



**INTERNATIONAL JOURNAL OF  
PHARMACEUTICAL SCIENCES**  
[ISSN: 0975-4725; CODEN(USA): IJPS00]  
Journal Homepage: <https://www.ijpsjournal.com>



## Research Article

# Evaluation Of the Effect of Kaempferol on Letrozole Induced Polycystic Ovarian Syndrome in Female Wistar Rats

Nayana Shaji\*, Sri Suku J., Surabhi M.

College of Pharmaceutical Sciences, Government Medical College, Thiruvananthapuram, Kerala, India.

### ARTICLE INFO

Published: 17 Mar. 2025

**Keywords:**

Polycystic ovarian syndrome, androgen, flavonoid, kaempferol, hormone, letrozole.

**DOI:**

10.5281/zenodo.15038182

### ABSTRACT

Polycystic ovarian syndrome (PCOS) is a complex endocrine as well as metabolic disorder in which ovaries produce an excessive amount of androgens (hyperandrogenism). It mainly affects women of reproductive age. Kaempferol belongs to the category of flavonoids and in this study, we evaluated the efficacy of kaempferol in PCOS condition. The animals were divided into 5 groups with each group containing 6 animals each. Letrozole, an aromatase inhibitor was used for PCOS induction. After disease induction the animals were divided into 4 groups that is disease control, metformin, kaempferol (80 and 200 mg/kg). Weekly body weight estimation was carried out. Blood glucose was estimated on the 0th, 21st, and 34th day of study. Animals were sacrificed on the terminal day and their ovaries were isolated and preserved for further histopathological studies. H&E staining confirmed the presence of cystic follicles and degraded ovarian tissues. Treatment normalised the disrupted histological features. Serum hormonal parameters such as estrogen, luteinizing hormone, and follicle-stimulating hormone were estimated. Lipid parameters (LDL-C, HDL-C, serum total cholesterol) were also checked. MTT assay was carried out in order to check the cytotoxicity of kaempferol on ovarian cell line (SKOV3 cell line). DPPH scavenging assay was carried out to find out the antioxidant potential of kaempferol. The percentage inhibition was calculated from the superoxide dismutase assay.

### INTRODUCTION

PCOS is a complex endocrine disorder with multiple environmental factors that impact around 10% of women of reproductive age globally and lead to infertility due to lack of ovulation in women. The manifestation of PCOS includes

excessive androgen levels, as well as halted follicular growth and the formation of atresia and cysts<sup>[1]</sup>. According to a study conducted by Irving Freiler Stein and Michael Leo Leventhal in 1935, it was found that amenorrhea was associated with the presence of bilateral polycystic ovaries and it

\*Corresponding Author: Nayana Shaji

Address: College of Pharmaceutical Sciences, Government Medical College, Thiruvananthapuram, Kerala, India.

Email : [nayana123ks@gmail.com](mailto:nayana123ks@gmail.com)

Relevant conflicts of interest/financial disclosures: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.



was observed in seven women. Three of these women were obese, five were hirsute, and one was acneic but slim. These scientists were the first ones to observe the clustering of amenorrhea, hirsutism, and polycystic ovaries<sup>[2]</sup>. This led to the discovery of PCOS, which was initially termed as Stein-Leventhal syndrome. Although the exact cause of PCOS is unknown, it is believed to be the result of a complex interaction between several environmental and inherited factors. This is a complicated disorder with different vulnerabilities among people, likely resulting from a mix of genetic and environmental risk elements.<sup>[3]</sup> The condition may primarily be biochemical (hyperandrogenaemia) or morphological (polycystic ovaries). The main hallmark of PCOS does often involve follicular cysts, but the syndrome itself is not defined solely by the presence of these ovarian cysts. Instead, PCOS is a complex endocrine disorder diagnosed based on a combination of symptoms and criteria, with cysts being only one possible feature. Rather it was confirmed by the presence of two out of three diagnostic criteria. These diagnostic criteria were defined several times – by the National Institute of Health (1990), by the European Society of Human Reproduction and Embryology (ESHRE), and the American Society for Reproductive Medicine (ASRM) in 2003 (also known as the Rotterdam criteria), by the Androgen Excess and PCOS Society (AE-PCOS) in 2006. In 2012, the National Institutes of Health approved the 2003 Rotterdam criteria for PCOS<sup>[7]</sup>. The prevalence of PCOS ranges from 6 – 21% in reproductive-aged women globally. According to various definitions, the prevalence of PCOS ranges from 5% to 20%<sup>[8]</sup>. Indian women have a high incidence of PCOS prevalence; the country's estimated pooled prevalence of the illness, per Rotterdam's criteria, is 11.34%. As per WHO, 116 million women are affected by PCOS globally. In India the prevalence rate is 3.7 to 22.5%, while if we consider the

Kerala it is found to be 30% (high prevalence in Trivandrum). PCOM results from ovulatory dysfunction and irregular follicular growth, both associated with excessive androgen production in instances of ovarian issues. Altering the follicular microenvironment and/or GnRH pulsation can exacerbate ovarian failure due to elevated levels of anti-Müllerian hormone (AMH), which are secreted by pre-/small antral follicles that accumulate in ovaries affected by PCOS. The disruption of pulsatile GnRH release linked to hyperandrogenism can be partially attributed to faulty progesterone and estrogen feedback mechanisms, resulting in heightened gonadotropin production, especially increased LH secretion. The disruption of follicular growth is aggravated by elevated LH levels, which also result in an imbalance of the LH/FSH ratio and excess androgen production from thecal cells. Another crucial element in the development of PCOS is insulin resistance. The degree of insulin resistance is heightened by increased androgen levels, and the conditions tied to hyperandrogenism are intensified by hyperinsulinemia, resulting from insulin resistance, which further boosts androgen production and prompts the liver to generate sex hormone-binding globulin (SHBG). This elevates the level of bioactive free testosterone present in the blood. The simplest interpretation of this complex and diverse condition is that insulin resistance triggers the onset of PCOS, while hyperandrogenism acts as a contributing factor. Insulin resistance brings about hyperinsulinemia, which in turn encourages androgen secretion from theca cells and alters the effects of gonadotropins on these cells. Hyperandrogenism and insulin resistance exacerbate each other. Excessive androgen production leads to visceral fat accumulation and dysfunction in adipocytes. Treatments for PCOS should be customized to meet the individual needs of each patient; they may include reducing hyperandrogenic symptoms,



stimulating ovulation, controlling menstruation, and averting cardiometabolic issues. Numerous treatment options could be advantageous for managing metabolic comorbidities in PCOS; nonetheless, it is crucial to understand that no individual medication can sufficiently tackle the range of metabolic irregularities in women with PCOS. Treatment is always personalized and modified to meet the needs of patients. Existing approaches for treating PCOS are constrained by the prevalence of contraindications in PCOS subjects, medication failure, and serious adverse effects. So the global attention have focused into the use of traditional remedies. The whole plant extract contains numerous compounds, some of them may have synergistic effects while some may have antagonistic effects. So our aim was to mainly focus on the use of certain specific constituents. Polyphenols have gained attention in the therapeutic management of PCOS condition. Polyphenols are again subdivided into phenolic acids, flavonoids, stilbenes and lignans. Among these flavonoids are of the following types, flavonols, flavanones, flavones, anthocyanins and isoflavones. Some of the flavonoids has been extensively studied. Isolated flavonoids can be more concentrated, offering higher doses of the active compound known to benefit conditions like insulin resistance, inflammation, or oxidative stress—all of which are major factors in PCOS. With isolated flavonoids, it is easier to standardize the dose of a specific compound to achieve therapeutic effects. Here in this study, we used the flavonoid kaempferol. Kaempferol is chemically 3,5,7-trihydroxy-2-(4-hydroxyphenyl)-4H-1 benzopyran-4-one. It is a yellow-colored solid having a molecular weight of 286.2 g/mol. It is found in significant quantities in tea, broccoli, apples, strawberries, grapes, tomatoes, potatoes, onions, cucumbers, and beans. Recent research indicates that kaempferol offers a range of nutritional and health advantages, including

antioxidant, anti-inflammatory, anti-cancer properties, and therapeutic effects for diseases such as diabetes mellitus, atherosclerosis, and osteoporosis. Its primary action is to boost the body's defense system against damaging free radicals and has been shown to affect various cellular functions. It has recently been confirmed that kaempferol provides neuroprotective benefits and also protects the liver and heart muscle. Kaempferol has been studied for its potential health benefits particularly its anti-inflammatory, antioxidant, and anticancer properties.

## **MATERIALS AND METHODS**

### **MATERIALS**

#### **Animal selection, maintenance, and care**

Female *Wistar* rats weighing between 100-200g were procured from the University of Kerala, Kariavattom Campus Thiruvananthapuram. They were kept properly in polypropylene cages and kept for acclimatization over 1 week. Rats were fed with a pellet diet and water *ad libitum*. Cages were changed and cleaned twice weekly.

#### **Ethical clearance**

The certificate of approval for the study protocol was issued by the Institutional Animal Ethical Committee on 30<sup>th</sup> January 2024. 30 young female *Wistar* rats were approved under the IAEC order no: 01/06/IAEC/2024/GMCT.

#### **Chemicals, reagents, and instruments**

##### **a) Chemicals and reagents**

The chemicals and drugs used for the study were HPLC/analytical grade or of pharmacopoeial standard, as the case may be. The standard drug metformin and the drug used for inducing polycystic ovarian syndrome (letrozole) were purchased from Retail Pharmacy Ullloor. The test drug kaempferol was purchased from Yarrow Chem Pharmaceuticals.

##### **b) Instruments**

The instruments used in the study were of analytical grade.



## METHODS

### Grouping of animals

Animals were divided into 5 groups where each group carrying 6 animals. These animals were observed daily from their vaginal smears to confirm the reproductive health of them and also to conclude whether they were following the normal pattern of estrous cycle. Group I served as the control, Group II served as the diseased group, Group III served as the standard group, Group IV served as the low dose treated group and finally Group VI served as the high dose treated group.

### Estrous cyclicity and examination of vaginal smear

To collect the vaginal smear, the rat was held around the thorax with the ventral area uppermost, and the tail was held in the same hand. The smear was collected between 10 a.m. and 12 p.m. on various days. The tip of a sterile cotton bud was delicately placed into the rat's vagina and rotated before the plug was transferred to a glass slide. Then it was fixed using a suitable fixing agent stained and finally observed under a microscope. Different types of cells were identified according to the phase of the estrous cycle. Initially, the smears were collected to examine whether these animals had normal reproductive cycle patterns. This process was continued until the disease was confirmed in these animals after the administration of letrozole. The smears were collected using a sterile cotton bud. The tip of the bud was moistened with normal saline and was gently inserted into the vaginal orifice.

### Biochemical estimation of blood

#### i) Estimation of serum hormonal parameters

All animals were anaesthetized by using chloroform and sacrificed by administering ketamine intraperitoneally on day 37 (i.e. 24 hours after the last dose of treatment). The blood of animals of each group will be collected by cardiac puncture on the last day of treatment. The

concentration of serum estrogen, luteinizing hormone, follicle-stimulating hormone, and testosterone was measured by using ELISA.

#### ii) Estimation of serum lipid profile

HDL-C, LDL-C, and serum total cholesterol were quantified after the terminal day of blood sampling.

#### iii) Estimation of blood glucose level

Fasting blood glucose level was measured on the day 0<sup>th</sup>, 21<sup>st</sup>, and 37<sup>th</sup> day of the study duration.

#### iv) Estimation of superoxide dismutase

##### Procedure

The reaction mixture consisted of 0.1ml 1.5 M sodium carbonate, 0.3ml methionine (0.13Mm), 0.3ml EDTA, 0.3ml NBT (0.65Mm), 0.3ml riboflavin (0.13Mm) and 0.1ml tissue homogenate (same volume of phosphate buffer in control tube). Phosphate buffer was added to each tube to bring the total volume to 3 millilitres. After thoroughly mixing and incubating for 30 minutes, measure the optical density at 560 nm. The quantity of superoxide dismutase that prevents 50% of NBT from being reduced is one unit.

##### Calculation

Percentage of inhibition =  $\{(Control-Test) / Control\} \times 100$

Enzyme units = Percentage inhibition / 50

##### Histopathology of ovary

After sacrifice, the ovaries were promptly isolated and separated. Overnight, the tissue was preserved in a 10% neutral buffered formalin solution (5 mL). It was diced, placed in the cassette, and processed by soaking it in various concentrations of isopropyl alcohol. Later, it was cleaned with xylene, embedded in paraffin wax, and sectioned with a microtome. Sections measuring 6-7  $\mu$ m thick were stained with Hematoxylin and Eosin and evaluated for histopathological alterations. Corpora lutea, cysts, graffian follicles, and the thickness of the peripheral theca and granulosa layer were all detected.



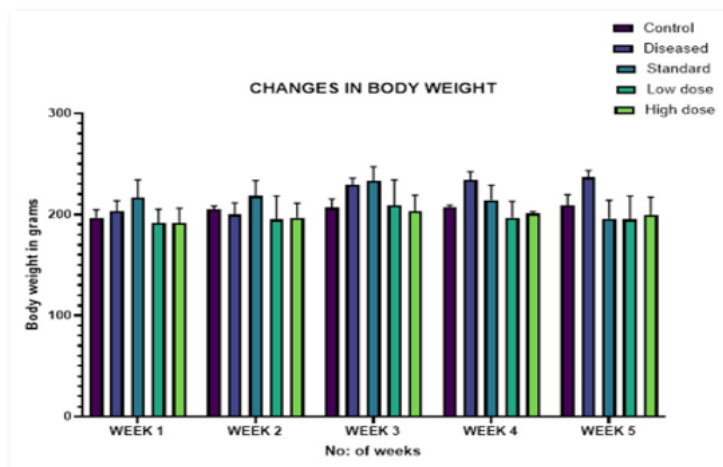
### Statistical evaluation

The data were presented as means  $\pm$  standard error of the mean (SEM). The collected data were subjected to one way ANOVA (Analysis of Variance) and two-way ANOVA followed by Tukey's multiple comparison test and Dunnet test.

$P$  value  $<0.05$  were considered as significant. The analysis was performed by using Graph pad prism software of version 10.

### RESULTS

#### 1) Effect of kaempferol on body weight

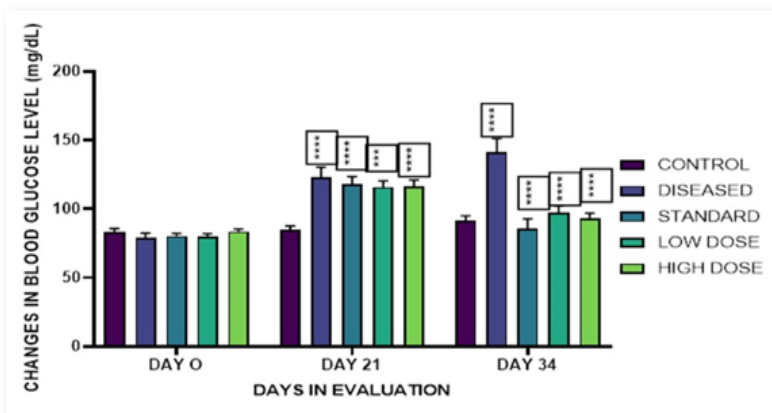


**Figure 1: Effect of drug on body weight. Results were expressed as mean  $\pm$  SEM(n=6). Analysis was carried out by two-way ANOVA followed by Tukey's multiple comparison tests. \*\*\*\* indicates p value  $<0.0001$ , \*\*\* indicates p value  $<0.001$ .**

It was discovered that there was a progressive rise in body weight in every group but the control group. Kaempferol therapy using both regular medication and high and low doses. In contrast, metformin significantly reduced the body weight of PCOS rats to the PCOS group. During the first 3 weeks of the study, letrozole was administered and after that from the 22<sup>nd</sup> day onwards standard

group received metformin whereas the treatment group received high and low doses of kaempferol respectively. From the 4<sup>th</sup> week onwards, it was noted that there was a slight decrease in the body weight of animals present in standard, high, and low dose groups.

#### 1) Effect of kaempferol on blood glucose levels

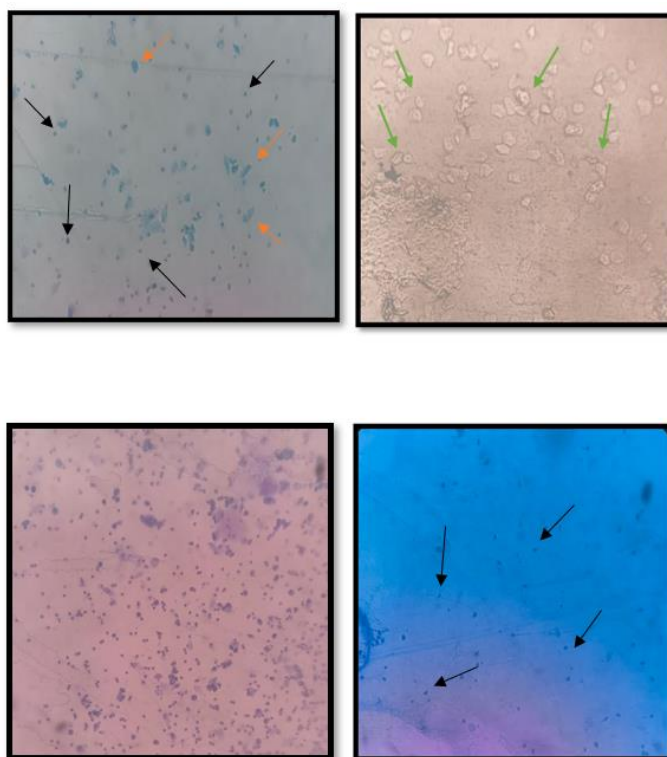


**Figure 2: Effect of drug on blood glucose level. Results were expressed as mean  $\pm$  SEM(n=6). Analysis was carried out by two-way ANOVA followed by Tukey's multiple comparison tests. \*\*\*\* indicates p value  $<0.0001$ , \*\*\* indicates p value  $<0.001$ .**

There was an increase in blood glucose level in all groups till day 21 (the last day of disease induction) when compared to control. The diseased group showed a gradual increase until the last day of treatment (day 37). But when we compare the other three groups (standard, low dose, high dose), these three groups started showing a decrease in the blood glucose level. Of

which standard group which received metformin showed the highest decrease in blood glucose level compared with the disease group. After treatment with standard and extract at low and high doses, there was a significant decrease in the blood glucose level when compared to the PCOS group.

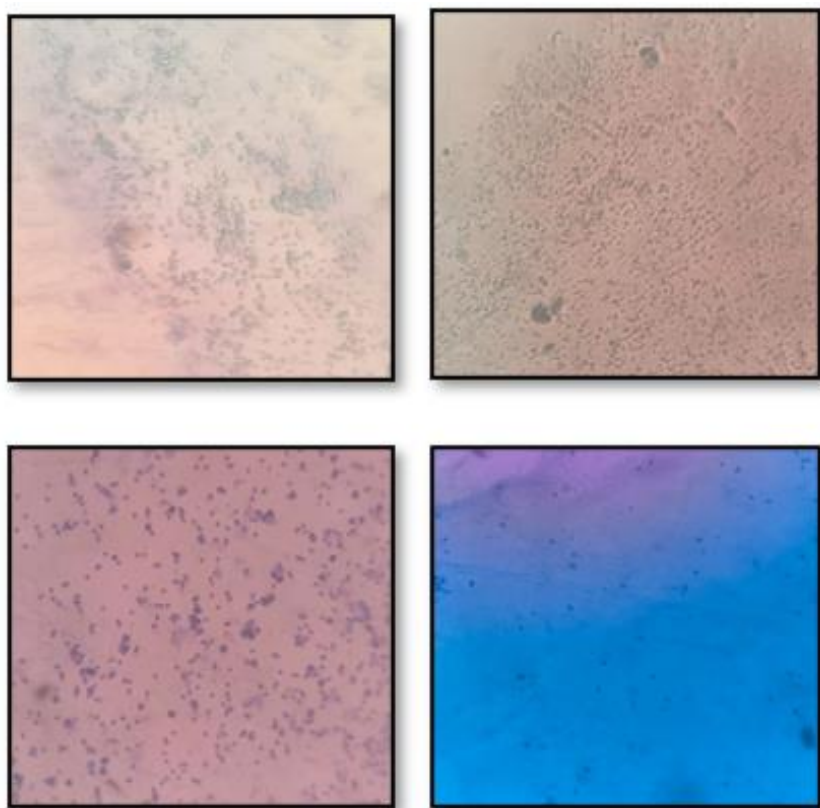
### Effect of Kaempferol on Estrous Cycle



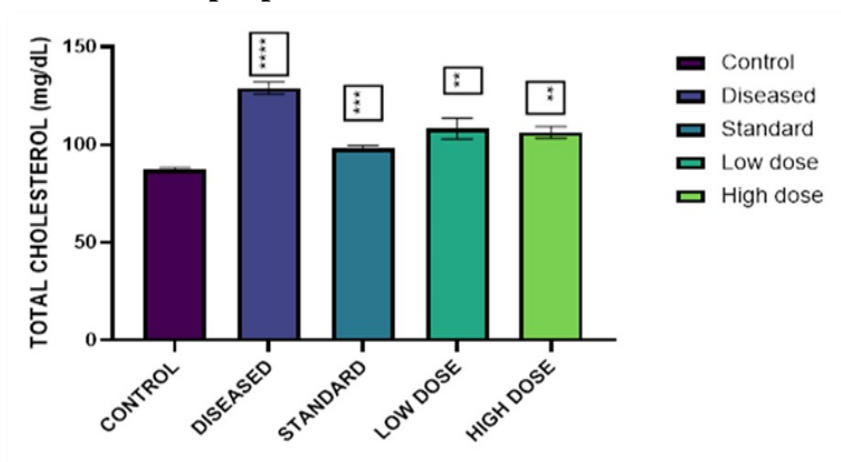
**Figure 3: Different stages of estrous cycle** a) Proestrus (nucleated cornified epithelial cells, numerous seen in cluster) b) Estrus (non nucleated epithelial cells with few cornified epithelial cells) c) Metestrus (cornified epithelium with and without nucleus with few neutrophils) d) Diestrus (>neutrophils and < cornified epithelial cells). All the phase are observed using Olympus Binocular Microscope and images are captured using Magnus analytic Mag Vision 3.7.8538 The oestrus cycle, consisting of the proestrus, oestrus, metestrus, and diestrus

phases, lasts for 4-5 days in female rats. Most of the rats exhibited a prolonged diestrus phase beginning on the seventh day after receiving letrozole therapy and ending with oestrus on the fifteenth. The rats were given varied treatments and discovered to be in the diestrus stage twenty-one days after the letrozole was administered.

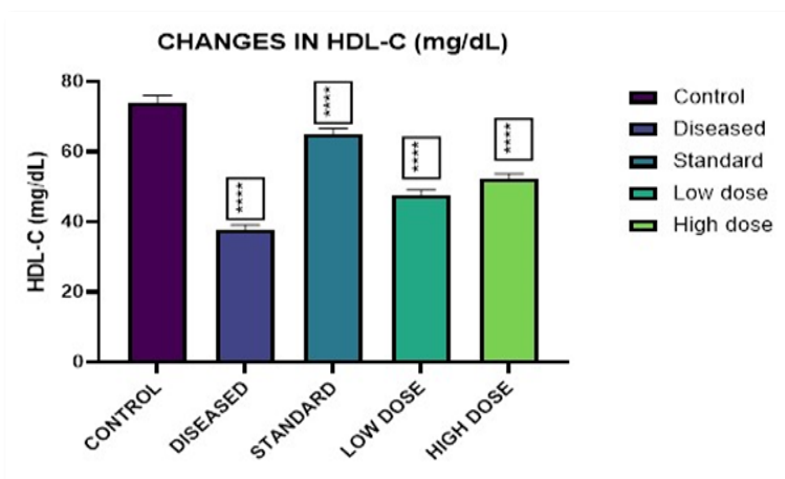
- Black arrows indicate neutrophils, orange arrows indicate nucleated cornified epithelial cells and blue arrows indicate anucleated epithelial cells



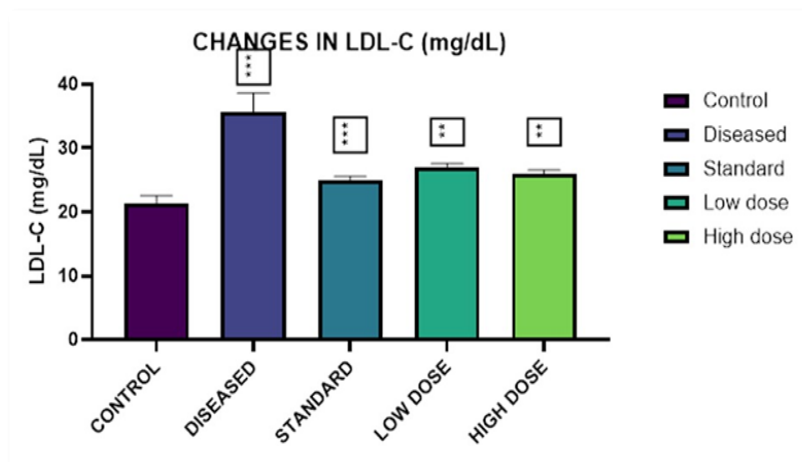
**Figure 4: Disease Group Showing Extended Diestrous Phase**  
**Effect of kaempferol on serum lipid profile**



**Figure 5: Effect of drug on serum total cholesterol. Results were expressed as mean  $\pm$  SEM(n=4). Analysis was carried out by one-way ANOVA followed by Bonferroni analysis. \*\* indicates p value <0.01, \*\*\* indicates p value <0.001, \*\*\*\* indicates p value <0.0001**



**Figure 6: Effect of drug on serum HDL-C. Results were expressed as mean  $\pm$  SEM(n=4). Statistical analysis was carried out by one-way ANOVA followed by Bonferroni analysis. \*\*\*\* indicates p value <0.0001**

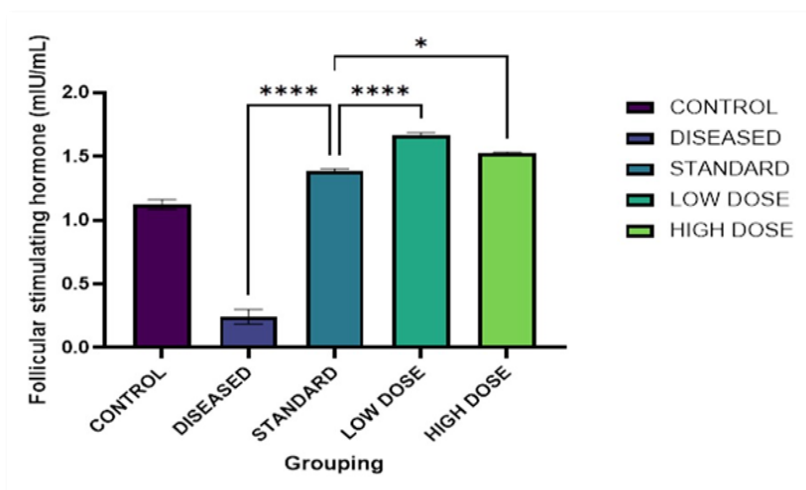


**Figure 7: Effect of drug on serum LDL-C. Results were expressed as mean  $\pm$  SEM(n=4). Analysis was carried out by one-way ANOVA followed by Bonferroni analysis. \*\* indicates p value <0.01, \*\*\* indicates p value <0.001**

Women with PCOS usually have lipid abnormalities, such as elevated levels of LDL, triglycerides, and low HDL. Because both high cholesterol and high glucose levels increase the risk of insulin resistance. Insulin resistance causes VLDL overproduction, which results in more TGs, lower HDL, and higher LDL [63]. Flavonoids are

said to have antihyperlipidemic properties, and thus we can conclude that kaempferol may also have some antihyperlipidemic properties which can be illustrated from the above plotted graphical representations.

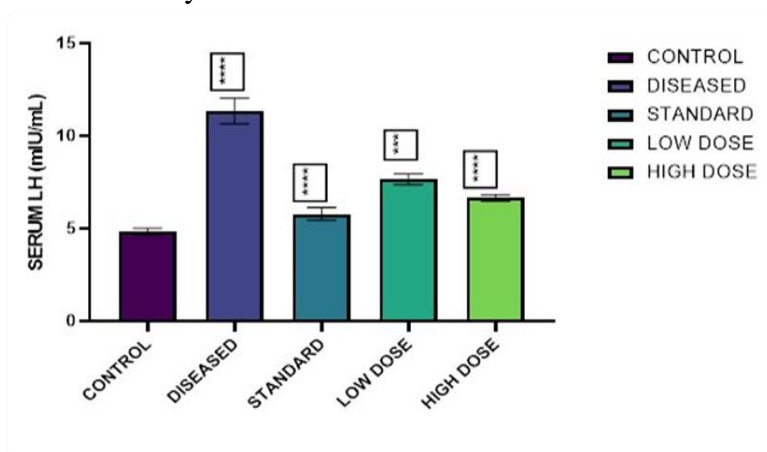
#### **Effect of kaempferol on serum hormonal levels**



**Figure 8: Effect of drug on serum follicle stimulating hormone concentration. Results were expressed as mean  $\pm$  SEM(n=4). Analysis was carried out by one-way ANOVA followed by Bonferroni analysis. \*\*\*\* indicates p value <0.0001, \*indicates p value <0.05**

Follicle-stimulating hormone (FSH) is an important hormone that is essential for follicular growth and maturation. The anterior pituitary gland produces follicle-stimulating hormone (FSH) in response to gonadotropin-releasing hormone (GnRH) from the hypothalamus [64]. The level of FSH is too low in PCOS subjects. A disruption in the secretion rhythm of the

gonadotrophin-releasing hormone (GnRH) causes a relative increase in LH to FSH release. Ovarian estrogen is responsible for activating an aberrant feedback process, increasing LH secretion [65]. Increased LH level along with the deficiency of FSH leads to an increased LH: FSH ratio which is an important hallmark feature of PCOS.

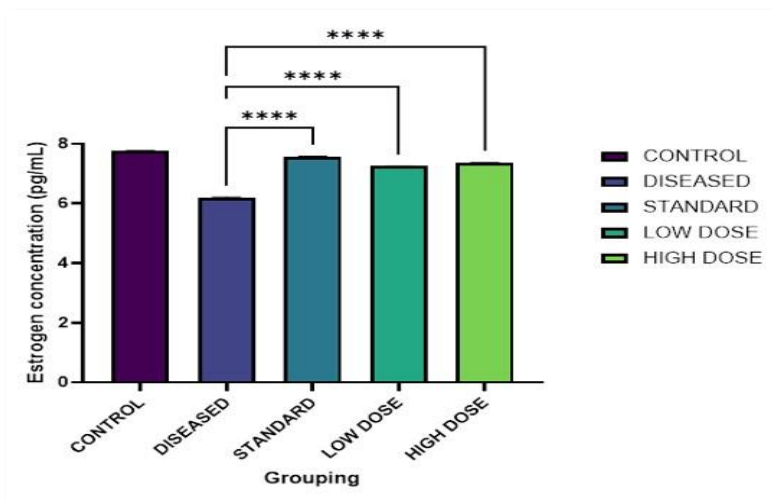


**Figure 9: Effect of drug on serum luteinizing hormone. Results were expressed as mean  $\pm$  SEM(n=4). Analysis was carried out by one-way ANOVA followed by Bonferroni analysis. \*\*\*\* indicates p value <0.0001.**

When it comes to the levels of LH in PCOS, it was observed that the diseased group shows increased serum LH levels. PCOS is characterized by increased levels of both total and free testosterone because luteinizing hormone (LH) and insulin

levels in the blood are too high in PCOS. LH stimulates the testes to release testosterone while IR stimulates androgen synthesis in the ovary and lowers the amount of free testosterone (FT) accessible to the body, which inhibits the

development of sex hormone-binding globulin (SHBG) in the liver [66]. Elevated LH to FSH ratio is seen but both levels fall within the normal.



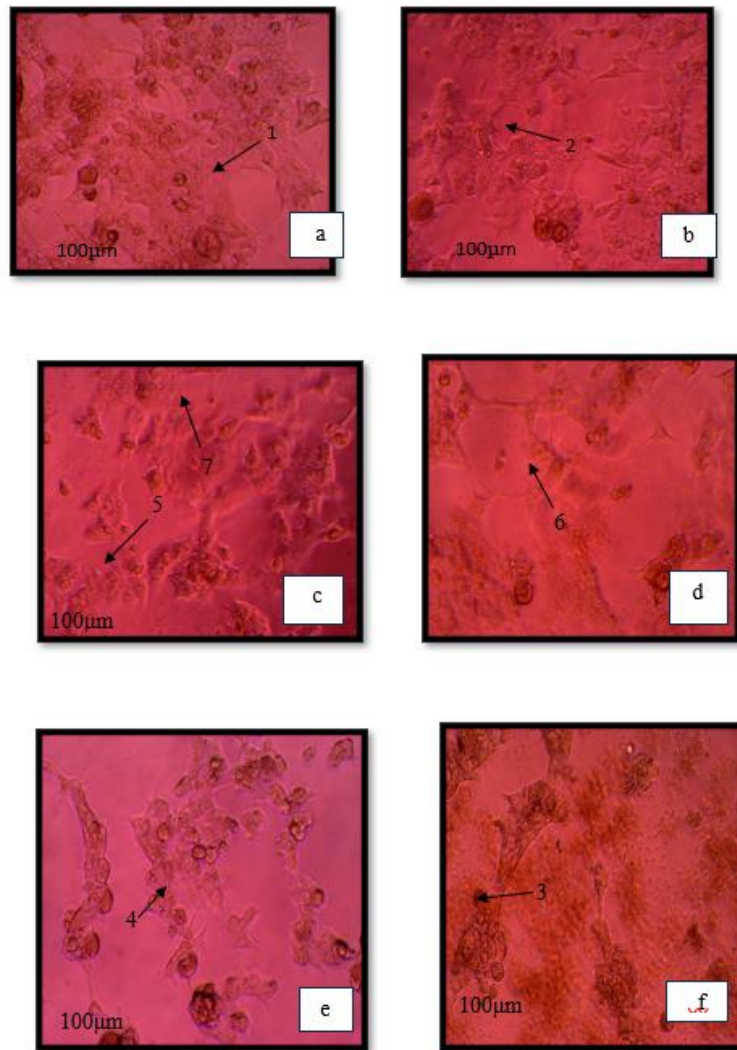
**Figure 10: Effect of drug on serum estrogen concentration. Results were expressed as mean  $\pm$  SEM(n=4). Analysis was carried out by one-way ANOVA followed by Bonferroni analysis. \*\*\*\* indicates p value <0.0001.**

The level of estrogen varies in different phases of the estrous cycle. It ranges between 10 – 100pg/mL. Estrogens have a physiological role through estrogen receptors (ER), activated by transcriptional receptors. Kaempferol may sometimes act as an estrogen agonist while sometimes it acts as an estrogen antagonist [69].

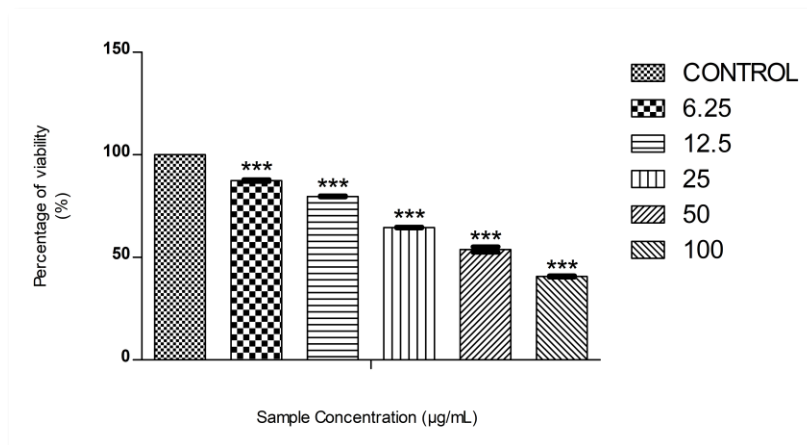
Here the data shows that after treatment with the test drug, a slight change was observed in the high-dose group which was somewhat similar to the standard group. However, more studies are needed to confirm its estrogenic activity in PCOS studies.

### ***In Vitro* Studies**

#### **MTT Assay**



**Figure 11: Effect of different concentration of kaempferol in SKOV (Human ovarian cancer) cell line. Arrows indicates (1) control cell, (3) condensed nuclei, (2) membrane blebbing, (4) cell shrinkage (5) apoptotic bodies (6) echinoid spikes (7) bubbling**



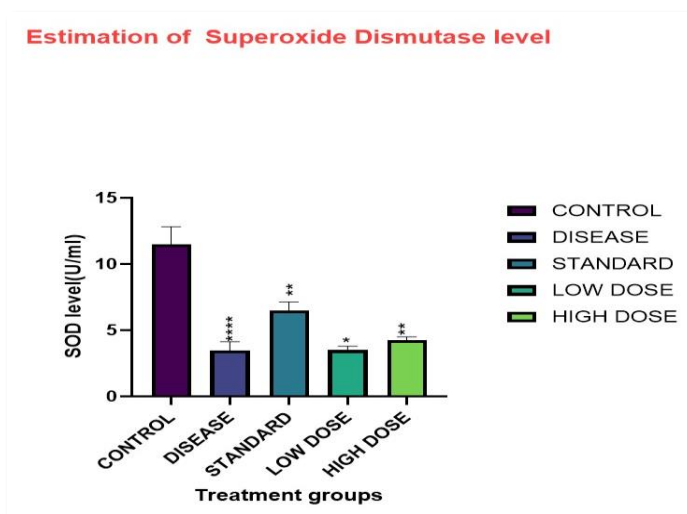
**Figure 12: Graphical representation depicting the effect of sample kaempferol on SKOV3 cell line by MTT assay.**

Y axis: Percentage viability, X axis: varied concentration of sample kaempferol. All experiments were done in triplicates and results represented as Mean $\pm$  SE. One-way ANOVA and Dunnett's test were performed to analyse data. \*\*\*p < 0.001 compared to control groups.

Graphical representation of MTT assay reveals that cytotoxic effect was found to have linear relation with the concentration, which explains the sequential reduction in viability of SKOV cells.

### Antioxidant Assays

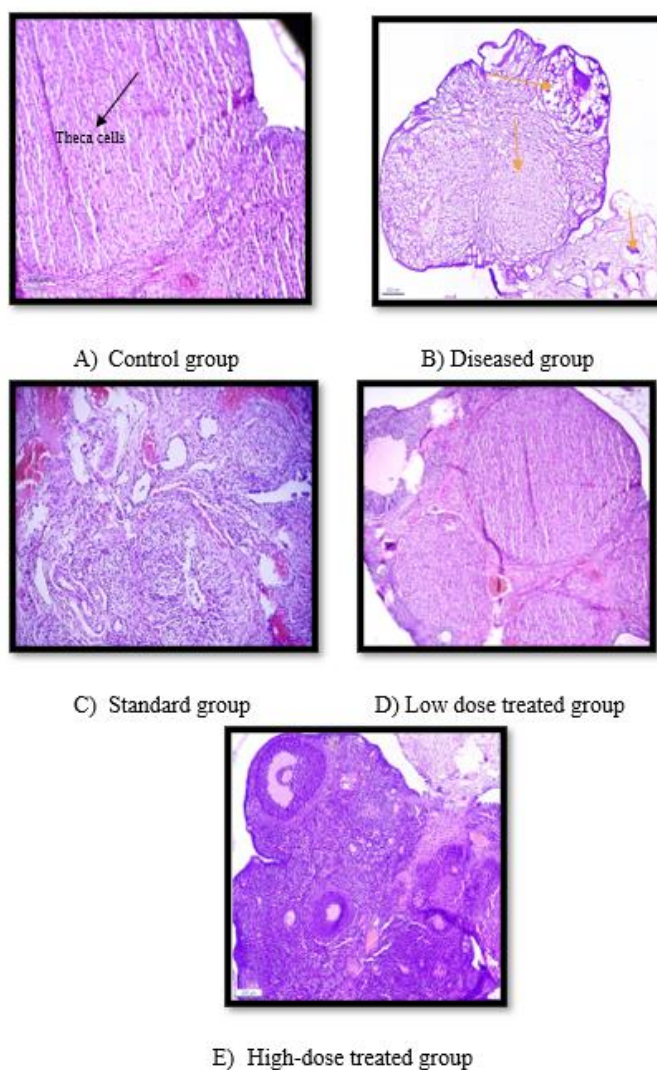
**a) Estimation of superoxide dismutase activity**  
SOD levels in the body diminish with age and pathological diseases such as PCOS, resulting in reduced inhibitory function. The percentage inhibition of superoxide anion by SOD was substantially higher in the drug sample treated group than in the induction group, indicating the drug's antioxidant efficacy.



**Figure 13: Effect of drug on superoxide dismutase level. Results were expressed as mean  $\pm$  SEM(n=2). Statistical analysis was carried out by one-way ANOVA followed by Bonferroni analysis. \*\*\* indicates p value <0.001, \*\* indicates p value <0.01, \* indicates p value <0.05.**

### Ex Vivo Studies

### Histopathology Of Ovarian Tissue



A

- i. Follicular cells – Present
- ii. Cyst -- Present

B

- i. Follicular cells – Absent
- ii. Cyst -- Present

C

- i. Follicular cells – Present
- ii. Cyst -- Absent
- iii. Corpus luteum – Present

D

- i. Follicular cells – Present
- ii. Cyst -- Present in lesser number

E

- i. Follicular cells – Present in more number
- ii. Cyst -- less number

In disease control group showed abnormal structure of ovarian tissue, increased number of cystic follicles and there is disruption between the granulosa cell layers. Also, degenerative changes in the theca layer observed. In treated group it showed improved morphology of ovarian tissue with very few cystic follicles and minimal degenerative changes in the granulosa layers. Hence, the test compound used here is showing beneficial effect against PCOS ovary in rats.

The ovarian morphology of animals in the disease group differed from that of the normal group, with several enlarged cysts, the absence of corpora lutea, thickening of the ovarian cortex, and an uneven structure. The kaempferol-treated group showed a visible improvement in the pathological

damage of ovarian tissue in letrozole-induced PCOS rats, i.e. the number of cystic follicles was reduced and numerous healthy follicles were discovered at a different stage of development, and the results were significantly similar to those of standard drug-treated animals.

## CONCLUSION

PCOS is one among the most relevant reproductive and metabolic disorder affecting a large number of females. Most of them remains undiagnosed because of their late onset of symptoms. The number of females affected by PCOS is gradually increasing and also it leads to many co-morbidities such as Type II diabetes mellitus, cardiovascular disorders and so on. The etiology of PCOS is complicated and an exact pathophysiology is still not understood. Many parameters are linked to this disorder and it is very difficult to treat it by targeting on the root cause. Subjects vary in symptoms as each one will be experiencing different types of symptoms. The most common symptoms are hirsutism, acne, irregular periods, amenorrhea and sometimes leading to infertility. Currently, available medications are mainly focused on symptomatic relief. They are personalized as symptoms vary from individual to individual. The medications which we are currently using have many adverse effects and limitations. Because of this reason, there is an immediate requirement to switch to herbal approaches. The major drawback of utilizing a whole plant extract is that it contains a large number of phytoconstituents, some of them have synergistic effects while others have antagonistic effects. So, it is very challenging to understand the exact constituent in charge for the action and also the major mechanism through which it manages the disease. Here the single constituent kaempferol has been studied. Some of the flavonoids such as curcumin, quercetin, apigenin, etc. have been studied in PCOS. When it comes to kaempferol, an *in-silico* study showed

that kaempferol has a better binding score than 19 other compounds, and also no *in vivo* studies have been carried out. Kaempferol is currently used as an anti-cancerous drug. In order to produce polycystic ovarian syndrome in healthy female *Wistar* rats, the aromatase inhibitor letrozole was utilized. The whole protocol was approved by IAEC. Here 1 mg/kg of letrozole was administered to rats daily and their vaginal smears were examined daily. On the 21<sup>st</sup> day it was observed that rats showed an extended diestrous phase in their estrous cycle. It was followed by two weeks of treatment where low and high doses of kaempferol were administered orally. Body weights were examined weekly and blood glucose levels were examined on 0<sup>th</sup>, 21<sup>st</sup>, and 36<sup>th</sup> day of study. On the terminal day blood samples were collected for examining the serum hormonal parameters and serum lipid profile. The results demonstrated that kaempferol administration ameliorated the disturbed parameters in letrozole induced female *Wistar* rats. Histopathological studies also revealed the effect of kaempferol in cystic follicles in the ovaries of rats. It reduced the number of cystic follicles and also healthy follicles were observed as compared with the standard drug-treated groups. This indicates that treatment with high dose of kaempferol showed a recovery of ovarian tissue and a low dose showed a slight difference but it was not that comparable to that of the high dose treated group.

## ACKNOWLEDGEMENT

I would like to acknowledge the Head of the Department, College of Pharmaceutical Sciences, Government Medical College, Thiruvananthapuram for providing all the necessary facilities in conducting laboratory work.

## Conflict Of Interest

All the authors have no conflict of interest.

## REFERENCES

1. Ganie MA, Vasudevan V, Wani IA, Baba MS, Arif T, Rashid A. Epidemiology, pathogenesis,



- genetics & management of polycystic ovary syndrome in India. *Indian Journal of Medical Research*. 2019 Oct 1;150(4):333-44.
2. Patel M. Polycystic Ovary Syndrome: An update from the 2023 international guideline. *African Journal of Obstetrics and Gynaecology*. 2024 Aug 1;2(2):4-7.
  3. Stener-Victorin E, Teede H, Norman RJ, Legro R, Goodarzi MO, Dokras A, Laven J, Hoeger K, Piltonen TT. Polycystic ovary syndrome. *Nature Reviews Disease Primers*. 2024 Apr 18;10(1):27.
  4. Bu N, Jamil A et.al. Phytochemical-based study of ethanolic extract of *Saraca asoca* in letrozole-induced polycystic ovarian syndrome in female adult rats. *ACS omega*. 2023 Nov 1;8(45):42586-97.
  5. Yang J, Chen C. Hormonal changes in PCOS. *Journal of Endocrinology*. 2024 Apr 1;261(1).
  6. PCOS: A Leading Cause of Infertility [Internet]. *Naturopathic Doctor News and Review*. 2022. Available from: <https://ndnr.com/womens-health/pcos-a-leading-cause-of-infertility/>
  7. Chang S, Dunaif A. Diagnosis of polycystic ovary syndrome: which criteria to use and when?. *Endocrinology and Metabolism Clinics*. 2021 Mar 1;50(1):11-23.
  8. Salari N, Nankali A, Ghanbari A, Jafarpour S, Ghasemi H, Dokaneheifard S, Mohammadi M. Global prevalence of polycystic ovary syndrome in women worldwide: a comprehensive systematic review and meta-analysis. *Archives of Gynecology and Obstetrics*. 2024 Sep;310(3):1303-14.
  9. Jain A, Neravi A, Kumar KS, Shirahatti RS, Oli AK. A Prospective Study on the Prevalence of Polycystic Ovary Syndrome at a Tertiary Care Hospital of North Karnataka, India. *Journal of Preventive, Diagnostic and Treatment Strategies in Medicine*. 2024 Jul 1;3(3):171-9.
  10. Salari N, Nankali A, Ghanbari A, Jafarpour S, Ghasemi H, Dokaneheifard S, Mohammadi M. Global prevalence of polycystic ovary syndrome in women worldwide: a comprehensive systematic review and meta-analysis. *Archives of Gynecology and Obstetrics*. 2024 Sep;310(3):1303-14.
  11. Percentage of women with physician-confirmed PCOS versus women without... [Internet]. *ResearchGate*. 2024 [cited 2024 Oct 7]. Available from: [https://www.researchgate.net/figure/Percentage-of-women-with-physician-confirmed-PCOS-versus-women-without-PCOS-among-users\\_fig3\\_349760149](https://www.researchgate.net/figure/Percentage-of-women-with-physician-confirmed-PCOS-versus-women-without-PCOS-among-users_fig3_349760149)
  12. Jain T, Negris O, Brown D, Galic I, Salimgaraev R, Zhaunova L. Characterization of polycystic ovary syndrome among Flo app users around the world. *Reproductive Biology and Endocrinology*. 2021 Dec;19:1-1.
  13. Rasouli F. Predicts factor in Polycystic Ovary Syndrome: An Evidence-based Study and Invitation from the Chief Editor. *Eurasian Journal of Chemical, Medicinal and Petroleum Research*. 2024 Jun 6;3(2):644-50.
  14. van der Ham K, Laven JS, Tay CT, Mousa A, Teede H, Louwers YV. Anti-Müllerian hormone as a diagnostic biomarker for Polycystic Ovary Syndrome (PCOS) and Polycystic Ovarian Morphology (PCOM): a systematic review and meta-analysis. *Fertility and Sterility*. 2024 Jun 27.
  15. Panda S, Das R, Konar L, Singh M. Causes of Polycystic Ovarian Syndrome.
  16. Siddiqui S, Mateen S, Ahmad R, Moin S. A brief insight into the etiology, genetics, and immunology of polycystic ovarian syndrome (PCOS). *Journal of assisted reproduction and genetics*. 2022 Nov;39(11):2439-73.
  17. Pasquali R, Gambineri A. Polycystic ovary syndrome: a multifaceted disease from adolescence to adult age. *Annals of the New*

- York Academy of Sciences. 2006 Dec;1092(1):158-74.
18. Kabakchieva P. Polycystic Ovary Syndrome: Diverse Clinical Presentations Across Adolescence, Reproductive Age, And Menopause. *Anti-Aging Eastern Europe*. 2024 Jun 27;3(2):78-86.
  19. Hart R, Hickey M, Franks S. Definitions, prevalence and symptoms of polycystic ovaries and polycystic ovary syndrome. *Best Practice & Research Clinical Obstetrics & Gynaecology*. 2004 Oct 1;18(5):671-83.
  20. WebMD. What Is PCOS? [Internet]. WebMD. WebMD; 2017. Available from: <https://www.webmd.com/women/what-is-pcos>
  21. Ltd HP. The Four Types Of PCOS: How Can You Identify Which One You Have? [Internet]. HealthMatch. 2023. Available from: <https://healthmatch.io/pcos/types-of-pcos>
  22. Sachdeva G, Gainer S, Suri V, Sachdeva N, Chopra S. Comparison of the different PCOS phenotypes based on clinical metabolic, and hormonal profile, and their response to clomiphene. *Indian journal of endocrinology and metabolism*. 2019 May 1;23(3):326-31.
  23. Malini NA, George KR. Evaluation of different ranges of LH: FSH ratios in polycystic ovarian syndrome (PCOS)—Clinical based case control study. *General and comparative endocrinology*. 2018 May 1;260:51-7.
  24. Sahmay S, Mathyk BA, Sofiyeva N, Atakul N, Azami A, Erel T. Serum AMH levels and insulin resistance in women with PCOS. *European Journal of Obstetrics & Gynecology and Reproductive Biology*. 2018 May 1;224:159-64.
  25. Le MT, Le VN, Le DD, Nguyen VQ, Chen C, Cao NT. Exploration of the role of anti-Mullerian hormone and LH/FSH ratio in diagnosis of polycystic ovary syndrome. *Clinical Endocrinology*. 2019 Apr;90(4):579-85.
  26. Bulsara J, Patel P, Soni A, Acharya S. A review: Brief insight into Polycystic Ovarian syndrome. *Endocrine and Metabolic Science [Internet]*. 2021 Jun 30;3(100085):100085. Available from: <https://www.sciencedirect.com/science/article/pii/S266639612100008X>
  27. Zeng X, Xie YJ, Liu YT, Long SL, Mo ZC. Polycystic ovarian syndrome: Correlation between hyperandrogenism, insulin resistance and obesity. *Clinica chimica acta*. 2020 Mar 1;502:214-21.
  28. Diamanti-Kandarakis E, Kandarakis H, Legro RS. The role of genes and environment in the etiology of PCOS. *Endocrine*. 2006 Aug;30:19-26.
  29. Sengupta P, Dutta S, Hassan MF. Polycystic ovary syndrome (PCOS) and oxidative stress. *Journal of Integrated Science and Technology*. 2024;12(3):752-.
  30. Chang KJ, Chen JH, Chen KH. The Pathophysiological Mechanism and Clinical Treatment of Polycystic Ovary Syndrome: A Molecular and Cellular Review of the Literature. *International Journal of Molecular Sciences*. 2024 Aug 20;25(16):9037.
  31. Chang KJ, Chen JH, Chen KH. The Pathophysiological Mechanism and Clinical Treatment of Polycystic Ovary Syndrome: A Molecular and Cellular Review of the Literature. *International Journal of Molecular Sciences*. 2024 Aug 20;25(16):9037.
  32. Ee C, Tay CT. Pharmacological management of polycystic ovary syndrome. *Australian Prescriber*. 2024 Aug;47(4):109.
  33. Marcondes FK, Bianchi FJ, Tanno AP. Determination of the estrous cycle phases of rats: some helpful considerations. *Brazilian journal of biology*. 2002;62:609-14.



34. Paccola CC, Resende CG, Stumpp T, Miraglia SM, Cipriano I. The rat estrous cycle revisited: a quantitative and qualitative analysis. *Animal Reproduction (AR)*. 2018 Jul 26;10(4):677-83.
35. Ajayi AF, Akhigbe RE. Staging of the estrous cycle and induction of estrus in experimental rodents: an update. *Fertility research and practice*. 2020 Dec;6:1-5.
36. Verma PK, Kumar S, Verma AK, Tripathi A, Shukla MK. Staging of Estrous Cycle in Rats using Vaginal Cytology and Visual Assessment. *Indian Journal of Animal Research.*;1:4.
37. Figure 1: Comparison of the estrous cycle of the rat and menstrual... [Internet]. ResearchGate. 2024 [cited 2024 Oct 7]. Available from: [https://www.researchgate.net/figure/Comparison-of-the-estrous-cycle-of-the-rat-and-menstrual-cycle-of-the-humana-The-4-day\\_fig1\\_7823587](https://www.researchgate.net/figure/Comparison-of-the-estrous-cycle-of-the-rat-and-menstrual-cycle-of-the-humana-The-4-day_fig1_7823587)
38. Yang H, Lee SY, Lee SR, Pyun BJ, Kim HJ, Lee YH, Kwon SW, Suh DH, Lee CH, Hong EJ, Lee HW. Therapeutic effect of *Ecklonia cava* extract in letrozole-induced polycystic ovary syndrome rats. *Frontiers in pharmacology*. 2018 Nov 19;9:1325.
39. Maliqueo M, Sun M, Johansson J, Benrick A, Labrie F, Svensson H, Lönn M, Duleba AJ, Stener-Victorin E. Continuous administration of a P450 aromatase inhibitor induces polycystic ovary syndrome with a metabolic and endocrine phenotype in female rats at adult age. *Endocrinology*. 2013 Jan 1;154(1):434-45.
40. Mukherjee AG, Wanjari UR, Nagarajan D, Vibhaa KK, Anagha V, Chakraborty R, Renu K, Dey A, Vellingiri B, Gopalakrishnan AV. Letrozole: Pharmacology, toxicity and potential therapeutic effects. *Life sciences*. 2022 Dec 1;310:121074.
41. Figure 2: Central mechanism of action (at pituitary level) of Letrozole... [Internet]. ResearchGate. 2024 [cited 2024 Oct 7]. Available from: [https://www.researchgate.net/figure/Central-mechanism-of-action-at-pituitary-level-of-Letrozole-in-ovulation-induction-22\\_fig3\\_338910387](https://www.researchgate.net/figure/Central-mechanism-of-action-at-pituitary-level-of-Letrozole-in-ovulation-induction-22_fig3_338910387)
42. Balasubramanian A, Pachiappan S, Mohan S, Adhikesavan H, Karupphasamy I, Ramalingam K. Therapeutic exploration of polyherbal formulation against letrozole induced PCOS rats: a mechanistic approach. *Heliyon*. 2023 May 1;9(5).
43. Kar TK, Sil S, Ghosh A, Barman A, Chattopadhyay S. Mitigation of letrozole induced polycystic ovarian syndrome associated inflammatory response and endocrinal dysfunction by *Vitex negundo* seeds. *Journal of Ovarian Research*. 2024 Apr 8;17(1):76.
44. Kshama DN. From nature to nurture: A review of herbal approaches to polycystic ovary syndrome (PCOS). In *Obstetrics and Gynaecology Forum* 2024 May 26 (Vol. 34, No. 3s, pp. 1142-1152).
45. Lakshmi JN, Babu AN, Kiran SM, Nori LP, Hassan N, Ashames A, Bhandare RR, Shaik AB. Herbs as a source for the treatment of polycystic ovarian syndrome: A systematic review. *BioTech*. 2023 Jan 3;12(1):4.
46. Irmak E, Sanlier NT, Sanlier N. Could polyphenols be an effective treatment in the management of polycystic ovary syndrome?. *International Journal for Vitamin and Nutrition Research*. 2024 Jan 17.
47. Kiyama R. Estrogenic flavonoids and their molecular mechanisms of action. *The Journal of Nutritional Biochemistry*. 2023 Apr 1;114:109250.
48. Tiwari P, Mishra R, Mazumder A, Mazumder R, Singh A. An insight into diverse activities

- and targets of flavonoids. *Current Drug Targets*. 2023 Jan 1;24(1):89-102.
49. Bangar SP, Chaudhary V, Sharma N, Bansal V, Ozogul F, Lorenzo JM. Kaempferol: A flavonoid with wider biological activities and its applications. *Critical Reviews in Food science and nutrition*. 2023 Nov 6;63(28):9580-604.
50. Figure 1. Structure of kaempferol. [Internet]. ResearchGate. 2024 [cited 2024 Oct 7]. Available from: [https://www.researchgate.net/figure/Structure-of-kaempferol\\_fig5\\_260253002](https://www.researchgate.net/figure/Structure-of-kaempferol_fig5_260253002)
51. Kaempferol [Internet]. Wikipedia. 2022. Available from: <https://en.wikipedia.org/wiki/Kaempferol>
52. Nezhad Salari AM, Rasoulizadeh Z, Shabgah AG, Vakili-Ghartavol R, Sargazi G, Gholizadeh Navashenaq J. Exploring the mechanisms of kaempferol in neuroprotection: Implications for neurological disorders. *Cell Biochemistry and Function*. 2024 Mar;42(2):e3964.
53. Hossain MA, Al Ashik SA, Mahin MR, Al Amin M, Rahman MH, Khan MA, Al Emran A. Systems biology and in silico-based analysis of PCOS revealed the risk of metabolic disorders. *Heliyon*. 2022 Dec 1;8(12).
54. Mahmoud AA, Elfiky AM, Abo-Zeid FS. The anti-androgenic effect of quercetin on hyperandrogenism and ovarian dysfunction induced in a dehydroepiandrosterone rat model of polycystic ovary syndrome. *Steroids*. 2022 Jan 1;177:108936.
55. Chen M, Xiao J, El-Seedi HR, Woźniak KS, Daglia M, Little PJ, Weng J, Xu S. Kaempferol and atherosclerosis: From mechanism to medicine. *Critical Reviews in Food Science and Nutrition*. 2024 Mar 22;64(8):2157-75.
56. Priyanka CL, Premkumar B. Exploring the therapeutic potential of phytoconstituents in treatment of polycystic ovarian syndrome: An In-Silico study.
57. Zhang J, Arshad K, Siddique R, Xu H, Alshammari A, Albekairi NA, Bazmi RR, Hussain L, Lv G. Phytochemicals-based investigation of *Rubia cordifolia* pharmacological potential against letrozole-induced polycystic ovarian syndrome in female adult rats: In vitro, in vivo and mechanistic approach. *Heliyon*. 2024 Jul 30;10(14).
58. Ghosh A, Sil S, Kar TK, Barman A, Chattopadhyay S. Management of metabolic and reproductive disturbances in letrozole induced polycystic ovarian syndrome by *Asparagus racemosus* roots.
59. Pratama G, Wiweko B, Asmarinah, Widyahening IS, Andraini T, Bayuaji H, Hestiantoro A. Mechanism of elevated LH/FSH ratio in lean PCOS revisited: a path analysis. *Scientific reports*. 2024 Apr 8;14(1):8229.
60. Mihanfar A, Nouri M, Roshangar L, Khadem-Ansari MH. Ameliorative effects of fisetin in letrozole-induced rat model of polycystic ovary syndrome. *The Journal of Steroid Biochemistry and Molecular Biology*. 2021 Oct 1;213:105954.
61. Zheng N, Wang C, Li Y, Fu H, Hu T. Myricetin ameliorates polycystic ovary syndrome in mice by brown adipose tissue activation. *Reproduction*. 2024 Jun 1;167(6).
62. Van Meerloo J, Kaspers GJ, Cloos J. Cell sensitivity assays: the MTT assay. *Cancer cell culture: methods and protocols*. 2011:237-45.
63. Hussain L, Aamir N, Hussain M, Asif M, Chauhdary Z, Manzoor F, Siddique R, Riaz M. Therapeutic investigation of standardized aqueous methanolic extract of bitter melon (*Momordica charantia* L.) for its potential against polycystic ovarian syndrome in experimental animals' model: in vitro and in

- vivo studies. Evidence-Based Complementary and Alternative Medicine. 2022;2022(1):5143653.
64. Dou L, Zheng Y, Li L, Gui X, Chen Y, Yu M, Guo Y. The effect of cinnamon on polycystic ovary syndrome in a mouse model. *Reproductive Biology and Endocrinology*. 2018 Dec;16:1-0.
65. Silva EG, Kim G, Bakkar R, Bozdag Z, Shaye-Brown A, Loghavi S, Stolnicu S, Hadareanu V, Bulgaru D, Cayax LI, Pitalua MC. Histology of the normal ovary in premenopausal patients. *Annals of Diagnostic Pathology*. 2020 Jun 1;46:151475.
66. Weydert CJ, Cullen JJ. Measurement of superoxide dismutase, catalase and glutathione peroxidase in cultured cells and tissue. *Nature protocols*. 2010 Jan;5(1):51-66.
67. Gulcin İ, Alwasel SH. DPPH radical scavenging assay. *Processes*. 2023 Jul 26;11(8):2248.
68. Dubey P, Reddy S, Sharma K, Johnson S, Hardy G, Dwivedi AK. Polycystic Ovary Syndrome, Insulin Resistance, and Cardiovascular Disease. *Current Cardiology Reports*. 2024 Apr 3:1-3.
69. Mičić B, Djordjevic A, Veličković N, Kovačević S, Martić T, Macut D, Vojnović Milutinović D. AMPK Activation as a Protective Mechanism to Restrain Oxidative Stress in the Insulin-Resistant State in Skeletal Muscle of Rat Model of PCOS Subjected to Postnatal Overfeeding. *Biomedicines*. 2023 May 30;11(6):1586.
70. Wahid S, Ramli MD, Fazleen NE, Naim RM, Mokhtar MH. Exploring the Therapeutic Potential of Natural Products in Polycystic Ovarian Syndrome (PCOS): A Mini-Review of Lipid Profile, Blood Glucose, and Ovarian Histological Improvements. *Life*. 2024 Jan 19;14(1):150.
71. Howles CM. Role of LH and FSH in ovarian function. *Molecular and cellular endocrinology*. 2000 Mar 30;161(1-2):25-30.
72. Malini NA, George KR. Evaluation of different ranges of LH: FSH ratios in polycystic ovarian syndrome (PCOS)—Clinical based case control study. *General and comparative endocrinology*. 2018 May 1;260:51-7.
73. Baskind NE, Balen AH. Hypothalamic–pituitary, ovarian and adrenal contributions to polycystic ovary syndrome. *Best Practice & Research Clinical Obstetrics & Gynaecology*. 2016 Nov 1;37:80-97.
74. Chen X, Tang H, Liang Y, Wu P, Xie L, Ding Y, Yang P, Long B, Lin J. Acupuncture regulates the autophagy of ovarian granulosa cells in polycystic ovarian syndrome ovulation disorder by inhibiting the PI3K/AKT/mTOR pathway through LncMEG3. *Biomedicine & Pharmacotherapy*. 2021 Dec 1;144:112288.
75. Wang Y, Zhang Y, Feng X, Tian H, Fu X, Gu W, Wen Y. Metformin inhibits mTOR and c-Myc by decreasing YAP protein expression in OSCC cells. *Oncology reports*. 2021 Mar 1;45(3):1249-60.
76. Bergsten TM, Li K, Lantvit DD, Murphy BT, Burdette JE. Kaempferol, a phytoprogestin, induces a subset of progesterone-regulated genes in the uterus. *Nutrients*. 2023 Mar 15;15(6):1407.

**HOW TO CITE:** Nayana Shaji\*, Sri Suku J., Surabhi M., Prasanna S., Rizvana B., Evaluation of the Effect of Kaempferol on Letrozole Induced Polycystic Ovarian Syndrome in Female Wistar Rats, *Int. J. of Pharm. Sci.*, 2025, Vol 3, Issue 3, 1528-1546. <https://doi.org/10.5281/zenodo.15038182>

