POTENTIAL ROLE OF ERIOCITRIN FOR THE TREATMENT OF POLYCYSTIC OVARIAN SYNDROME IN LETROZOLE INDUCED RAT MODEL

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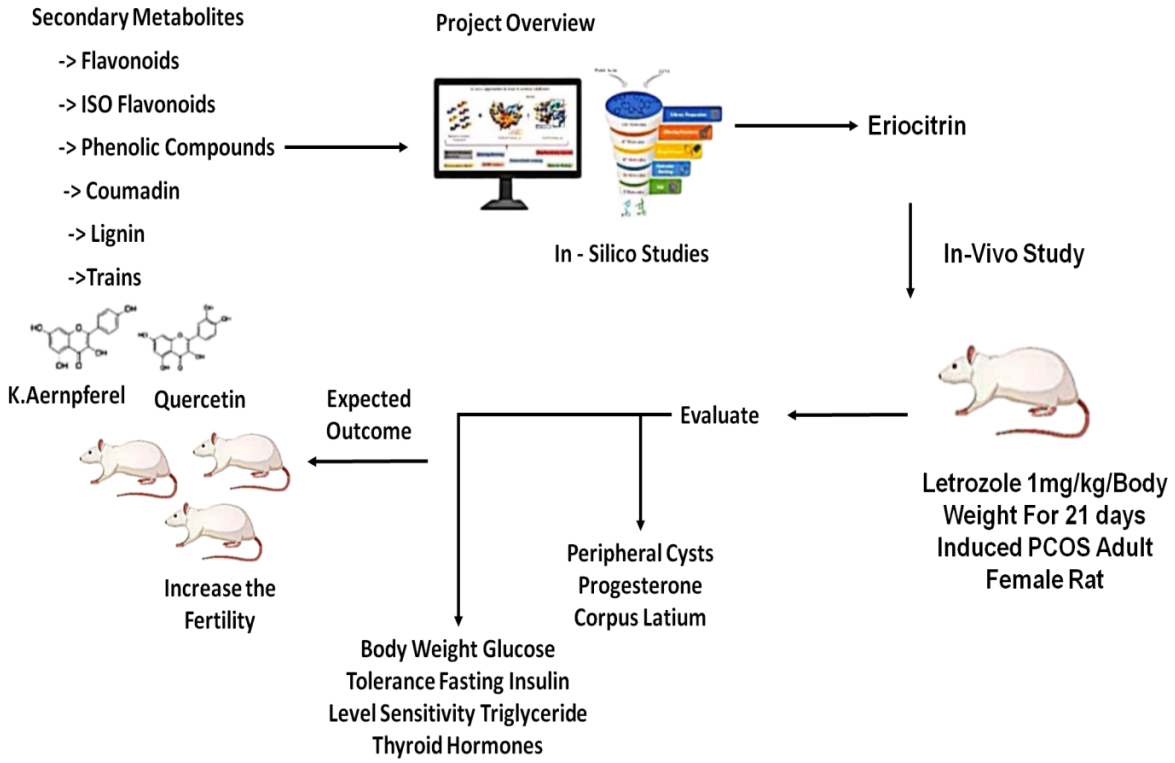
Graphical Abstract



Abstract

The study investigates eriocitrin can be used to treat Polycystic ovarian syndrome (PCOS) in a letrozole-induced rat model. Docking analysis was performed using the glides model (Schrodinger suit -2023), and the MMGBSA Study was performed using the Prime model. Based on the in-silico results, we chose eriocitrin as the top-hit compound. The evaluation of eriocitrin at doses of 5 mg and 10 mg/kg orally used a letrozole-induced animal model, including biochemical parameters, steroidal hormonal assessment, and histopathological examination of the ovary. *In-silico* result showing molecular docking range (-6.34 to -1.4 Kcal /mol). MMGBSA show the binding free energy range of (-3.45- 0.63 Kcal /mol). The glucose tolerance test showed that Eriocitrin (5 mg/kg and 10 mg/kg) significantly reduced blood glucose levels and decreased serum insulin compared to the negative control and letrozole-treated group. Eriocitrin also lowered total cholesterol, LDL, and HDL levels compared to letrozole. While letrozole reduced steroidal hormones (FSH, LH, estrogen), both Eriocitrin and metformin increased these levels. However, Eriocitrin significantly reduced progesterone and testosterone compared to metformin 20mg/kg used as standard drugs. Eriocitrin significantly reduced body weight, while letrozole increased it and caused tissue damage, which Eriocitrin and metformin mitigated.

Keywords: PCOS; Eriocitrin; Molecular docking; Metformin; Letrozole

1. Introduction

Polycystic ovarian syndrome (PCOS) is a hormonal or endocrinology condition which impacts the reproductive system in women, leading to fluid-filled sacs and increased risk of obesity, type 2 diabetes, endometrial cancer, dyslipidemia, hypertension, infertility, and cardiovascular disease. Endometrial cancer, depression, sleep apnoea, obesity, metabolic syndrome, poor glucose tolerance, cardiovascular risk, and nonalcoholic fatty liver disease are only a few of the morbidities that it is linked to[1,2]. A few indicators are larger ovaries, high testosterone levels, and irregular menstruation. PCOS affects 5% to 15% of girls globally, with 5 million United States (US) females suffering from the condition[3]. It costs $4 billion annually for diagnosis and treatment. Over 2 billion people worldwide are overweight or obese, accounting for 44% of all adults. Obesity poses a significant public health risk, especially in low-income or middle-income countries (LMICs), where under-nutrition is prevalent[4]. Addressing obesity in LMICs is crucial to prevent harmful health effects, as it is a significant predictor of cardiovascular disease, polycystic ovarian syndrome, type 2 diabetes, and other metabolic diseases[5–7].PCOS is a condition characterized by an excess of androgen in the ovaries and ovulation, which can be caused by various factors. An irregularity in insulin synthesis and action, which results in hyperinsulinemia and insulin resistance, has been linked to the pathophysiology of PCOS [8–11]. The increased LH pulse frequency and amplitude are a result of mainneuro-endocrine detection, while a malfunction in androgen synthesis leads to increased androgen production in the ovaries[12–14]. However, current understanding of the metabolic ovarian pituitary circuitry suggests that it is maximally intertwined[15].

Herbal remedies are increasingly used in medical practices to treat PCOS, a condition characterized by sperm production. These plant-based medications have a significant therapeutic impact and are less harmful than conventional treatments[16–18]. They play a crucial role in recovery, prevention, and treatment. Herbal medications can have antagonistic and synergistic interactions, making them less likely to cause side effects than allopathic medications[19]. Regular use of herbs can be safer and more effective in treating PCOS, reducing cyst formation. The research aims to cure PCOS with letrozole by inducing eriocitrin in a rat model. The study investigated the impact of *B. integerrima* extract resveratrol and B. integerrima treatment dramatically lowered levels of triglycerides, low-density lipoprotein, tumour necrosis factor-alpha, and malondialdehyde contents, decreased insulin resistance, increased antioxidant capacity, and superoxide dismutase[20]. The research examined the effects of vitamin C and black cohosh ethanolic extract on oxidative response and ovarian functioning in rats with hyperandrogenism-induced PCOS [21]. Oral letrozole was given daily for 21 days, while extracts were administered for 28 days. Assessments included hormone concentrations, antioxidant capacity, histological examination, immune histo-chemical scrutiny, cellular proliferation, and aromatase gene expression ratio. Secondary metabolites were also analyzed [22] with a negative control, PCOS, and therapy groups were among the five groups into which the rats were split. Each treatment group received clomiphene citrate and CSE for 28 days following a 21-day PCOS induction period. Hormonal and metabolic profiles, Cyp11a1 mRNA expression, histological

analysis, and antioxidant tests were performed on the ovaries and uteri. The underlying mechanisms are examined by[23] and the four groups (n = 8) of female SD rats were randomly assigned; the control group received 1within a period of 21 days. At the conclusion of the research, the Rha therapy reversed the effects of letrozole on body weight, visceral adipose tissue weight, and relative ovarian weights.

PCOS caused by letrozole (LTZ) and F. deltoidea are investigated. The rats were given LTZ for 21 days, antioxidant enzyme levels and reproductive hormone levels were measured, and histomorphometric alterations in the uterus and ovaries were evaluated. The purpose of [24] was to determine if red clover may be treated with saline given to the control group in every case for thirty days following PCOS induction. Red clover extracts and clomiphene citrate were shown to lower testosterone levels and raise estrogen levels in comparison to the PCOS-induced group, based on histopathological investigations. The effectiveness of Fagoniaindica ethanol extract is helpful in treating young adult female rats with PCOS[25]. The amount of flavonoid and phenols in the extract after the disease was introduced; the rats were divided into four groups. Proanthocyanidins (PCs) impacted ovarian fibrosis in rats with letrozole-induced PCOS [26]. The PCOS rats' body weight (BW) and relative ovarian weight were both successfully decreased by PC treatment. To look into the possible advantages of naringenin in treating PCOS using human granulose cells and a Sprague Dawley rat model [27]. Naringenin or metformin were administered to rats that had PCOS caused by letrozole in combination. The rats underwent glucose sensitivity tests, estrous cycle evaluations, and hormonal analysis. Naringenin therapy increased ovulation chances, decreased androgen levels, and increased cystic follicles. To evaluate the lipid and hormone profiles and vaginal smear were examined [28]. They assessed the shape of ovarian tissue, oxidative indicators, and inflammatory condition.

2. Materials and Methods

In–silico study maximum throughput virtual screening and molecular docking

The glide module included in the Schrödinger suite 2022-3 was used to do in-silico maximum throughput virtual screening (VHTS). The 3D structures that were constructed and inserted into the catalytic pocket to boost binding affinity were obtained from super natural databases and Pubchem. A virtual screening approach was put into practice, using filters such drug similarity, toxicity, terrible stance, ADMET, and PAINS. Next, glide SP docking will be performed on the top ligands from the VHTS, which were categorised as top virtual hits based on their docking score. Subsequently, the top-scoring molecules from standard precision (SP) docking are analysed to see whether they have maximum glide scores and whether they have hydrophobic or hydrogen bond formation in the catalytic pocket[29]. Using energetic-based glide in extra-precision mode, the top virtual hits of PCOS treatment were chosen based on glide g score and interaction pattern, and they were then docked into the catalytic pocket.

In–silico MM-GBSA by prime model

Compute binding free energy using the prime/molecular mechanics Generalized Born Surface Area (MM-GBSA) approach. MM-GBSA methods were used to the OPLS3 force field to determine the inherent energy of bonding of certain ligands. Prime had been used to decrease the protein-ligand intricate, whereas the VSGB version 2.0 energy frameworks could be used to simulate the process without placing any restrictions on the flexible residues that maintained the partial charges of the input ligand. This energy model at the moment includes an optimized implicit solvation model as well as corrections based on hydrophobic physics, self-contact interaction, and hydrogen bonding[30,31].

In vivo evaluation of polycystic ovarian syndrome selection of Animals

The animal housing was home to 150–200 g Wistar rats of the female sex. After being chosen, the animals were kept in groups and kept in polypropylene cages with standard lighting and dark cycles at 23±2o and 12:12 hours. Libitum, food, and water were all freely available to the animals. The animals were kept in regular, sanitary laboratory settings one week before the tests. Animal research complied with the authorized procedures of the institution's Animal Protection Commission experimental protocols IAEC NO: JKKMMRAFCP/1158/PO/S/07/CPSCEA.

Animal Model of letrozole induced polycystic ovarian syndrome

Examining vaginal smears every day allowed researchers to keep an eye on six-week-old female rats (mean body weight, 150–200 g) with regular 4-day oestrus cycles. In this investigation, we utilized only mice that showed two consecutive regular 4-day periods. A 28-day course of treatment was followed. Group 1 is the control group; normal saline was administered at 0.9% in 1 ml (p.o). After letrozole treatment, the standard drug METFORMIN (20mg/kg) (p.o) was administered to the next group of animals for 28 days. Group 2 is negative control; Letrozole was administered 1mg /Kg/ BW– (p.o) for 28days for disease induction. Test drug Eriocitrin at low dose (5mg/kg/p.o) and maximum doses (10mg /kg/p.o) was administered for 28 days. Every day throughout this time, vaginal samples were taken and analysed under a microscope using Giemsa staining to determine the estrus cycle. All animals were decapitated and slaughtered twenty-four hours after receiving their final letrozole dosage[24,32,33]. Utilizing stump bloodstream, several hormone concentrations have been measured: progesterone, testosterone, oestrogen, follicle-stimulating hormone (FSH), luteinizing hormone (LH), and estradiol. The spikes were refrigerated at 20°C. Uteri and ovaries were weighed to determine how the test chemical affected the animals' endocrine balance. The ovaries were divided along their longest longitudinal axis, preserved in 10% neutral formalin, sectioned at intervals of 4 µm, and stained with H and E. Duration of study: 28 days and blood collection: retro-orbital puncture and cardiac puncture.

Biochemical parameters

OGTT and Serum insulin level

The glucose tolerance testing was performed after fasting for 15 hours. Fasting blood glucose was measured. Glucose (2 g/kg/p.o) was fed 30 min after administering the test drug. The animal was allowed to fast for 12 hours. Inject the mouse intraperitoneally with 10µl/g BW of insulin injection solution. Take note of the injection time point on the record sheet.

Total cholesterol levels

Triglycerides, HDL, LDL and total cholesterol: Blood was drawn into a red-topped Vacationer® tube at room temperature for 45 minutes to coagulate and fully retract the clot. At this point, the tube was sealed, and the samples were centrifuged for 30 minutes at 40ºC at 1,500 x g; a refrigerator-style centrifuge was used. Following centrifugation, the samples were kept at 2–40 degrees Celsius in an ice bath. Samples were kept in a non-self-defrosting freezer at -20ºC until they were sent to the lab. Using a series of connected processes, glycerol oxidase oxidized glycerol, and H_2O_2 were measured to determine blood triglyceride levels enzymatically. 500 nm was used to measure absorbance.

Steroidal hormone levels: An immune assay kit measured progesterone and other hormone levels, including oestrogen. Testosterone, FSH, and LH blood levels were quantitatively measured.

FSH and LH

The kit contains one micro-ELISA plate with an antibody specific to rat FSH and LH pre-coated on it. After standards or samples are added, the biotinylated detection antibody is added to the plate wells. The only components of the wells that turn blue are the biotinylated detection antibody, the Avidin-HRP conjugate, the rat FSH and LH, and the stop solution, which is used to halt the reaction. Rat FSH and LH optical densities (OD) are measured at 450 nm ± 2 nm using spectrophotometry. By comparing the OD with the standard curve, one may calculate the sample concentration, which is proportional to the OD value.

Estrogen

An eppendorf tube was filled with blood extracted from the retro-orbital plexus using a micro-capillary, and the tube was centrifuged for ten minutes at 3000 rpm. Half a litre of serum was obtained, and then 500 microlitters of uranyl acetate were added during the deproteinized process. The mixture was taken from the supernatant after it had been centrifuged for five minutes at 3000 rpm. Rat/Mouse Estradiol ELISA kit technique was used to evaluate the levels of oestrogen hormone concentrations. The findings were reported in pg./mL and measured at 450 nm wavelength using a spectrophotometer.

Testosterone

Utilizing the competitive binding concept, the testosterone of the rat/mouse ELISA kit is a solid phase ELISA. There is competition between a preset quantity of the hormone testosterone attached to mustard and the unidentified quantity of testosterone in the specimen for the attachment locations of the testosterone antiserum covered in the micro plate's holes peroxides. Four washings of the micro-plate are performed following an hour-long incubation on a shaker. Testosterone concentration is inversely related to observing optical density upon adding substrate solution.

Progesterone

The quantitative measurement of progesterone in rat serum or plasma is usedwith the progesterone ELISA. The competitive ELISA of this phase is strong. The samples are placed into the wells coated with anti-progesterone monoclonal antibody and the progesterone enzyme conjugate. The sample's progesterone competes for binding sites with a progesterone enzyme conjugate. The progesterone enzyme conjugate and unbound progesterone are removed using a washing buffer. The amount of progesterone in the samples negatively correlates with the color intensity when the substrate is added. A reference curve between color intensity and progesterone concentration is created.

Thyroid hormone

Thyroid hormones, primarily thyroxin and triodothyronine, regulate metabolism, growth, and development. They impact various physiological processes, including heart function, brain development, and other endocrine glands. It usesletrozole, an aromatase inhibitor, to study PCOS in rat models. Eriocitrin, a flavonoid glycoside found in citrus fruits, has antioxidant, anti-inflammatory, and potential endocrine-modulating effects. These properties suggest that eriocitrin could be therapeutic in managing PCOS by reducing oxidative stress and inflammation, improving insulin resistance, and lowering androgen levels. Studying eriocitrin's effects on letrozole-induced PCOS could provide insights into its efficacy and mechanisms of action, potentially leading to new therapeutic approaches for managing PCOS and related endocrine dysfunctions.

Vaginal smears to evaluate oestrous cycle

Every animal was chosen, and the aperture within the vagina became apparent. The rat's vaginal cavity had been filled with an inch of moist cotton net that had been bathed in water. A piece of cotton had been removed after softly spinning for quite a while. A streak was produced on a plate of glass that had been cleansed and cleared of oil using this wool wipe. Following drying by air and water from distillation, 10% methanol and the methylene blue dye were used to clean the slide.

Weight changes and histopathology of rat ovaries to visualize follicular cysts

The rats were weighed daily during the experiment to determine the mean change in body weight. Removed ovaries were left in Bouin's solution to repair them. They were immersed in paraffin wax that melted at 60 degrees Celsius, cleaned in xylene, and dehydrated a succession of alcohol. Serial slices were placed on slides coated with 3-aminopropyl triethsilane and allowed to dry at 37°C for a full day. After deparaffinising, hydrating, and staining the sections on the slides after being dried and mounted for histology, the cells were dyed with Mayer, a mixture of eosin. The photographic apparatus in Scope Picture 3.0 was presented to view the ovaries at a magnification of 40x. The cystic follicles' thickness and diameter were assessed. A conspicuous outer theca and interior layer characterized the thicker, fibrotic cortex of the cystic follicles.

3. Results and Discussion

In this section, we have examined whether eriocitrin, a potent antioxidant, anti-inflammatory, and endocrine-modulating agent, has potential as a treatment for PCOS. We evaluated its efficacy using a letrozole-induced rat model.

Molecular docking

When contrasted to standard drug metformin glide scores2.37 kcal/mol. Thepolyphenoliccompound shows good ligand drug receptor interaction. Here, the top 10 compound results were mentioned. Eriocitrin – 7.33 kcal/mol, Orient in – 6.34 kcal/mol, Procyanidin – 6.24 kcal/mol, Aloes in – 5.87 kcal/mol, Baicalin-5.35 kcal/mol, Aloe emodin-4.94 kcal/mol, Nicotiflorin- 4.88 kcal/mol, Baicalein – 4.88 kcal/mol, Amentoflavone-3.56 kcal/mol, Purpurin – 3.61kcal/mol[34]. Table 1 represents the numerical value of polyphenolic compound vs. 2IOG PDB.

Table 1: Polyphenolic compound Vs 2IOG PDB

Comp Name	Comp Code	G_H Bond	G_score	G_ecoul	G_evdw	G_e model	G_energy
Psoralidin	5281806	-0.7	-4.61	-4.34	-25.53	-35.28	-29.87
Mauritianin	10919701	-3.57	-4.33	-4.817	-33.17	-40.75	-37.99
Pectolinarigenin	5320438	-3.98	-0.48	-24.92	-1.22	-26.15	-27.67
Aloenin	162305	-2.32	-5.14	-8.86	-26.58	-41.31	-35.44
Anacardic acid	167551	-3.7	-2.02	-16.49	-9.76	-26.25	-27.89
Orientin	5281675	-3.45	-6.34	-8.22	-27.59	-43.76	-35.82
Leucocyanidi	71629	-2.07	-4.6	-5.778	-20.26	-30.12	-26.04

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Baicalin	64982	-2.88	-5.35	-8.82	-19.04	-36.33	-27.87
Tamoxifencitrate (std)	2733525	-1.48	- 3.13	-4.03	-19.69	-29.24	-46.72
Nicotiflorin	5318767	-3.69	-4.88	-14.29	-26.85	-46.12	-41.14
Aloesin	160190	-3.16	-5.87	-6.42	-22.69	-34.49	-29.12
Aloe emodin	10207	-2.07	-4.94	-3.55	-20.14	-29.78	-23.69
Wogonin	5281703	-0.84	-4.45	-3.23	-23.55	-30.8	-26.79
Metformin (Std)	4091	-1.34	-2.37	-0.45	-26.23	-18.96	-43.28
Broussonol E	10343070	-1.92	-4.16	-4.06	-25.85	-33.82	-29.92
Procyanidin	107876	-3.08	-6.32	-8.89	-30.41	-51.71	-39.31
Biorobin	15944778	-3.67	-3.35	-23.07	-10.97	-34.09	-40.56
Macluraxanthone	5281646	-1.92	-4.67	-9.254	-18.48	-34.38	-27.73
Catechol	289	-1.92	-4.35	-6.58	-10.16	-18.65	-16.75
Glabridin	124052	-0.7	-4.05	-5.02	-21.27	-31.63	-26.29
Theaflavanin	1.35E+08	-2.02	-4.8	-4.566	-19.27	-29.06	-23.83
Epicatechin	72276	-1.62	-4.3	-8.52	-16.64	-27.9	-25.16
Hyperoside	5281643	-3.67	-3.28	-27.64	-7.18	-34.83	-40.38
Kaempferol 3-neohesperidoside	5318761	-3.86	-4.34	-6.19	-24.81	-35.09	-31
Eriocitrin	83489	-2.76	-7.33	-7.54	-28.54	-49.21	-32.97
Dihydrotanshinone	5316743	-3.46	0	-22.81	0.86	-21.94	-25.13
Mangiferin	5281647	-1.92	-4.09	-4.45	-25.66	-36.01	-30.11
Aloin A	12305761	-1.9	-4.65	-6.29	-20.46	-32.43	-26.75
beta-Lapachone	3885	-2.86	0	-19.93	-0.46	-20.4	-23.95
Cryptotanshinone	160254	-3.6	0	-23.7	-0.53	-24.24	-30.24
Baicalein	5281605	-2.4	-4.87	-7.65	-18.62	-31.92	-26.27
Abyssinones Iii	10408069	-3.31	0	-27.31	-0.83	-28.14	-35.17
Isorhamnetin	5281654	-2.8	-1.34	-23.2	-1.84	-25.04	-24.08
Clitorin	11592917	-2.76	-2.67	-21.83	-7.24	-29.07	-37.09
Bavachinin	1033721	-3.52	0	-25.16	-1.06	-26.22	-33.18

	1						
chloroxine	2722	-2.78	-0.7	-15.89	-2.12	-18.01	-22.03
Amentoflavone	5281600	-3.57	-0.96	-30.54	-3.54	-34	-44.32
Tryptanthrin	73549	-2.8	0	-20.61	-0.37	-20.95	-26.37
Tanshinone I	114917	-3.59	0	-21.49	-0.17	-21.67	-27.68
Sophoridine	165549	-2.72	0	-19.42	-0.81	-20.23	-25.51
Sophoraflavanone G	72936	-2.72	-0.82	-18.64	-11.57	-30.21	-40.47
tolmetin	5509	-2.81	0	-17.48	1.08	-16.4	-19.7
Azanium	78224690	-2.61	0	-29.72	-4.89	-34.62	-46.94
Astilbin	119258	-2.87	-2.88	-23.44	-8.83	-32.28	-39.75
beta-Himachalene	11586487	-2.65	0	-18.72	-0.029	-18.75	-22.1
Pinostrobin	73201	-3.39	-0.96	-19.69	-4.03	-23.72	-29.24
Nimbin	108058	-2.58	-0.64	-23.62	-2.63	-26.25	-32.61
Disulfiram	3117	-2	0	-20.39	0.18	-20.2	-26.06
Agaric acid	12629	-1.61	-1.62	-18.48	-7.89	-26.37	-24.08
Tricin	5281702	-2.45	-0.83	-23.6	-1.1	-24.7	-27.01
Silymarin	5213	-2.1	-0.96	-27.39	-3.31	-30.71	-35.78
Daidzein	5281708	-0.76	-0.7	-17.36	-3.76	-21.12	-25.37
Avasimibe	166558	-2.44	0	-28.05	0.64	-27.4	-35.67
Proanthocyanidin	108065	-2.28	-1.18	-22.36	-4.41	-26.77	-30.72
Kolaflavanone	155169	-2.29	-1.98	-24.8	-7.22	-32.03	-38.4
Tamarixetin	5281699	-2.09	-0.96	-22.68	-0.24	-22.92	-23.26
Myricetin	5281672	-2.31	-2.34	-18.45	-7.25	-25.71	-31.77
candesartan	2541	-1.4	0	-29.86	-5.34	-35.25	-47.8
Chrysophanic acid	10208	-2.37	0	-18.18	-0.22	-18.41	-22.2
Metformin (Std)	4091	-2.37	-1.34	-26.23	-0.45	-43.28	-18.96
Juglanin	5318717	-2.48	-1.44	-24.66	-5.74	-30.41	-34.84
Oleanolic Acid	10494	0	-1.49	-20.21	-5.53	-25.74	-31.63
Aloeresin A	5317657	0.09	-1.44	-27.24	-5.1	-32.34	-41.97
Umbelliferone	5281426	0.19	0	-14.36	0.19	-14.164	-16.74
Neobavaisoflavone	5320053	-0.83	-0.7	-19.44	-5.81	-25.25	-29.65

Lithium;4-[2-(diethylamino) ethylcarbamoyl]-2-iodobenzoate	24801580	-1.7	0	-21.62	-6.24	-33.63	

Interactions of maximum hit compounds

All the top hit compounds showing good amino acid residue intermolecular interaction. Figure 2 shows diagrammatic representation of the 2D structure of (a) Eriocitrin and (b) Metformin.

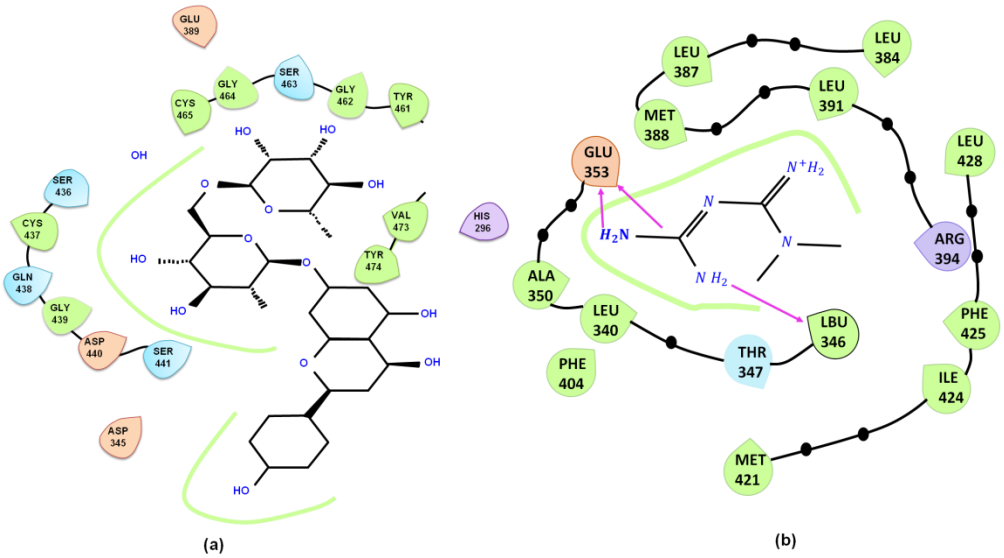

Figure 2: 2D structure of (a) Eriocitrin and (b) Metformin

***In-vivo* evaluation of PCOS animal model and serum insulin level**

It was discovered that there was a substantial difference (***p<0.001) between the glucose-controlling animal group and the usual control group. At doses of 5 and 10 mg/kg, eriocitrin significantly (p<0.001) reduced blood glucose levels in mice with PCOS compared to control groups. The glucose volume was decreased in the rats receiving 5 mg/kg and 10 mg/kg of eriocitrin compared to the PCOS control group. The mice under the disease management group had considerably (***p<0.001) higher insulin levels than those under standard control. When contrasted to the PCOS control animals, the effects of eriocitrin on blood insulin levels were substantial ($p < 0.001$) at dosages of 5 and 10 mg/kg. In contrast to the PCOS control group, the insulin volume was lower in the rats receiving 5 mg/kg and 10 mg/kg of eriocitrin. The glucose tolerance test showed that Eriocitrin (5 mg/kg and 10 mg/kg) significantly reduced blood glucose levels and decreased serum insulin compared to the negative control and letrozole-treated group.[35]Eriocitrin also lowered total cholesterol, LDL, and HDL levels compared to letrozole. While letrozole reduced steroidal hormones (FSH, LH,

estrogen), both Eriocitrin and metformin increased these levels, though Eriocitrin significantly reduced progesterone and testosterone compared to metformin. Additionally, Eriocitrin significantly reduced body weight, while letrozole increased it and caused tissue damage, which Eriocitrin and metformin mitigated.

Table 2 depicts the numerical outcomes of OGTT and serum insulin levels. Figure 3 represents the (a) OGTT and (b) Serum insulin levels.

Table 2: OGTT and Serum insulin level.

S.NO	GROUP	BLOOD GLUCOSE LEVEL(mg/dL)	INSULIN LEVEL (mcU/mL)
1	CONTROL GROUP (Normal saline 0.9 %)	114.16± 1.8***	16±1.06**
2	LETROZOLE TREATE GROUP (2 mg/kg)	193.6± 2.1###	20.5±0.56###
3	STANDARD DRUG (Metformin 20 mg/kg)	158± 1.4***	12.5±0.76**
4	TEST DRUG (Eriocitrin 5mg/kg)	159± 1.5***	13.3±0.3**
5	TEST DRUG (Eriocitrin 10mg/kg)	156± 1.9***	13.6±0.3**

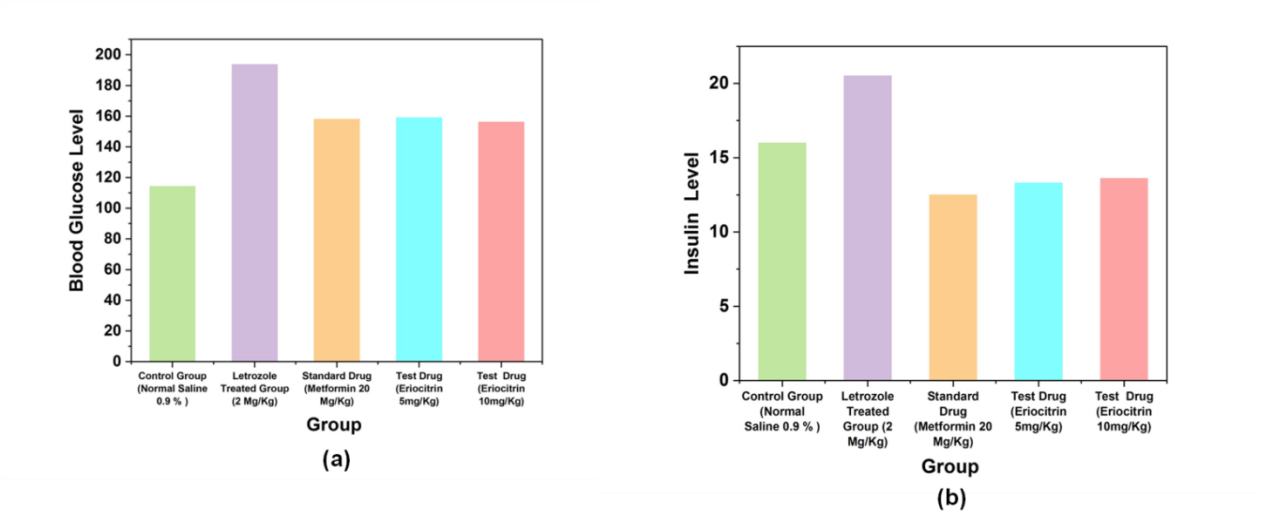

Figure 3: Graphical representation (a) Oral glucose test, (b) Serum insulin level

Total cholesterol level and higher density lipoprotein

In the animal group that controls illness, the total cholesterol level was found to be significantly different ($*** p < 0.001$) from animals in the standard control group. When contrasted to the PCOS control animals, the effects of eriocitrinon cholesterol levels were substantial ($P < 0.001$) at dosages of 5 and 10 mg/kg. Compared to the PCOS control group, the rats receiving 5 mg/kg and 10 mg/kg of eriocitrin had minimum cholesterol volumes. When contrasted to normal control animals, the HDL level in the illness-control animal group was significantly higher ($*** P < 0.001$). Eriocitrin at 5 and 10 mg/kg had significant ($P < 0.001$) impacts on HDL levels when compared to the PCOS control animals. The rats given eriocitrin at doses of 5 mg/kg and 10 mg/kg had a reduced volume of maximum-density lipoprotein compared to the PCOS control group. The ranges for total cholesterol and maximum density lipoprotein are displayed in Table 3. The levels of (a) cholesterol and (b) higher-density lipoprotein are shown in Figure 4.

Table 3: Total cholesterol level and higher density lipoprotein

S.NO	GROUP	CHOLESTEROL LEVEL(mg/dL)	HDL(mg/dL)
1	CONTROL GROUP (Normal saline 0.9 %)	125.16±1.3**	57.3±0.88***
2	LETROZOLE TREATED GROUP (Letrozole 2mg/kg)	168.8±1.07###	102.4±0.91###

3	STANDARD DRUG (Metformin 20 mg/kg)	158±1.4**	43.5±1.105***
4	TEST DRUG (Eriocitrin 5mg/kg)	161±1.06**	47.3±1.105***
5	TEST DRUG (10mg/kg)	156.8±1.04**	44.4±0.4***

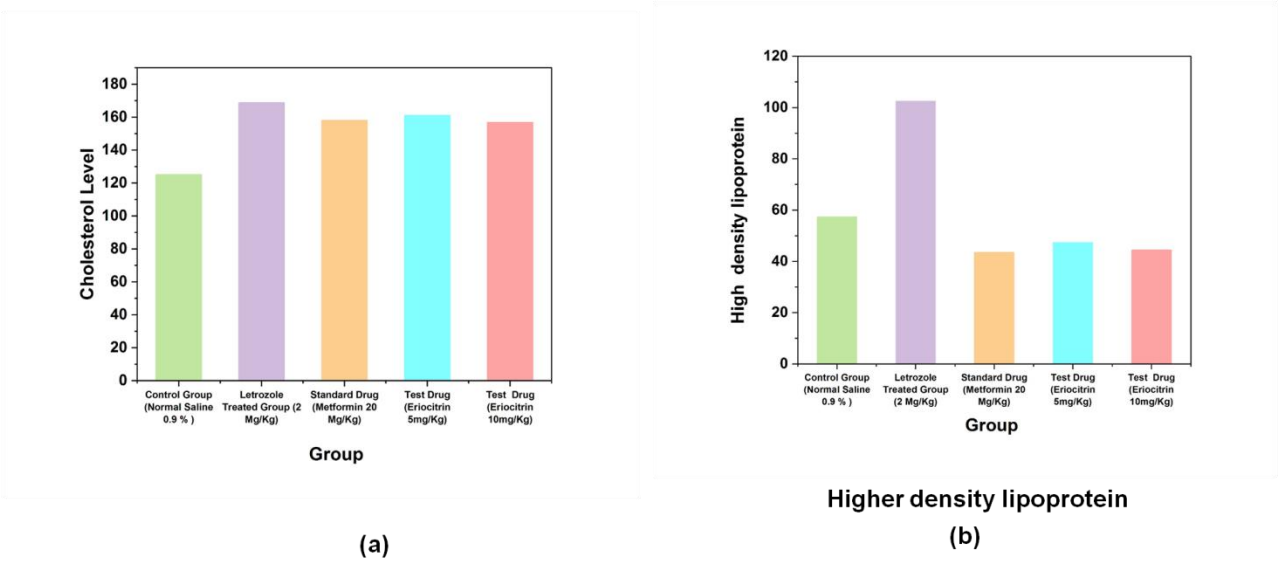

Figure 4: Graphical representation of (a) Total cholesterol level, (b) Higher density lipoprotein.

Lower density lipoprotein and follicular stimulating hormone

The LDL level in the disease control animal group was found to be considerably higher than that of standard control animals($*** p < 0.001$). There were significant impacts of eriocitrin on LDL level as compared to the PCOS control animals ($p < 0.001$) both at 5 and 10 mg/kg doses. The rats receiving 5 mg/kg and 10 mg/kg of eriocitrin had a smaller volume of low-density lipoprotein in comparison to the PCOS control group. The FSH level was considerably higher in the disease control animal group compared to normal control animals($*** p < 0.001$). In comparison to the animals under PCOS control, eriocitrin significantly affected the amount of FSH ($p < 0.001$) in amounts between 5 and 10 mg/kg. The rats administered eriocitrin at dosages of 5 mg/kg and 10 mg/kg exhibited the highest quantities of FSH in comparison to the PCOS control group. The levels of follicular stimulating hormones alongside low-dense lipoprotein are displayed in Table 4. Figure 5 shows the (a) lower-density lipoprotein and (b) follicular stimulating hormone.

Table 4: Lower density lipoprotein and Follicular stimulating hormone

S.NO	GROUP	LDL(mg/dL)	FSH(mIU/mL)
1	CONTROL GROUP (Normal saline 0.9 %)	102.0±0.8***	11.7±0.8***
2	LETROZOLE TREATED GROUP (Letrozole 2mg/kg)	168±0.7###	12.13±0.25***
3	STANDARD DRUG (Metformin 20 mg/kg)	156±0.5***	13.2±0.3
4	TEST DRUG (Eriocitrin 5mg/kg)	166.5±0.76***	4.8±0.08###
5	TEST DRUG (Eriocitrin 10mg/kg)	162.98±0.5***	11.3±0.21***

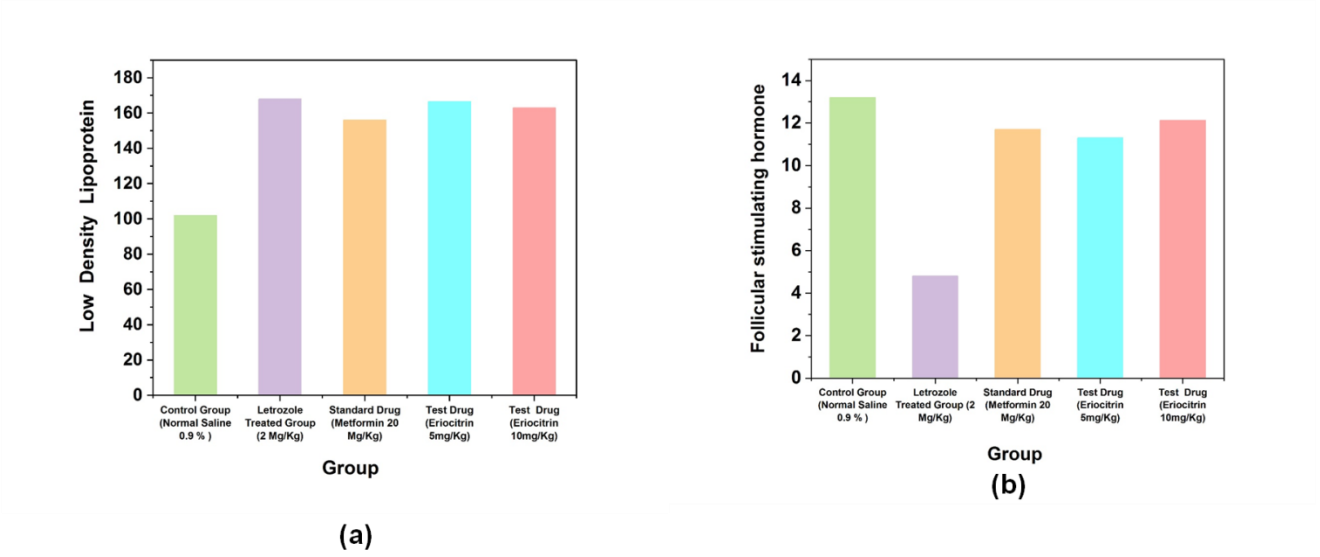

Figure 5: Graphical representation of (a) Lowerdensity lipoprotein (b) Follicular stimulating hormone.

Estrogen, testosterone, progesterone and body weight

Comparing the illness control animal group's oestrogen level to that of the normal control animals revealed a significant difference ($*** p < 0.001$). Comparing the 5 and 10 mg/kg dosages of eriocitrin to the PCOS control animals revealed substantial ($p < 0.001$) impacts on the amount of oestrogen. Comparing the eriocitrin 5 mg/kg and 10 mg/kg animals , the level of oestrogen hormones in the test subjects increased ompared to the PCOS control group. When testosterone levels of the disease control's animal group were compared to those of the normal control animals, there was a notable difference ($*** p < 0.001$). Comparing the 5 and 10 mg/kg dosages of eriocitrin to the PCOS control animals revealed substantial ($p < 0.001$) impacts on the levels of testosterone. Comparing the PCOS control group's testosterone hormone volume with the animals given Eriocitrin at doses of 5 mg/kg and 10 mg/kg produced a reduction. Comparing the progesterone llevels of the illness control animal group to that of the normal control animals revealed a significant difference in that of the normal control animals revealed a significant difference($*** p < 0.001$). When comparing the 5 and 10 mg/kg dosages of eriocitrin to the PCOS control animals, the effects on progesterone levels were considerable($p < 0.001$). Comparing the Eriocitrin 5 mg/kg and 10 mg/kg animals to the PCOS control group revealed an increase in the amount of progesterone hormone. Comparing the 5 and 10 mg/kg dosages of eriocitrin to the PCOS control rats revealed substantial ($p < 0.001$) impacts on body weight. When comparing the rats receiving 5 mg/kg and 10 mg/kg of eriocitrin to the PCOS control group, the animals' body weight fell. Table 5 determines the numerical outcomes of estrogen, testosterone, progesterone, and body weight. Figure 6 presents the graphical representation of (a) estrogen,(b) testosterone, (c)progesterone and (d) body weight.

Table 5: Numerical outcomes of estrogen and Testosterone.

S.NO	**GROUP**	**ESTROGEN(pg/mL)**	**TESTOSTERONE(ng/dL)**	**PROGESTERONE(ng/mL)**	**WEIGHT CHANGES(g)**
1	TEST DRUG (Eriocitrin 5mg/kg)	141.5±0.7***	127.3±0.66###	5.65±0.11**	198.16±34.2**
2	TEST DRUG (Eriocitrin10 mg/kg)	147±0.7***	101.48±0.77***	6.33±0.10***	227±1.01**

3	STANDARD DRUG (Metformin 20 mg/kg)	154±0.3***	92.85±0.46***	4.33±0.023	221±2.08**
4	LETROZOLE TREATED GROUP (Letrozole 2mg/kg)	74.35±0.5###	64±0.8***	6.75±0.13***	221.1±1.1**
5	CONTROL GROUP (Normal saline 0.9%)	160.5±0.7***	98.31±0.40***	1.98±0.16###	245±1.02###

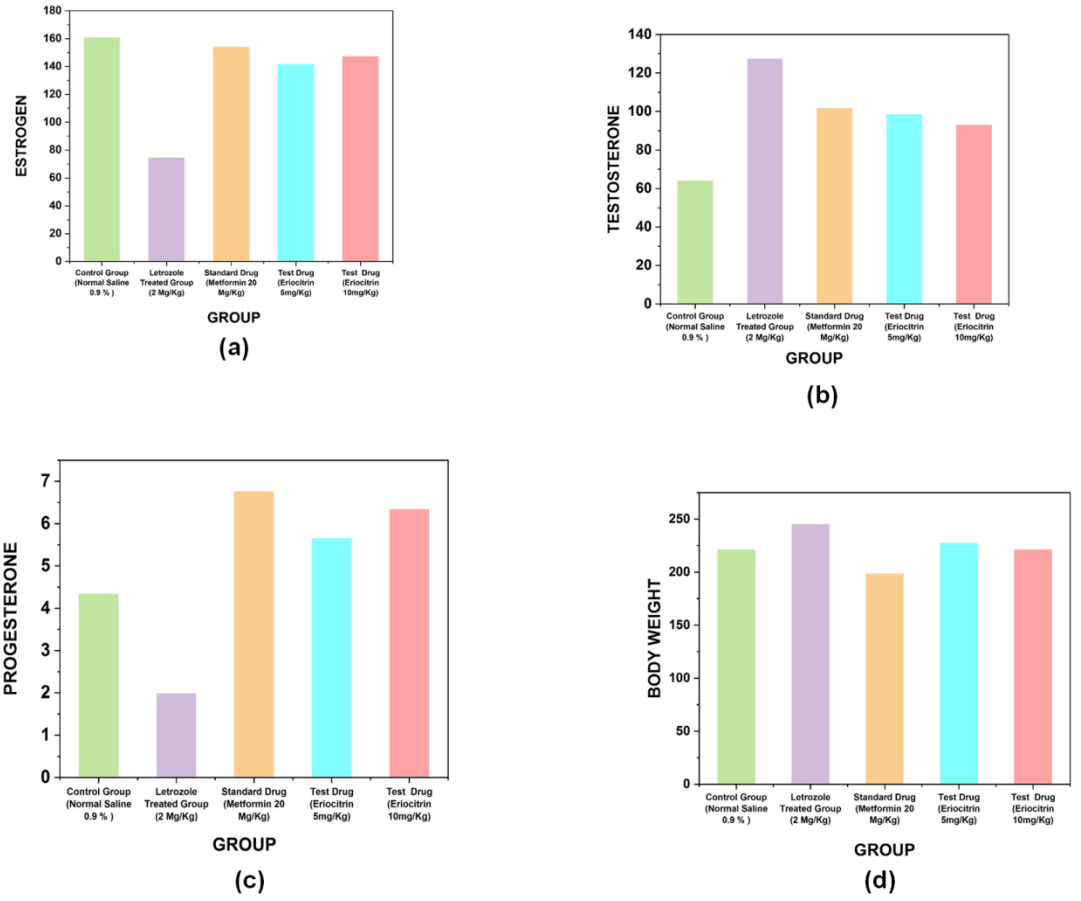

Figure 6: Graphical representation of (a) Estrogen (b) Testosterone(c) Progesterone (d) and body weight.

Vaginal smears for PCOS model by microscopically view and histopathology of rat uterus

Figures 7 and 8 show the microscopic images of (a) GROUP – I, (b) GROUP II, (c) GROUP III, (d) GROUP IV and (e) GROUP V of the vaginal smears for PCOS model by microscopically view and histopathology of rat uterus.

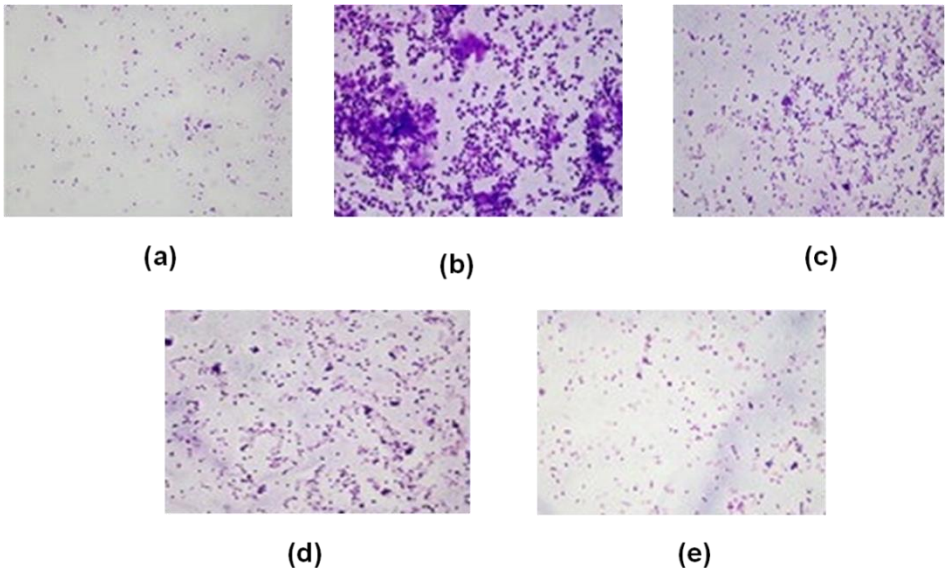

Figure 7: (a) GROUP - I – Smear of animal treated with normal saline (0.9%) represents the rat vaginal smears is estrus, (b) GROUP II –Smear of the animal treated with Negative control (Letrozole 2mg/kg) represents the rats vaginal smears is diestrus, (c) GROUP III– Smear of the animal treated with Standard drug (Metformin 20mg/kg) represents the rat vaginal smears is Proestrus, (d) GROUP IV – Smear of the animal treated with test drug (Eriocitrin 5mg/kg) represents the rat vaginal smears is proestrus, and (e) GROUP V - Smear of animal treated with test drug (Eriocitrin 10 mg/kg) represents the rat vaginal smears is proestrus

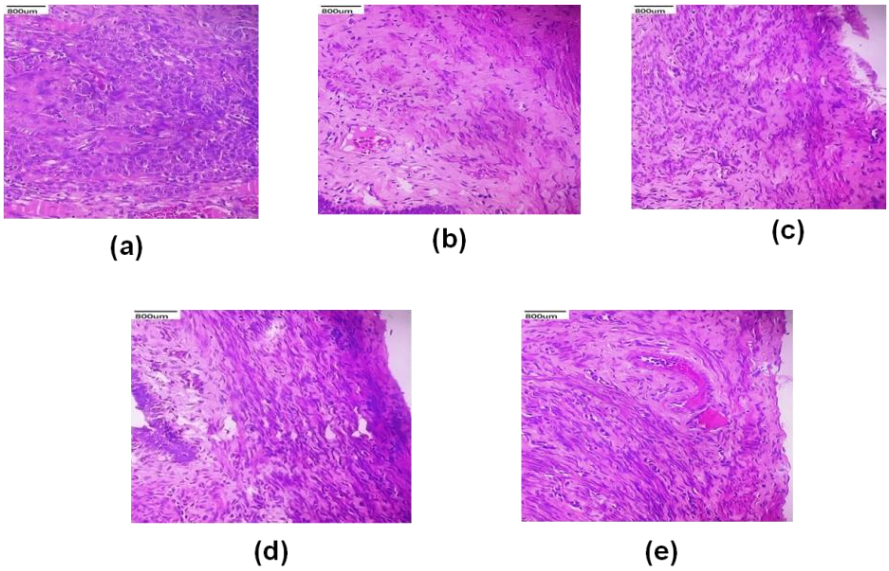

Figure 8: (a) GROUP I– Section of uterus from normal rate showing the presence of antral follicles,corpus leuteum and oocytes sorrounded by granulosa cells and theca cell layer, (b) GROUP II- section of uterus from PCOS (Letrozole 2mg/kg) induced group many cystic degenerating follicles with degrated granulosa layer, (c) GROUP III – PCOS treated with metformin (20mg/kg) as standard drug showing the presence of normal follicles with clear antrum and oocytes in granulosa layer, (d) GROUP IV – animals treated with Eriocitrin 5mg/kg shows mild degeneration and absence of cystic and antra follicles, and (e) GROUP V – Animals treated with Eriocitrin (10 mg/kg) shows developing regenerating follicles and corpus leutum with granulosalayer

4. Conclusion

In a rat model of Polycystic Ovary Syndrome (PCOS) generated by letrozole, the study examined the possible therapeutic advantages of eriocitrin, a flavonoid glycoside. According to research findings, Eriocitrin outperforms metformin in terms of binding affinity to critical PCOS-related receptors. In vitro research has shown that eriocitrin administered at 5 and 10 mg/kg dosages significantly improved blood glucose and insulin levels, addressing insulin resistance and several other PCOS indicators. Additionally, compared to the PCOS control group, it decreased total cholesterol levels and better lipid profiles. Furthermore, eriocitrin restored normal reproductive function by favorably influencing the oestrous cycle. A decrease in follicular cysts was seen in the ovarian tissues examined histopathologically, indicating that eriocitrin may be able to relieve the structural irregularities that are frequently observed in PCOS. The results indicate that eriocitrin's antioxidant, anti-inflammatory, and endocrine-modulating qualities make it a promising treatment agent for PCOS. To fully understand the therapeutic uses of eriocitrin and its effectiveness in treating PCOS, more investigation is required.

Acknowledgement

We acknowledge Department of Pharmacology, JKKMMRFs Annai JKK Sampoorani Ammal College of Pharmacy, B. Komarapalayam, Namakkal (D.T), Tamil Nadu, India.

Conflict of interest: The authors declared that there was no conflict of interest.

Funding: Not applicable.

Ethics approval: Animal research complied with the authorized procedures of the institution's Animal Protection Commission experimental protocols IAEC NO: JKKMMRAFCP/1158/PO/S/07/CPSCEA.

Conflicts of interest: The authors declare that they have no conflict of interests.

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