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these findings suggested the improvement in ovarian architecture following bergapten treatment. Moreover, ovarian histological assessment in the present study was qualitative which is a study limitation and future quantitative morphometric analysis would strengthen these observations. Oxidative stress and chronic low-grade inflammation are recognized contributors to the pathophysiology of obesity-associated PCOS (Zhou *et al*., 2021; Li *et al*., 2020). In the present study, disease control animals exhibited altered expression of oxidative stress–related genes, characterized by reduced Nrf2 and increased Keap1 expression, together with elevated inflammatory cytokines. Bergapten treatment modulated these expression patterns, including increased Nrf2 and reduced Keap1 expression, alongside suppression of TNF-α, IL-6 and IL-8. These findings are consistent with previous reports describing antioxidant and anti-inflammatory properties of bergapten and related compounds (Liang *et al*., 2021; Phucharoenrak and Trachootham, 2024). However, interpretation of these molecular findings should be made cautiously. Since gene expression was assessed at the mRNA level only, definitive confirmation of Nrf2/Keap1 pathway activation would require protein-level evidence, including nuclear translocation studies and downstream antioxidant targets such as HO-1 and NQO1. Therefore, the present findings are more appropriately interpreted as changes in gene expression consistent with modulation of oxidative stress–related pathways rather than direct pathway activation. Bergapten treatment also improved expression of steroidogenesis-related genes, characterized by increased CYP19A1 and reduced CYP11A1 expression, changes that may be associated with improved hormonal balance in PCOS (Xiao *et al*., 2023). Additionally, partial restoration of PPAR-γ expression at higher bergapten doses was consistent with the observed improvements in metabolic parameters, given the recognized role of PPAR-γ in lipid metabolism and insulin sensitivity (Liu *et al*., 2018). Nevertheless, these mechanistic interpretations remain preliminary and require confirmation through tissue-specific and protein-level investigations. In parallel, modulation of pathways involved in lipid metabolism, inflammation and steroidogenesis may contribute to the observed metabolic and endocrine improvements in bergapten-treated animals (Xia *et al*., 2022). The greater efficacy observed with bergapten at 20 mg/kg across several biochemical and molecular endpoints suggests a dose-dependent therapeutic response. Although intermediate responses at 10 mg/kg were not consistently superior to lower doses for all parameters, the underlying reasons remain unclear and may involve pharmacodynamic or bioavailability-related factors that were not investigated in the present study. Overall, the findings suggest that the beneficial effects of bergapten in obesity-associated PCOS may be related to its antioxidant, anti-inflammatory and metabolic regulatory properties, consistent with previous reports in metabolic and reproductive disorders (Rudrapal *et al*., 2024). Several limitations of the present study should be acknowledged. Gene expression analysis was limited to mRNA assessment and protein-level validation through techniques such as western blotting or immunohistochemistry was not performed. Future studies incorporating downstream molecular targets would strengthen mechanistic interpretation. In addition, steroidogenic gene expression was evaluated from blood samples rather than ovarian tissue, which may not fully reflect local ovarian activity. Fasting insulin and insulin resistance indices such as HOMA-IR were not assessed, limiting comprehensive metabolic characterization. Furthermore, histomorphometric quantification of ovarian follicles and corpora lutea, as well as direct assessment of ovulation, were not performed. Addressing these limitations in future studies would strengthen the mechanistic and translational relevance of bergapten in PCOS management.

CONCLUSION

In conclusion, the present study demonstrates that bergapten ameliorates metabolic, hormonal, inflammatory and oxidative stress–related alterations in a high-fat diet and letrozole-induced rat model of PCOS. These improvements were accompanied by restoration of ovarian and hepatic histoarchitecture, indicating beneficial effects on both reproductive and metabolic dysfunctions.

The observed effects were dose-dependent, with higher doses showing greater efficacy. These findings are associated with modulation of pathways related to oxidative stress, inflammation and steroidogenesis; however, the underlying mechanisms require further validation. Acute toxicity study data revealed that LD_{50} of bergapten was more than 2000 mg/kg. Despite these promising results, the findings should be interpreted within the limitations of a preclinical animal model and short study duration. In addition, safety evaluation was limited to acute exposure and long-term toxicity remains to be established.

Future studies should focus on detailed mechanistic validation, long-term safety assessment and clinical investigations to determine the translational potential of bergapten in PCOS. Overall, bergapten represents a promising bioactive compound for further exploration, although its clinical applicability remains to be established.

Acknowledgments

The preprint of this manuscript has been presented in "research square" and accessible with link: https://assets-eu.researchsquare.com/files/rs-7807066/v1/46dad2c3-3877-4c66-a7eb-2cd6525cca5c.pdf?c=1761667964.

Authors' contributions

This research is part of PHD thesis of Miss Abida Hussain. Abida Hussain: Experimentation and analysis, performed literature review, Histological analysis and writing – original draft. Ammara Saleem and Muhammad Furqan Akhtar: Conceptualization, visualization, review,

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supervision, study design, writing, reviewing and editing the manuscript.

Funding
We did not get any funding from the government or private agency.

Data availability statement
Authors declare that all the data supporting the findings of this study are included in the article.

Ethical approval
The study protocol was approved by the Research Ethical Committee of Government College University Faisalabad (Approval No. GCUF/ERC/633). All procedures involving animals were conducted in accordance with the National Research Council guidelines for the care and use of laboratory animals. The study was designed, conducted and reported in compliance with the ARRIVE 2.0 guidelines. Every effort was made to minimize animal suffering and reduce the number of animals used. Animals were monitored daily for signs of pain, distress, illness and changes in feeding behavior or abnormal activity. This study was performed in adherence with the ARRIVE guidelines. See supplementary file for the ARRIVE checklist.

Conflict of interest
The authors declare that they have no conflict of interest.

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