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ORIGINAL ARTICLE

PROTECTIVE ROLE OF KAEMPFEROL AGAINST ESTRADIOL VALERATE-INDUCED POLYCYSTIC OVARY SYNDROME IN RATS

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ABSTRACT : Polycystic ovary syndrome (PCOS) is a prevalent endocrine-metabolic disorder characterized by hormonal imbalance and ovarian dysfunction, necessitating the exploration of novel therapeutic agents. Kaempferol, a natural flavonoid, possesses documented antioxidant and anti-inflammatory properties, but its efficacy in PCOS remains underexplored. This study investigated the protective role of kaempferol against estradiol valerate (EV)-induced PCOS in rats. Thirty female Wistar rats were divided into five groups (n=6): Control (vehicle), PCOS control (EV, 4 mg/rat single dose), Standard (EV + Metformin, 500 mg/kg/day), and two treatment groups (EV + Kaempferol at 50 or 100 mg/kg/day). PCOS was induced via a single intramuscular EV injection, followed by a 30-day treatment period. Parameters assessed included serum levels of luteinizing hormone (LH), follicle-stimulating hormone (FSH), testosterone, estradiol, and progesterone; ovarian oxidative stress markers (SOD, CAT, GSH, MDA); and histopathological examination of ovarian tissues. EV induction successfully established a PCOS phenotype, manifesting as elevated LH, testosterone, and LH/FSH ratio, decreased FSH and progesterone, increased oxidative stress (lowered SOD, CAT, GSH; elevated MDA), and cystic ovarian morphology. Kaempferol treatment, particularly at the 100 mg/kg dose, significantly reversed these alterations. It normalized gonadotropin and steroid hormone levels, restored antioxidant enzyme activities, reduced lipid peroxidation, and markedly improved ovarian histoarchitecture by reducing cystic follicles and promoting corpus luteum formation. The efficacy of high-dose kaempferol was comparable to metformin. The findings demonstrate that kaempferol effectively attenuates EV-induced PCOS in rats by restoring hormonal balance, mitigating oxidative stress, and reversing ovarian pathology. Its therapeutic potential is comparable to first-line treatment metformin, suggesting kaempferol as a promising complementary phytotherapeutic agent for PCOS management, likely mediated through its antioxidant and endocrine-modulating activities.

Key words : Polycystic ovary syndrome, Kaempferol, estradiol xalerate, oxidative stress, rat model, metformin, hormonal balance.

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INTRODUCTION

Polycystic Ovary Syndrome (PCOS) is one of the most prevalent endocrine disorders among women of reproductive age, affecting approximately 5-10% globally and characterized by a complex interplay of hormonal imbalances, metabolic dysfunctions and reproductive irregularities (Azziz *et al*, 2016; Huang *et al*, 2019). The syndrome is clinically defined by oligo-anovulation, hyperandrogenism, and polycystic ovarian morphology, often accompanied by insulin resistance, obesity, and increased risk of type 2 diabetes and cardiovascular complications. Despite its high prevalence, the exact

etiology of PCOS remains multifactorial and not fully elucidated, involving genetic, hormonal and environmental factors that disrupt the hypothalamic-pituitary-ovarian axis (Norman *et al*, 2007). Current therapeutic approaches for PCOS primarily focus on symptom management rather than cure, including hormonal contraceptives to regulate menstrual cycles, anti-androgens for hirsutism and insulin sensitizers like metformin to improve metabolic parameters (Legro *et al*, 2013; Park *et al*, 2018). However, these treatments often have limitations such as side effects, contraindications and inadequate response in a subset of patients, underscoring the need for novel, well-tolerated therapeutic agents, particularly those derived from natural sources with multi-target potential. In preclinical research, the estradiol valerate (EV)-induced rat model has been established as a reliable and reproducible method to mimic human PCOS, exhibiting key features such as cystic follicles, anovulation, hyperandrogenism, and hormonal disturbances comparable to the clinical phenotype (Kafali *et al*, 2004; Wang *et al*, 2020). A single intramuscular injection of EV in rodents leads to a sustained disruption of steroid feedback mechanisms, resulting in polyfollicular ovaries and elevated luteinizing hormone (LH) levels, thus providing a validated platform for evaluating potential therapeutic compounds (Maliqueo *et al*, 2013;Yao *et al*, 2019). Among natural compounds, kaempferol a dietary flavonoid abundantly present in fruits, vegetables, and medicinal plants such as tea, broccoli, and Ginkgo biloba has garnered significant attention due to its broad pharmacological profile, including antioxidant, anti-inflammatory, insulin-sensitizing, and anti-androgenic properties (Calderon-Montano *et al*, 2011). Experimental studies have demonstrated kaempferol's ability to modulate key signaling pathways implicated in PCOS, such as PI3K/AKT and AMPK, improve glucose homeostasis, reduce oxidative stress, and inhibit testosterone synthesis in ovarian theca cells (Huang *et al*, 2019; Wang *et al*, 2020). These multifaceted actions position kaempferol as a promising candidate for PCOS management, potentially addressing both reproductive and metabolic abnormalities concurrently. Nevertheless, despite compelling preliminary evidence, there remains a significant research gap regarding the systematic evaluation of kaempferol in a well-characterized EV-induced PCOS model, particularly focusing on its dose-dependent effects on hormonal profiles, ovarian histoarchitecture, and oxidative stress markers in comparison to standard therapy. Most existing studies have explored other flavonoids like quercetin or myricetin, leaving kaempferol's specific therapeutic efficacy and mechanism in PCOS insufficiently investigated (Neisy *et al*, 2019). Therefore, this study hypothesizes that kaempferol administration will attenuate EV-induced PCOS manifestations in rats by restoring hormonal balance, mitigating ovarian cyst formation, and reducing oxidative damage in ovarian tissues. The primary aim of this research is to evaluate the protective role of kaempferol against EV-induced PCOS in a female rat model. Specific objectives include: (1) assessing the impact of kaempferol on serum levels of reproductive hormones (LH, FSH, testosterone, estradiol, progesterone); (2) examining its effects on ovarian morphology and folliculogenesis through histopathological analysis; (3) evaluating its influence on oxidative stress parameters (SOD, CAT, GSH, MDA) in ovarian tissue; and (4) comparing its efficacy with metformin, a first-line insulin-sensitizing drug used in PCOS management.

MATERIALS AND METHODS

Chemicals and drugs

Kaempferol (purity ≥95%, HPLC-grade) was procured from Sigma-Aldrich (CAS Number: 520-18-3). Estradiol valerate (Progynon Depot®) was obtained from German Remedies Ltd. Metformin hydrochloride was purchased from Sun Pharmaceutical Industries Ltd. All other chemicals, including solvents, ELISA kits for hormonal assays, and reagents for oxidative stress analysis, were of analytical grade and sourced from Sisco Research Laboratories and Sigma-Aldrich.

Experimental animals and Ethical approval

The study utilized thirty (30) healthy adult female Wistar albino rats (weighing 180-220 g, aged 10-12 weeks). The animals were procured from the institutional animal house and housed in polypropylene cages under standard laboratory conditions (temperature: 22±2°C, relative humidity: 50-60%, and a 12-hour light/dark cycle). They were provided with a standard pellet diet and water *ad libitum*. All experimental procedures were strictly conducted in accordance with the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India.

Induction of PCOS using Estradiol valerate

Polycystic ovary syndrome was induced in the rats following a well-established protocol (Kafali *et al*, 2004). After a two-week acclimatization period, the rats received a single intramuscular injection of estradiol valerate (4 mg/rat, dissolved in 0.2 ml of sesame oil) into the hind limb. The control group received an equivalent volume of the vehicle (sesame oil) only. The development of PCOS was confirmed after 60 days of injection. The primary indicator was the induction of persistent estrus,

determined by daily vaginal cytology. Vaginal smears were obtained using a saline-moistened cotton swab, stained with methylene blue, and examined under a light microscope. The presence of cornified epithelial cells for ten consecutive days confirmed anovulation and the PCOS-like state (Maliqueo *et al*, 2013).

Experimental Design and grouping

After confirmation of the PCOS phenotype, the rats were randomly allocated into five experimental groups, each consisting of six animals (n=6), to ensure statistical robustness and minimize bias. The grouping was designed to systematically evaluate the therapeutic potential of kaempferol against the induced pathology.

Group 1: Control (Vehicle)

This group served as the healthy, non-PCOS baseline. Rats received a single intramuscular injection of the vehicle (0.2 ml sesame oil) and were subsequently administered 1% carboxymethyl cellulose (CMC) suspension orally via gavage daily for the 30-day treatment period. CMC acted as the vehicle for the drug treatments in other groups.

Group 2: EV-induced PCOS (Disease Control)

Rats in this group received the estradiol valerate (EV) injection to induce PCOS. Following the confirmation of PCOS (persistent estrus), they were administered only the 1% CMC vehicle orally for 30 days. This group was critical for establishing the pathological profile against which all treatments were compared.

Group 3: EV + Metformin (Standard)

This group served as the positive control for pharmacological intervention. After successful PCOS induction, rats received a daily oral dose of metformin hydrochloride (500 mg/kg body weight, suspended in 1% CMC) for 30 days. This dose is well-established in rodent models for its insulin-sensitizing and therapeutic effects on PCOS symptoms (Maliqueo *et al*, 2013).

Group 4: EV + Kaempferol (Low Dose)

The PCOS-induced rats in this experimental group were treated with a daily oral dose of kaempferol at 50 mg/kg body weight (suspended in 1% CMC) for 30 days. This dose was selected based on preliminary sub-acute toxicity studies and previous research demonstrating its significant antioxidant and anti-inflammatory efficacy without adverse effects in rodent models (Calderon-Montano *et al*, 2011).

Group 5: EV + Kaempferol (High Dose)

The PCOS-induced rats in this group received a higher daily oral dose of kaempferol at 100 mg/kg body weight (suspended in 1% CMC) for 30 days. This dose was chosen to investigate a potential dose-dependent therapeutic response and to further assess the safety and maximal efficacy window of kaempferol in the PCOS model.

Treatment protocol and duration

The treatment phase commenced immediately after the confirmation of PCOS (Day 60 post-EV injection). All oral treatments (vehicle, metformin, or kaempferol) were administered once daily between 9:00 and 10:00 AM for a consecutive period of 30 days. Compounds were freshly prepared each morning as suspensions in 1% w/v carboxymethyl cellulose (CMC) solution. The administration volume was standardized at 5 ml/kg body weight and delivered via oral gavage using a stainless-steel feeding needle. Body weights were recorded weekly to monitor general health and adjust dosing volumes accordingly. Throughout the treatment period, vaginal cytology was continued on a weekly basis to track any changes in the estrous cycle.

Sample collection and processing

Twenty-four hours after the final treatment dose, all animals were fasted overnight with free access to water. The rats were then euthanized under deep anesthesia induced by an intraperitoneal injection of ketamine (80 mg/kg) and xylazine (10 mg/kg) (Maliqueo *et al*, 2013). Blood was collected via cardiac puncture using sterile syringes, allowed to clot at room temperature for 30 minutes and then centrifuged at 3000 rpm for 15 minutes at 4°C to separate the serum. The clear serum aliquots were stored at -80°C until analyzed for hormonal assays.

Immediately after blood collection, a midline laparotomy was performed. The ovaries and uteri were carefully dissected out, trimmed of surrounding adipose tissue, and blotted dry. Their wet weights were recorded using a precision analytical balance. The left ovary from each rat was fixed in Bouin's solution for 24 hours for subsequent histopathological processing. The right ovary was snap-frozen in liquid nitrogen and stored at -80°C for biochemical analysis of oxidative stress markers. Representative sections of the liver and kidney were also harvested and fixed in 10% neutral buffered formalin for potential histopathological screening of treatment-related toxicity.

Biochemical and Hormonal assays

Serum Hormone Analysis (LH, FSH, Testosterone, Estradiol, Progesterone) via ELISA

The concentrations of key reproductive hormones luteinizing hormone (LH), follicle-stimulating hormone (FSH), testosterone, estradiol and progesterone were quantitatively determined in the collected serum samples using commercially available, rat-specific Enzyme-Linked Immunosorbent Assay (ELISA) kits (Elabscience Biotechnology Inc., Wuhan, China). All assays were performed strictly according to the manufacturer's protocols. Briefly, standards and prediluted serum samples were added to the antibody-precoated microtiter plate wells. After incubation and washing steps to remove unbound substances, a horseradish peroxidase (HRP)-conjugated detection antibody was added to form an antibody-antigen-enzyme complex. Following another wash, a tetramethylbenzidine (TMB) substrate solution was added, which developed a blue colour proportional to the amount of bound hormone. The reaction was stopped with a stop solution, changing the colour to yellow. The absorbance of each well was immediately measured at 450 nm using a microplate reader (Thermo Fisher Scientific, Multiskan GO). Hormone concentrations were calculated by interpolating absorbance values against the standard curve generated for each assay. The intra- and inter-assay coefficients of variation for all kits were less than 10%, ensuring reliability and precision.

Oxidative Stress Markers in Ovarian Tissue (SOD, CAT, GSH, MDA)

To evaluate the ovarian oxidative stress status, a 10% (w/v) tissue homogenate was prepared from the snap-frozen right ovary in ice-cold 0.1 M phosphate buffer (pH 7.4) using a glass homogenizer. The homogenate was centrifuged at 10,000 × g for 15 minutes at 4°C and the resulting supernatant (post-mitochondrial fraction) was used for the following spectrophotometric assays:

- **Superoxide Dismutase (SOD) Activity:** SOD activity was estimated by measuring its capacity to inhibit the auto-oxidation of pyrogallol, as described by Marklund and Marklund (1974). One unit of SOD activity was defined as the amount of enzyme required to cause 50% inhibition of pyrogallol auto-oxidation per minute per milligram of protein.
- **Catalase (CAT) Activity:** CAT activity was assayed by measuring the decomposition of hydrogen peroxide (H_2O_2) at 240 nm according to the method of Aebi (1984). Activity was expressed as micromoles of H_2O_2 consumed per minute per milligram of protein.
- **Reduced Glutathione (GSH) Level:** The level of GSH, a key non-enzymatic antioxidant, was determined using Ellman's reagent [5,52 - dithiobis(2-nitrobenzoic acid) or DTNB] as per the method of Ellman (1959). The yellow-coloured product formed was read at 412 nm, and GSH concentration was calculated using a standard curve and expressed as nanomoles per milligram of protein.
- **Lipid Peroxidation (MDA level):** The extent of lipid peroxidation was assessed by measuring the concentration of malondialdehyde (MDA), a thiobarbituric acid reactive substance (TBARS). The method of Ohkawa *et al* (1979) was followed, where MDA reacts with thiobarbituric acid (TBA) to form a pink chromogen, the absorbance of which was measured at 532 nm. Results were expressed as nanomoles of MDA per milligram of protein. The total protein content in the supernatant was determined using Bradford's method with bovine serum albumin as the standard, to normalize all oxidative marker values

Histopathological Examination of Ovaries

For qualitative and quantitative histopathological assessment, the left ovarian tissues fixed in Bouin's solution were processed using a standard dehydration protocol. The tissues were embedded in paraffin wax, and serial sections of 5 µm thickness were cut using a rotary microtome (Leica RM2235). The sections were mounted on glass slides and stained with routine Hematoxylin and Eosin (H&E) stain (Bancroft and Gamble, 2008). Stained slides were examined under a light microscope (Olympus BX53) equipped with a digital camera (Olympus DP27). The histopathological evaluation was performed in a blinded manner by an experienced pathologist. Key parameters assessed included:

- **Follicular Count and Classification:** Numbers of primordial, primary, secondary, antral and atretic follicles were counted in every fifth section to avoid double counting. Follicles were classified based on established morphological criteria (Myers *et al*, 2004).
- **Cystic Follicle characterization :** The number of cystic follicles (defined as follicles with a thin granulosa cell layer, a large antral cavity, and a diameter >500 µm) was recorded. The mean diameter of cystic follicles and the thickness of the granulosa cell layer were measured using image analysis software (ImageJ, NIH).
- **Corpus Luteum Quantification:** The total

number of corpora lutea per ovary section was counted as an indicator of ovulatory activity.

- **Stromal Assessment:** The ovarian stroma was evaluated for hyperplasia, fibrosis, and inflammatory cell infiltration.

Statistical analysis

All quantitative data are presented as mean±standard error of the mean (SEM) for six animals per group (n=6). Statistical analysis was performed using GraphPad Prism software (Version 9.0). The normality of data distribution was confirmed using the Shapiro-Wilk test. For comparisons among all five experimental groups, one-way analysis of variance (ANOVA) was applied, followed by Tukey's post hoc test for multiple comparisons. Differences were considered statistically significant at a p-value of less than 0.05 ($p < 0.05$). For histological count data, non-parametric Kruskal-Wallis test followed by Dunn's post hoc test was employed. Correlation analysis between hormonal parameters and oxidative stress markers was performed using Pearson's correlation coefficient.

RESULTS AND DISCUSSION

Effect on Body weight and Ovarian weight

The estradiol valerate (EV)-induced PCOS model resulted in significant metabolic and morphological alterations. The data for final body weight and ovarian weight are summarized in Table 1.

As shown in Table 1, induction of PCOS with EV (G2) led to a significant increase in both final body weight and absolute ovarian weight compared to the healthy control group (G1) ($p < 0.01$ and $p < 0.001$, respectively). Treatment with Metformin (G3) significantly attenuated the EV-induced increase in ovarian weight ($p < 0.01$ vs. G2) and showed a trend towards reducing body weight. Kaempferol treatment demonstrated a dose-dependent protective effect. While the low dose (G4) significantly reduced ovarian weight compared to the PCOS control ($p < 0.05$), the high dose of Kaempferol (G5) was markedly more effective (Fig. 1a & 1b). The high dose not only normalized ovarian weight to a level comparable to the healthy control, but also significantly reduced the PCOS-associated increase in body weight ($p < 0.05$ vs. G2). These findings indicate that Kaempferol effectively counteracts the metabolic and morphological disturbances characteristic of the EV-induced PCOS model.

Effect on Hormonal Parameters

The induction of PCOS with estradiol valerate caused significant disruption in the reproductive hormone profile,

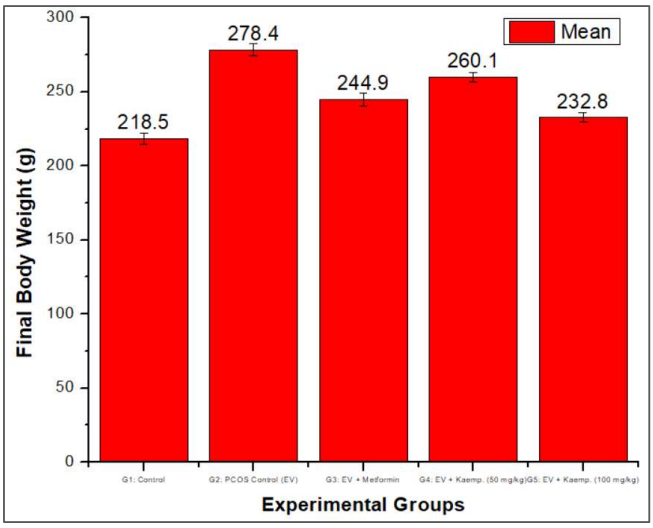

Fig. 1a : Effect of Kaempferol treatment on final body weight in EV-induced PCOS rats.

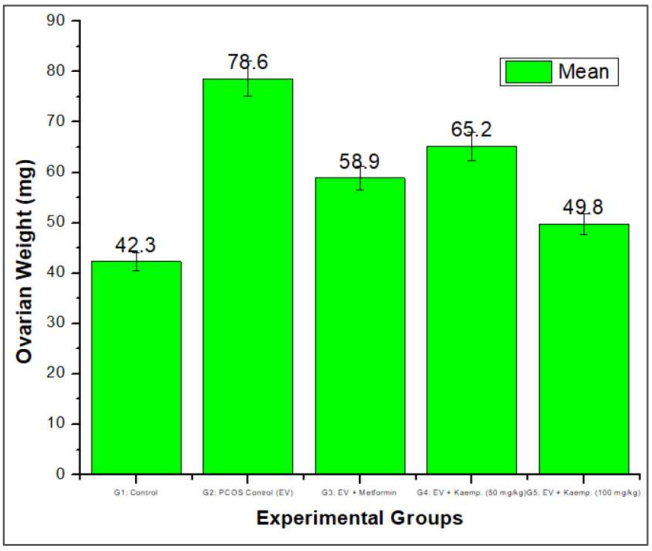

Fig. 1b : Effect of Kaempferol treatment on ovarian weight in EV-induced PCOS rats.

which was systematically assessed.

Luteinizing Hormone (LH) and Follicle-Stimulating Hormone (FSH) levels

The serum levels of LH and FSH, along with the calculated LH/FSH ratio, are presented in Table 2. A hallmark of the EV-induced PCOS model was a significant elevation in LH and a suppression of FSH, leading to a drastically increased LH/FSH ratio in the PCOS control group (G2) compared to the healthy controls (G1).

Metformin treatment (G3) effectively normalized both LH and FSH levels. Kaempferol exhibited a clear dose-dependent efficacy. The low dose (G4) partially corrected the imbalance, significantly lowering LH and raising FSH compared to G2. Notably, the high dose of kaempferol (G5) was highly effective, restoring LH and FSH levels and, consequently, the LH/FSH ratio to values

Table 1 : Effect of Kaempferol on body weight and ovarian weight in EV-induced PCOS rats.

Group	Final Body weight (g) (Mean ± SEM)	Ovarian weight (mg) (Mean ± SEM)
G1: Control (Vehicle)	218.5 ± 5.2	42.3 ± 1.8
G2: PCOS Control (EV)	278.4 ± 7.1a**	78.6 ± 3.5a***
G3: EV + Metformin (500 mg/kg)	245.8 ± 6.3*	58.9 ± 2.4b**
G4: EV + Kaempferol (50 mg/kg)	260.1 ± 5.9a**	65.2 ± 2.8b*
G5: EV + Kaempferol (100 mg/kg)	232.7 ± 4.8b	49.8 ± 2.1b

Statistical significance: **a* p<0.001, *a* p<0.01, *a* p<0.05 vs. Control (G1); **b* p<0.001, *b* p<0.01, *b* p<0.05 vs. PCOS Control (G2).
(One-way ANOVA followed by Tukey's post-hoc test).

Table 2 : Effect of Kaempferol on serum gonadotropin levels in EV-induced PCOS rats.

Group	LH (mIU/ml) (Mean ± SEM)	FSH (mIU/ml) (Mean ± SEM)	LH/FSH Ratio (Mean ± SEM)
G1: Control (Vehicle)	1.8 ± 0.2	4.5 ± 0.3	0.40 ± 0.05
G2: PCOS Control (EV)	8.5 ± 0.6a***	1.9 ± 0.2a***	4.47 ± 0.35a***
G3: EV + Metformin (500 mg/kg)	3.2 ± 0.4b***	3.8 ± 0.3b**	0.84 ± 0.09b***
G4: EV + Kaempferol (50 mg/kg)	6.1 ± 0.5a**, b*	2.8 ± 0.3a**, b*	2.18 ± 0.20a**, b***
G5: EV + Kaempferol (100 mg/kg)	2.9 ± 0.3b***	4.1 ± 0.4b***	0.71 ± 0.08b***

Statistical significance: **a* p<0.001, *a* p<0.01 vs. Control (G1); **b* p<0.001, *b* p<0.01, *b* p<0.05 vs. PCOS Control (G2).

Table 3 : Effect of Kaempferol on serum steroid hormone levels in EV-induced PCOS rats.

Group	Testosterone (ng/ml) (Mean ± SEM)	Estradiol (pg/ml) (Mean ± SEM)	Progesterone (ng/ml) (Mean ± SEM)
G1: Control (Vehicle)	0.32 ± 0.03	35.2 ± 2.1	12.8 ± 0.9
G2: PCOS Control (EV)	0.89 ± 0.07a***	18.6 ± 1.5a***	5.4 ± 0.6a***
G3: EV + Metformin (500 mg/kg)	0.48 ± 0.05b***	31.5 ± 2.3b***	10.9 ± 0.8b***
G4: EV + Kaempferol (50 mg/kg)	0.71 ± 0.06a***, b*	24.3 ± 1.9a**, b**	7.1 ± 0.7a***, b**
G5: EV + Kaempferol (100 mg/kg)	0.41 ± 0.04b***	33.8 ± 2.5b***	11.3 ± 0.9b***

Statistical significance: **a* p<0.001, *a* p<0.01 vs. Control (G1); **b* p<0.001, *b* p<0.01, *b* p<0.05 vs. PCOS Control (G2).

that were statistically indistinguishable from both the healthy control (G1) and the metformin-treated group (G3).

Testosterone, Estradiol and Progesterone levels

The impact on steroid hormone levels is summarized in Table 3. The PCOS control group (G2) exhibited a significant hyperandrogenic state (elevated testosterone), along with decreased levels of estradiol and progesterone.

Metformin (G3) significantly ameliorated all three steroid hormone abnormalities. Kaempferol treatment again showed a dose-responsive effect. The low dose (G4) produced a moderate, but significant reduction in testosterone and an increase in estradiol and progesterone. The high dose of kaempferol (G5) demonstrated potent activity, effectively normalizing testosterone, estradiol, and progesterone levels to those comparable with the healthy control and metformin-treated groups.

Effect on Oxidative Stress Markers

Oxidative stress in ovarian tissue was assessed by measuring key enzymatic and non-enzymatic antioxidants along with a marker of lipid peroxidation. The data, presented in Table 4, demonstrate a state of significant oxidative imbalance induced by EV, which was ameliorated by treatment.

Ovarian tissue from the PCOS control rats (G2) exhibited a pronounced oxidative stress profile, characterized by a significant decrease in the activity of antioxidant enzymes superoxide dismutase (SOD) and catalase (CAT), a depletion of the key antioxidant glutathione (GSH) and a concomitant increase in malondialdehyde (MDA), a marker of lipid peroxidation ($p < 0.001$ vs. G1 for all).

Metformin treatment (G3) significantly restored the antioxidant defense system, elevating SOD, CAT and GSH levels, while markedly reducing MDA concentration ($p < 0.001$ vs. G2). Kaempferol administration exerted a potent and dose-dependent antioxidant effect. The low

Table 4 : Effect of Kaempferol on ovarian oxidative stress markers in EV-induced PCOS rats.

Group	SOD (U/mg protein) (Mean ± SEM)	CAT (U/mg protein) (Mean ± SEM)	GSH (nmol/mg protein) (Mean ± SEM)	MDA (nmol/mg protein) (Mean ± SEM)
G1: Control (Vehicle)	22.5 ± 1.2	18.8 ± 1.0	25.4 ± 1.5	1.05 ± 0.08
G2: PCOS Control (EV)	11.3 ± 0.9a***	9.6 ± 0.7a***	12.1 ± 1.1a***	3.42 ± 0.21a***
G3: EV + Metformin (500 mg/kg)	19.8 ± 1.1b***	16.9 ± 0.9b***	22.7 ± 1.4b***	1.58 ± 0.12b***
G4: EV + Kaempferol (50 mg/kg)	15.6±1.0a**, b***	13.2±0.8a**, b***	17.9±1.2a**, b***	2.41±0.17a**, b***
G5: EV + Kaempferol (100 mg/kg)	21.1 ± 1.3b***	17.5 ± 1.1***b	24.0 ± 1.6b***	1.31 ± 0.10b***

Statistical significance: **a* p<0.001, *a* p<0.01 vs. Control (G1); **b* p<0.001, *b* p<0.01 vs. PCOS Control (G2).

Table 5 : Qualitative histopathological assessment of ovarian morphology.

Group	Follicular Architecture	Granulosa Cell Layer	Stroma	Corpus Luteum Presence
G1: Control (Vehicle)	Normal, various stages present (Primordial to Antral)	Intact, multi-layered	Normal cellularity, no fibrosis	Numerous, well-developed
G2: PCOS Control (EV)	Dominated by large cystic follicles (>500 µm)	Markedly thin/attenuated	Hyperplastic, mildly fibrotic	Very few or absent
G3: EV + Metformin	Reduced cysts, restored antral & secondary follicles	Improved thickness	Reduced hyperplasia	Moderately present
G4: EV + Kaempferol (50 mg/kg)	Moderate cysts, increased pre-antral follicles	Moderately thin	Mild hyperplasia	Few present
G5: EV + Kaempferol (100 mg/kg)	Rare cysts, abundant healthy follicles at various stages	Near-normal, well-organized	Near-normal cellularity	Abundant, indicating ovulation

dose (G4) significantly improved all oxidative stress parameters compared to the PCOS control. The high dose of Kaempferol (G5) was exceptionally effective, normalizing the levels of SOD, CAT, GSH and MDA to values that were not statistically different from those in the healthy control group (G1) and were comparable to the metformin standard. These results strongly indicate that the protective effects of Kaempferol against PCOS pathology are mediated, at least in part, through the attenuation of ovarian oxidative stress.

Histopathological findings

Microscopic examination of ovarian tissues provided critical structural evidence supporting the biochemical and hormonal findings. Representative photomicrographs and quantitative analyses are presented below.

Ovarian Morphology and Follicular development

Qualitative assessment of H&E-stained sections revealed distinct morphological differences among the groups, as summarized in Table 5.

The ovaries of the healthy control group (G1) exhibited normal folliculogenesis with follicles at various developmental stages (primordial, primary, secondary, and antral) and multiple corpora lutea (Fig. 2). In stark contrast, the PCOS control group (G2) showed a polycystic morphology characterized by numerous large, fluid-filled cystic follicles with a markedly thin granulosa cell layer and a virtually complete absence of corpora lutea, confirming anovulation (Fig. 2). Treatment with Metformin (G3) and Kaempferol, especially at the high dose (G5), reversed these pathological changes. The high-dose Kaempferol group showed ovarian architecture closest to normal, with a significant reduction in cystic structures, reappearance of healthy antral follicles, and the presence of multiple corpora lutea, indicating the restoration of ovulatory cycles.

Quantification of Cystic Follicles and *Corpora lutea*

Morphometric analysis provided quantitative support for the qualitative observations, as detailed in Table 6.

The PCOS control group (G2) had a significantly high number of cystic follicles and a near-total absence of corpora lutea ($p < 0.001$ vs. G1). Both Metformin and Kaempferol treatments significantly reduced the cystic follicle count and increased the number of corpora lutea in a dose-dependent manner. The quantitative data for the high-dose Kaempferol group (G5) were statistically comparable to those of the Metformin-treated (G3) and healthy control (G1) groups, confirming its efficacy in restoring normal ovarian histoarchitecture and function.

Comparative Efficacy: Kaempferol vs. Metformin

A direct comparative analysis of the therapeutic outcomes reveals the relative efficacy of Kaempferol

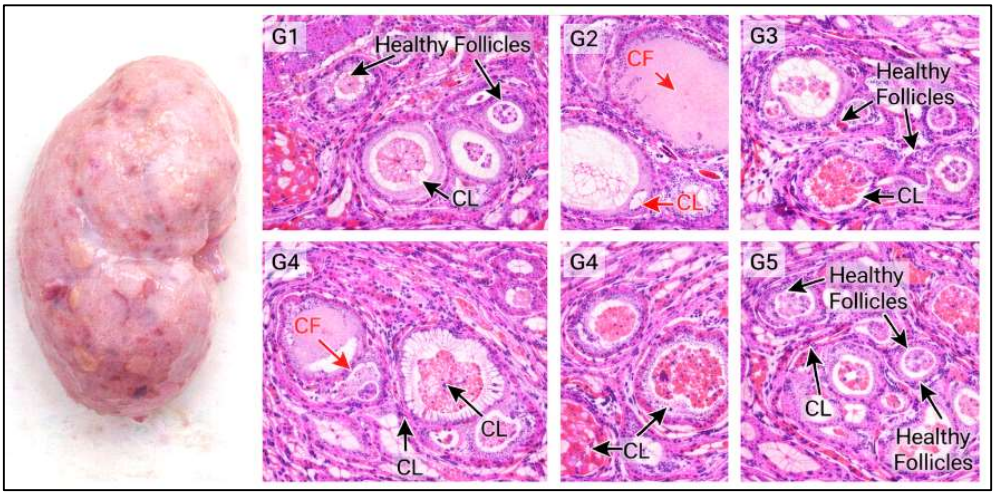

Fig. 2 : Representative histopathological evaluation of ovarian tissue (H&E Staining).

Table 6 : Quantitative analysis of ovarian follicles and corpora lutea.

Group	Cystic Follicles Count / Ovary Section (Mean ± SEM)	Corpus Luteum Count / Ovary Section (Mean ± SEM)
G1: Control (Vehicle)	0.3 ± 0.2	4.8 ± 0.5
G2: PCOS Control (EV)	8.5 ± 0.7a***	0.2 ± 0.1a***
G3: EV + Metformin (500 mg/kg)	2.1 ± 0.4b***	3.5 ± 0.4b***
G4: EV + Kaempferol (50 mg/kg)	4.8 ± 0.5a***, b***	1.6 ± 0.3a***, b***
G5: EV + Kaempferol (100 mg/kg)	1.4 ± 0.3b***	4.1 ± 0.5b***

Statistical significance: *a* $p<0.001$ vs. Control (G1); *b* $p<0.001$ vs. PCOS Control (G2).

against the standard drug Metformin in ameliorating EV-induced PCOS. The data from all assessed parameters (Tables 1-6) indicate that high-dose Kaempferol (100 mg/kg) produced effects that were statistically comparable to Metformin across most key metrics. Hormonal Restoration: Both Metformin (G3) and high-dose Kaempferol (G5) effectively normalized the disrupted gonadotropin axis (LH, FSH, LH/FSH ratio) and restored steroid hormone balance (Testosterone, Estradiol, Progesterone). Statistical analysis showed no significant difference ($p > 0.05$) between these two groups for any of these hormonal parameters, indicating equivalent potency in correcting the central endocrine disturbances of PCOS. Oxidative Stress Amelioration: In reversing ovarian oxidative stress, high-dose Kaempferol demonstrated exceptional activity. The restoration of SOD, CAT, and GSH levels, along with the reduction of MDA, was not statistically different between the G5 (Kaempferol 100 mg/kg) and G3 (Metformin) groups. In fact, the mean values for MDA in the Kaempferol high-dose group were marginally lower than in the Metformin group, suggesting a potentially strong antioxidant effect inherent to the flavonoid.

CONCLUSION

This study demonstrates that kaempferol, a natural dietary flavonoid, exerts significant protective effects against estradiol valerate-induced polycystic ovary syndrome (PCOS) in a rat model. The therapeutic efficacy of kaempferol was evident across hormonal, metabolic, oxidative, and histopathological parameters. Administration of kaempferol, particularly at the higher dose (100 mg/kg), effectively restored the disrupted gonadotropin balance by normalizing elevated LH levels, increasing suppressed FSH, and correcting the aberrant LH/FSH ratio. Concurrently, it ameliorated hyperandrogenism by reducing testosterone levels and restored the concentrations of estradiol and progesterone, indicating a recovery of ovarian steroidogenesis. Furthermore, kaempferol treatment markedly attenuated ovarian oxidative stress, as evidenced by the significant upregulation of antioxidant enzymes (SOD, CAT), increased glutathione levels, and reduction in lipid peroxidation (MDA). Critically, histopathological analysis confirmed the reversal of PCOS morphology; kaempferol reduced cystic follicle formation, promoted healthy folliculogenesis, and restored the presence of corpora lutea, indicating the resumption of ovulatory cycles. Notably, the high-dose kaempferol exhibited efficacy comparable to the standard drug metformin across most assessed endpoints.

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