**Educational Administration: Theory and Practice**
2024, 30(6)(s), 250 - 262
ISSN: 2148-2403
https://kuey.net/

**Research Article**

# Beneficial Effect Of Manilkara Hexandra Extract On Letrozole-Induced Polycystic Ovary Syndrome (PCOS) In Female Rats

Dr. Jignesh I. Patel[1*], Parth N. Surana[2], Adarsh V. Kevdiya[3]

[1*]B.K.Mody Government Pharmacy College, Rajkot, India. Email: jigneshmpharma@yahoo.com.
[2]Email: parthsurana11@gmail.com.
[3]Email: adarshkevdiya@gmail.com.

**Citation**: Dr. Jignesh I. Patel, et.al (2024) Beneficial Effect Of Manilkara Hexandra Extract On Letrozole-Induced Polycystic Ovary Syndrome (PCOS) In Female Rats, Educational *Administration: Theory And Practice, 30(6)(s), 250 - 262*
*Doi: 10.53555/kuey.v30i6(S).5364*

**ARTICLE INFO**

**ABSTRACT**

This study inves gates the therapeu c poten al of the methanolic extract of Manilkara hexandra leaves (MEMH) in managing letrozole-induced polycys c ovary syndrome (PCOS) in female rats. The rats were divided into six groups, with varying letrozole and MEMH doses administered over 66 days. Daily vaginal smears, food, water measurements, and weekly assessments of blood glucose and body weight were conducted. At the study's conclusion, various parameters including hormonal levels, lipid profile, liver profile, and histopathology were evaluated. Letrozole induc on resulted in hormonal imbalance, elevated blood sugar, and lipid levels, mimicking PCOS. Clomiphene citrate, the standard drug, showed posi ve effects. MEMH administra on demonstrated dose-dependent improvements, decreasing testosterone levels, eleva ng progesterone and estrogen, and regula ng glucose and lipid levels. The methanolic extract exhibited poten al therapeu c benefits a ributed to phytoestrogens like querce n and myrice n. The research highlights MEMH's posi ve impact on letrozole-induced PCOS, indica ng its poten al as a novel therapeu c agent for PCOS.

**Keywords:** Polycys c ovary syndrome (PCOS), Letrozole, methanolic extract of Manilkara hexandra leaves (MEMH), clomiphene citrate

## Introduction

Polycys c ovary syndrome (PCOS) is a prevalent endocrine disorder affec ng a significant propor on of women globally, with a par cularly high incidence of approximately 35% among women of fer lity age in India. (Sawant A, 2017) In 1935, Stein and Leventhal were pioneers in the modern medical era, providing the first descrip on of this condi on as "young married peasant women, moderately obese and infer le, with two larger than normal ovaries, bumpy, shiny, and whi sh, just like pigeon eggs". (Homburg, 2008) The Ro erdam 2003 criteria define PCOS based on the presence of any 2 of 3 key criteria: oligoovula on and anovula on, hyperandrogenism, and polycys c ovaries (PCOS). (Farquhar, 2007) This complex disorder is associated with a spectrum of adverse health outcomes, including menstrual dysfunc on, infer lity, hirsu sm, acne, obesity, and an increased risk of type 2 diabetes and cardiovascular disease. (Whitaker, 2011), (Soumya, 2021)

Current pharmacological interven ons for PCOS primarily focus on managing clinical signs and symptoms but fall short of providing a defini ve cure. Commonly prescribed medica ons, including clomiphene citrate, me ormin, thiazolidinediones, spironolactone and oral contracep ves (OCPs), come with their own set of side effects ranging from gastrointes nal issues to poten al risks of cardiovascular disease, hepa c toxicity, and bone density loss. (Whitaker, 2011), (Soumya, 2021)

Amidst the exis ng pharmaceu cal approaches, there is a growing interest in exploring alterna ve treatments for PCOS. Several studies, including randomized controlled trials, case studies, and animal experiments, have delved into the poten al of herbal drugs in managing PCOS. Rodent models, notably the Letrozole-Induced PCOS Model, have played a crucial role in comprehending the biochemical and histological aspects of PCOS. (Soumya, 2021), (Kafali Hasan, 2004), (Shi, 2012)

Letrozole, an aromatase inhibitor, induces PCOS in rats by decreasing aromatase ac vity, resul ng in increased ovarian androgen produc on and decreased estrogen levels, mirroring the hormonal imbalance observed in human PCOS. This model closely replicates the histological and biochemical findings seen in PCOS pa ents. (Soumya, 2021)

One herbal candidate under scru ny for its poten al in PCOS management is Manilkara hexandra. Belonging to the sapotaceae family and na ve to India and Southeast Asian countries, Manilkara hexandra has been tradi onally used to treat various ailments and is noted for its an -inflammatory, an -diabe c, an oxidant, an ulcer, an -arthri c, and an -diabe c proper es. (Shastri, 1994) The leaves of Manilkara hexandra boast a diverse chemical composi on, including sterols, tannin, saponin, triterphin alcohol, and flavonoids such as querce n and myrice n. (Jahan, 2018), (Hu, 2018). These flavonoids, known for their posi ve ac vity against PCOS, par cularly in restoring healthy follicles and regula ng the menstrual cycle, make Manilkara hexandra a compelling candidate for further inves ga on.

This study aims to evaluate the effects of Manilkara hexandra extract on letrozole-induced PCOS in female rats. Leveraging the plant's rich chemical composi on and focusing on its flavonoid content, especially querce n and myrice n, which have shown posi ve effects against PCOS, this research endeavors to provide valuable insights into the poten al therapeu c benefits of Manilkara hexandra in PCOS management. The study seeks to address the current limita ons of pharmacological approaches and pave the way for future therapeu c interven ons in the management of PCOS.

## 2 Materials and methods

### 2.1. Collec on and authen ca on of the plant material

Fresh leaves of Manilkara hexandra collected from Dhanvantari Botanical Garden located at B.k. Mody Government Pharmacy College, Rajkot, Gujarat. Plant authen ca on was done by Dr. V.S. Thaker (Department of Bioscience), Saurashtra University, Rajkot and specimen submission number is SU/BIO/44339.

### 2.2. Prepara on of extracts of MEMH leaves

Manilkara hexandra leaves underwent a 7-10 day drying process, followed by grinding to obtain powdered consistency. The powder was sieved through no. 40 and no. 80 sieves, yielding a fine flower powder stored for extrac on. For methanolic extrac on, 100 gm of dried powder was used in a Soxhlet apparatus with 500 ml methanol at 65 °C. Filtra on through Whatman filter paper No.1 and subsequent methanol evapora on produced a dark green, solid extract. This served as the research study's founda on for further analyses. (Monisha SI, 2017)

### 2.3. Phytochemical analysis of plant extracts

All the prepared extracts underwent a ba ery of qualita ve tests to iden fy the presence of dis nct phytocons tuents, including alkaloids, flavonoids, saponins, carbohydrates, sterols, terpenoids, anthraquinone glycosides, and flavonoids. (Monisha SI, 2017). Thin-layer chromatography (TLC) was conducted to iden fy the chemical cons tuents present in the methanolic extract of Manilkara hexandra leaves. (Meena MC, 2008). In total phenolic content assessment, 1 ml of 300 µg/ml sample reacted with 5 ml Folin-Ciocalteu reagent, followed by 4 ml 7.5% w/v $Na_2CO_3$. A er 30 minutes, the blue reac on color was measured at 765 nm against Gallic Acid. Total phenolic content was expressed as mg of Gallic acid equivalent per gram of dry weight, calculated using a calibra on curve. In total flavonoid assessment, a 100 µg/ml sample was mixed with $H_2O$, followed by sequen al addi ons. The solu on, adjusted to 10 ml, developed a pink color. Absorbance at 510 nm was measured. Flavonoid content was expressed in mg of querce n per gram of dry leaves. The procedure was conducted in triplicate. (Shanmugapriya, 2011)

### 2.4. Animal Protocol Proposal and selec on of animals

All experiments and protocol described in present study was submi ed for the approval to the Ins tu onal Animal Ethics Commi ee (IAEC) and with permission from Commi ee of Control and Supervision of Experiments on Animals (CCSEA), Ministry of Social Jus ce and Empowerment, Government of India. Proposal Approval No. BKMGPC/IAEC29/RP98/2022 Female, non-pregnant, 4-5 days regular estrous cycle Sprague Dawley rats (100200 gm) were used and maintained under standardized condi on (12hr light/dark cycle, 35 to 60% humidity) and provided free excess to pelleted diet and drinking water.

### 2.5. Selec on of doses

In accordance with OECD Guidelines 420, the study protocol entailed administering MEMH at 2000 mg/kg to the test group comprising 5 female rats, while the vehicle control group (5 female rats) received 0.5 CMC%. Monitoring for toxic signs and mortality was conducted at 1, 2, 4, and 6 hours post-oral MEMH treatment, followed by daily assessments for 14 days. Rats that survived were sacrificed at the study's conclusion, and necropsy, along with pathological examina ons of major internal organs, was performed. Notably, a single oral dose of MEMH at 2000 mg/kg did not result in fatality during the observa on period, and no signs of toxicity or behavioral changes were evident. Subsequent doses of 100 mg/kg (low), 200 mg/kg (medium, 1/10th of the

highest dose), and 400 mg/kg (high) were chosen for further inves ga on, ensuring a comprehensive explora on of poten al effects.

### 2.6. Experimental design

Female rats were divided into six groups (N=6) for a 66-day study. Group 1 received the vehicle only throughout the experiment. Group 2 rats were administered 1 mg/kg letrozole (P.O.) for 21 days, followed by vehicle from day 22 to 66. In Group 3, rats were given 1 mg/kg letrozole for 21 days and then 1 mg/kg clomiphene citrate dissolved in 0.5% CMC for the subsequent 45 days. Groups 4, 5, and 6 were administered 1 mg/kg letrozole for 21 days, followed by low dose (100 mg/kg), intermediate dose (200 mg/kg), and high dose (400 mg/kg) of MEMH for the next 45 days, respec vely. The study dura on was 66 days, encompassing the en re experimental protocol. (Kakadia, 2019)

### 2.7. Blood collec on

Following 21 days of treatment, animals in the normal and disease control groups were anesthe zed in accordance with CCSEA guidelines. Blood samples were obtained via retro-orbital puncture, collected in plain sterile Eppendorf tubes, and le to clot at room temperature. Serum was isolated through centrifuga on at 4°C and 5000 RPM for the assessment of hormones, lipid levels, and liver parameters. The iden cal procedure was replicated a er the comple on of the 66-day treatment period for all animals in both standard and treatment groups, ensuring consistency in the research protocol.

### 2.8. Parameters to be assessed

#### 2.8.1. Physical parameters

The body weight of each animal was assessed at the ini a on of the study, and subsequent changes were documented on the 7th, 14th, 21st, 28th, 35th, 42nd, 49th, 56th, and 66th days. This periodic recording of body weight allowed for a comprehensive analysis of weight fluctua ons throughout the study dura on, contribu ng valuable data to the research inves ga on.

#### 2.8.2. Vaginal smear test

For estrous cycle assessment, clean earbuds were immersed in saline or dis lled water. The rat was gently held with one hand around the thorax, ventral surface uppermost, while the other hand restrained the tail for addi onal support and to minimize animal resistance. The earbud p was then gently inserted into the vagina at a depth of 2-5 mm, and a small amount of the cell suspension was expelled onto a labelled glass slide. The four fundamental stages of the estrous cycle—estrus, metestrus, diestrus, and proestrus, denoted as E, M, D, and P—were iden fied by examining the presence, absence, or propor onal quan es of epithelial cells (two types), cornified (kera nized) cells, and leucocytes on the glass slide. This method facilitated a detailed understanding of the rat's reproduc ve cycle stages, enabling comprehensive observa ons for the research study. (Cora MC, 2015)

#### 2.8.3. Biochemical parameters es ma on

##### 2.8.3.1. Serum hormonal assay

The concentra ons of serum estrogen, progesterone, testosterone, luteinizing hormone (LH), and follicle-s mula ng hormone (FSH) were evaluated using Electrochemiluminescent Immunoassay on the Cobas e 411 analyser (Roche Diagnos cs GmbH, Germany) at Sanjivani Laboratory, Ahmedabad. This analy cal method allowed for precise measurement of hormone levels, contribu ng to the scien fic rigor and reliability of the research study.

##### 2.8.3.2. Lipid profile assessment

Low-density lipoproteins (LDL), high-density lipoproteins (HDL), very low-density lipoproteins (VLDL), triglycerides (TGs), and total cholesterol levels were quan fied using diagnos c kits from Biosystem Diagnos c Ltd.

##### 2.8.3.3. Liver profile assessment

Serum glutamic pyruvic transaminase (SGPT), serum glutamic oxaloace c transaminase (SGOT), and serum alkaline phosphatase (ALP) levels were measured u lizing diagnos c kits sourced from Biosystem Diagnos c Ltd.

##### 2.8.3.4. Blood glucose level

The baseline blood glucose levels of each animal were determined at the commencement of the study, and subsequent changes were documented on the 7th, 14th, 21st, 28th, 35th, 42nd, 49th, 56th, and 66th days.

#### 2.8.4. Histopathology of the ovary and uterus

A er blood collec on, the animals were euthanized, and the ovaries and uterus were excised, cleaned, air-dried, weighed, and the length of the uterus was measured. The harvested ovaries and uterus were preserved in 10% formalin for subsequent histopathological examina on. Haematoxylin–eosin-stained sec ons were prepared and analyzed under a light microscope, adhering to established research protocols. This method facilitated a detailed histopathological assessment of the ovaries and uterus in the study.

### 2.9. Sta s cal analysis

The results are expressed as mean ± SEM. The sta s cal significance of the data was determined by one and two-way analysis of variance (ANOVA) followed by Dunnet post hoc test. The level of significance was set at $p < 0.05$. The sta s cal analysis of data was performed using Prism 8.0 so ware (Graphpad So ware Inc., California, USA).

## 3. Result and Discussion

### 3.1. % Yield of methanolic extract of Manilkara hexandra leaves

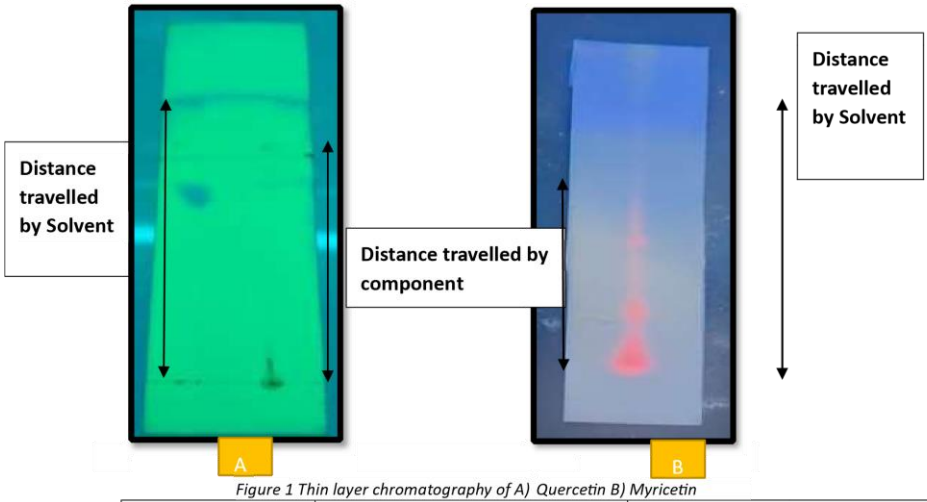



*Figure 1 Thin layer chromatography of A) Quercetin B) Myricetin*

| Compound | Quercetin | Myricetin |
|---|---|---|
| Identification | Yellowish green spot | Brown spo |
| $R_f$ value | 0.79 | 0.67 |
| Standard $R_f$ value | 0.82 | 0.73 |

*Table 1 Reported standard value of quercetin and myricetin.*

The percentage yield of the methanolic extract from 100 grams of dried powdered Manilkara hexandra was determined to be 16.86% w/w.

### 3.2. Result of Preliminary Phytochemical Screening

Preliminary phytochemical screening of methanolic extract of Manilkara hexandra leaves showed various phytocons tuents such as flavonoids, steroids, tannins, proteins, terpenoids, saponins and glycoside.

3.3. Result of Thin layer chromatography for detec on of phytocons tuents The methanolic extract of Manilkara hexandra exhibited Rf values for querce n and myrice n closely matching standard values, confirming the presence of these phytocons tuents. Chromatographic analysis through TLC revealed bands at Rf values of 0.79 and 0.67, corresponding to querce n and myrice n, respec vely, indica ng the presence of flavonoids in the leaf extract.

### 3.4. Result of total phenolic content and total flavonoids content

Total phenolic content in methanolic extract of Manilkara hexandra leaves was 138.46±4.92 mg/gm equivalent to gallic acid. Total flavonoid content in methanolic extract of Manilkara hexandra leaves was 148.11±3.69 mg/gm equivalent to querce n.

### 3.5. Effect of MEMH on estrus cycle of female rats

Phases of estrus cycle were obtained during 66 days study in female rats. Which are shown in figure 2.

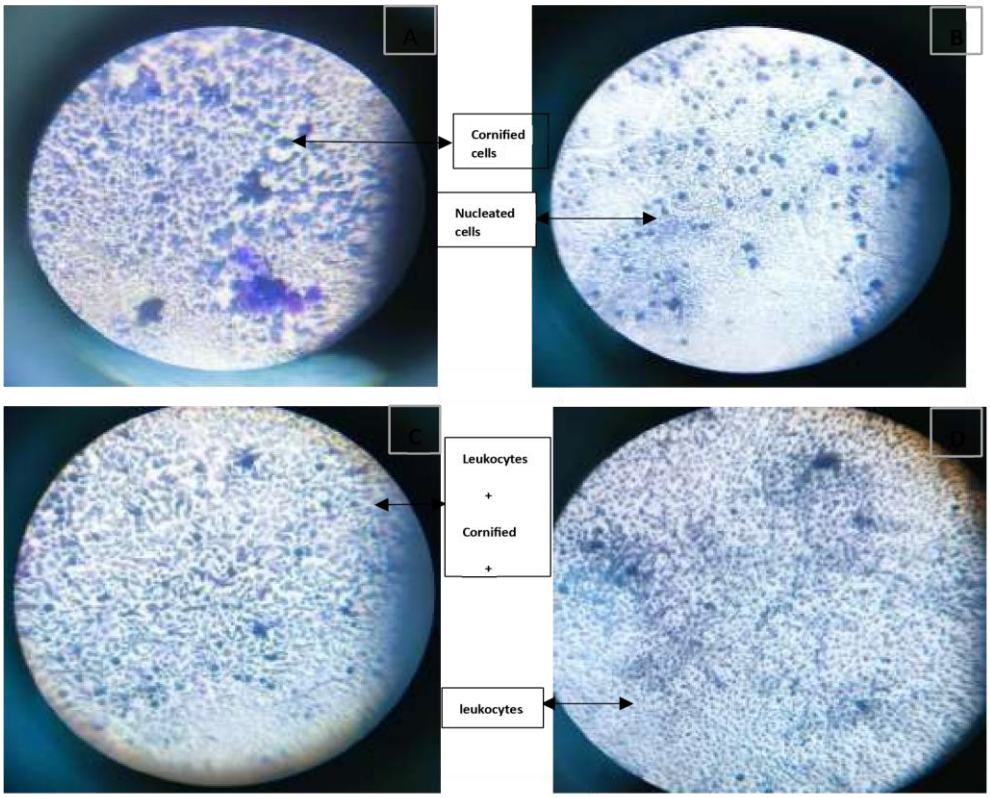



Figure 2 A) Estrus phase B) Proestrus phase C) Metestrus phase D) Diestrus phase

Administra on of letrozole (1 mg/kg P.O.) to female rats disrupted estrus cycle regula on, leading to an elevated frequency of diestrus phase observed through vaginal smear assessment over 66 days. The irregular estrus cycle, termed acyclic estrus, was quan fied by evalua ng diestrus phase frequency in each group. Figure 3 illustrates the observed diestrus phases in female Sprague Dawley rats throughout the study, providing a visual representa on of the disrupted estrus cycle.

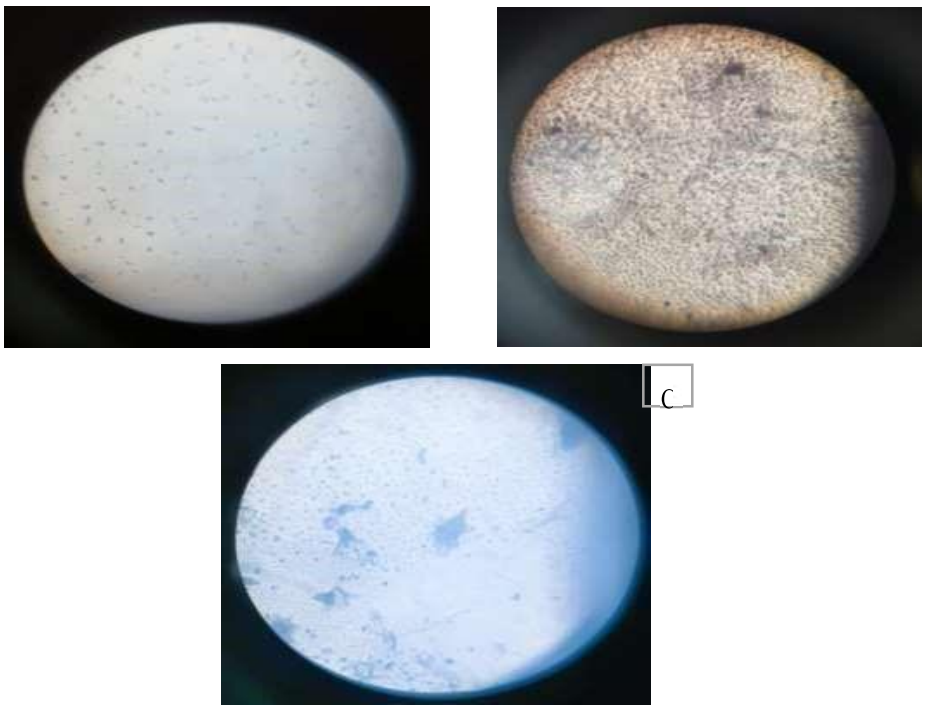



Figure 3 A) Diestrus phase in normal rat (Day 1) B) Diestrus phase in disease control rat (Day 21) C) Diestrus phase in treatment control rat (Day 66)

Letrozole induced irregular estrus cycles in the disease control group, eleva ng diestrus phase frequency significantly a er 66 days (40.83 ± 0.79). Clomiphene citrate in the standard control group reduced diestrus

phase frequency (29 ± 0.86). MEMH at 100 mg/kg, 200 mg/kg, and 400 mg/kg demonstrated a dose-dependent reduc on in diestrus phase frequency (34 ± 0.58, 29.17 ± 0.31, 28.17 ± 0.74, respec vely), indica ng poten al for normalizing the estrus cycle in female rats.

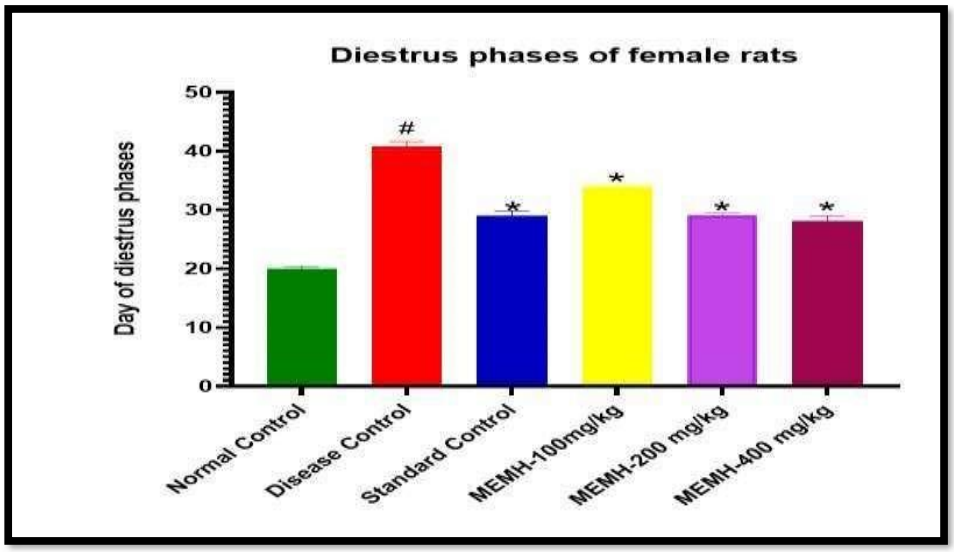



Figure 4 Effect of MEMH on frequency of diestrus phase of female rats

All values represent Mean ± SEM; (n=6)
* - Significant different from disease control (P<0.05)
# - Significant different from normal control (P<0.05)

### 3.6. Effect of MEMH on body weight of female rats

Administra on of letrozole (1 mg/kg P.O.) for 21 days significantly increased body weight in PCOS-induced rats compared to the normal control group. This weight gain is a ributed to hormonal changes causing insulin resistance, increased testosterone, decreased estrogen and progesterone, and elevated blood lipid levels. Clomiphene citrate (1 mg/kg P.O.) for 45 days in the standard control group reduced body weight by normalizing testosterone levels and addressing insulin resistance and hyperlipidemia following PCOS induc on in female rats. Treatment with low dose of MEMH (100mg/kg P.O), Intermediate dose of MEMH (200mg/kg p.o.) and Higher dose of MEMH (400mg/kg P.O.) for 45 days shown sta s cally significant difference in body weight compared to disease control female rats.

### 3.7. Effect of MEMH on blood glucose level of female rats

Letrozole (1mg/kg P.O.) for 21 days in the disease control group significantly increased blood glucose levels (7th day: 144.67±6.89, 14th day: 145.67±10.99, 21st day: 151.00±19.97, 66th day: 179±8.72) compared to the normal control group (7th day:
135.33±2.03, 14th day: 128.33±2.40, 21st day: 123.00±2.65, 66th day: 122.67±0.88). Clomiphene citrate (1mg/kg P.O.) for 45 days in the standard control group significantly decreased blood glucose at the 66th day (118.67±2.19) compared to the disease control (66th day: 179±8.72). A er letrozole, low dose MEMH (100mg/kg P.O.), intermediate dose MEMH (200mg/kg P.O.), and higher dose MEMH (400mg/kg
P.O.) for 45 days showed variable effects on blood glucose. On the 66th day, two MEMH groups demonstrated a significant decrease compared to the disease control. MEMH's flavonoids and phenolic compounds likely contribute to its dose-dependent hypoglycemic ac vity. Detailed blood glucose comparisons are in Figure 5.

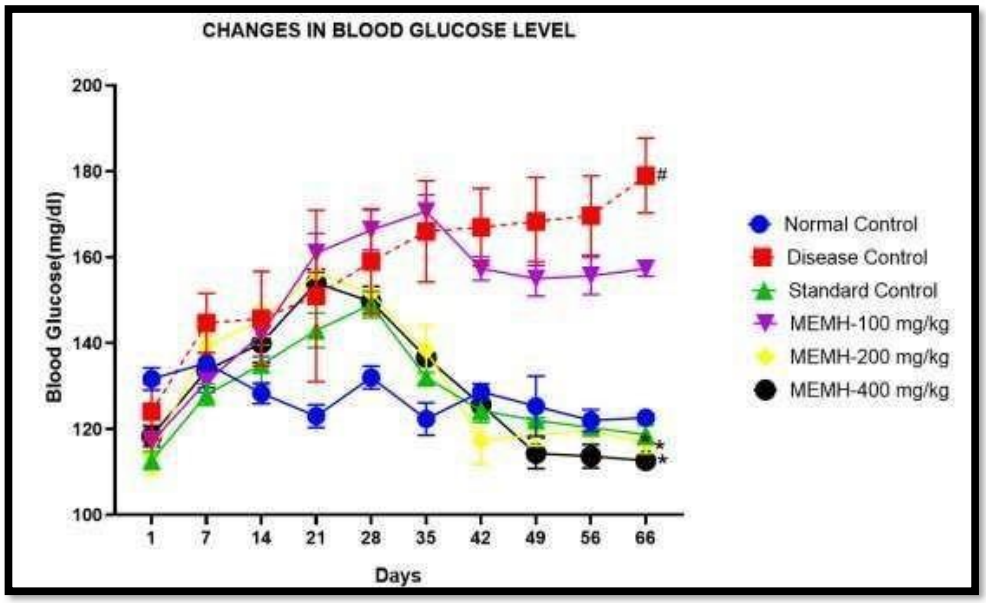



Figure 5 Effect of MEMH on blood glucose level in female rats

All values represent Mean ± SEM; (n=6)
* - Significant different from disease control (P<0.05)
# - Significant different from normal control (P<0.05)

3.8. Effect of MEMH on serum estrogen level in female rats

Following 21 days of letrozole (1 mg/kg P.O.) administra on, blood estrogen levels significantly decreased in the disease control group (77.95±4.81) compared to the normal control group (112.75±4.38) in female rats. The standard control group, treated with Clomiphene citrate (1 mg/kg P.O.) for 45 days, exhibited an increase in serum estrogen levels (134.89±1.87) compared to the disease control group (77.95±4.81). In contrast to the disease control group, treatment with low, intermediate, and high doses of MEMH (100 mg/kg P.O., 200 mg/kg P.O., and 400 mg/kg P.O., respec vely) led to an increase in serum estrogen levels (98.02±2.77, 112.29±3.15, and 123.71±3.80, respec vely) in a dose-dependent manner. These results are illustrated in Figure 6 of the research ar cle.

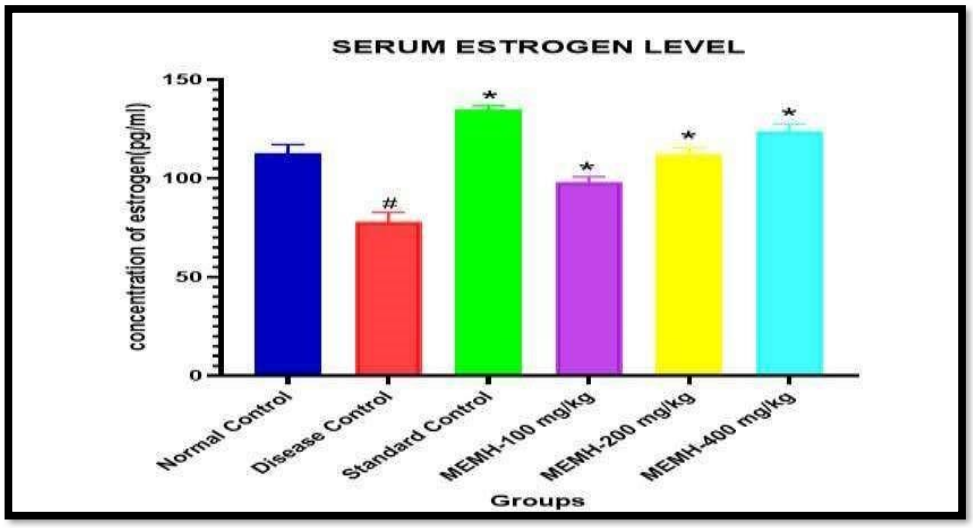



Figure 6 Effect of MEMH on serum estrogen level in female rats

All values represent Mean ± SEM; (n=6)
* - Significant different from disease control (P<0.05)
# - Significant different from normal control (P<0.05)

Increase in serum estrogen level in treatment groups is due to the present of phytoestrogen such as, myrice n and querce n in methanolic extract of Manilkara hexandra leaves which blocks the estrogen receptors in our body and which induced nega ve feedback mechanism and increased the secre on of estrogen in female rats.

### 3.9. Effect of MEMH on serum progesterone level in female rats

Following 21 days of letrozole administra on (01 mg/kg P.O.), a significant decrease in serum progesterone levels was observed in female rats of the disease control group (12.51±1.52) compared to the normal group (31.49±1.92). The standard control group, treated with Clomiphene citrate (1 mg/kg P.O.) for 45 days post-PCOS induc on, exhibited an increase in serum progesterone levels (33.48±1.70) compared to the disease control group (12.51±1.52). Rela ve to the disease control group, treatment with low doses of MEMH (100 mg/kg P.O.), intermediate doses of MEMH (200 mg/kg P.O.), and high doses of MEMH (400 mg/kg P.O.) resulted in a dose-dependent increase in serum progesterone levels (21.68±1.73, 25.15±2.31, and 30.4±1.8, respec vely). These findings are illustrated in Figure 7 of the research article.

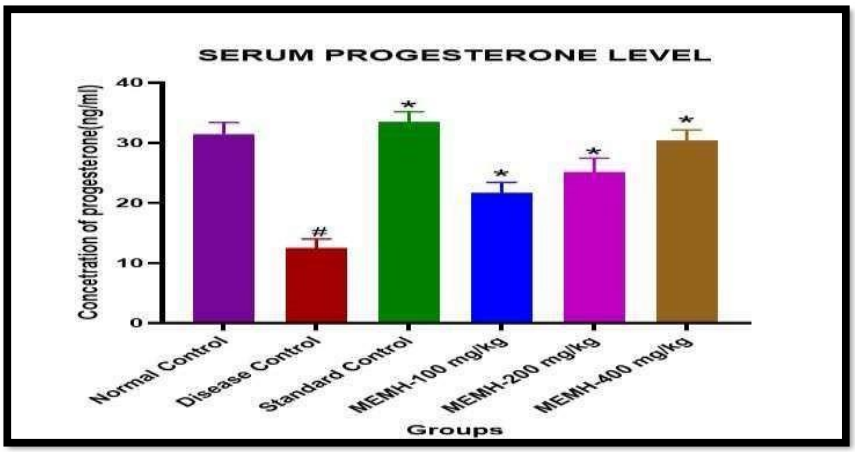



Figure 7 Effect of MEMH on serum progesterone level in female rats

All values represent Mean ± SEM; (n=6)
* - Significant different from disease control (P<0.05)
# - Significant different from normal control (P<0.05)

### 3.10. Effect of MEMH on serum testosterone level in female rats

A er 21 days of letrozole administra on (01 mg/kg P.O.), a significant reduc on in serum progesterone levels was observed in female rats of the disease control group (12.51±1.52) compared to the normal group (31.49±1.92). The standard control group, receiving Clomiphene citrate (1 mg/kg P.O.) for 45 days post-PCOS induc on, demonstrated an eleva on in serum progesterone levels (33.48±1.70) rela ve to the disease control group (12.51±1.52). In comparison to the disease control group, treatment with low, intermediate, and high doses of MEMH (100 mg/kg P.O., 200 mg/kg P.O., and 400 mg/kg P.O., respec vely) resulted in a dose-dependent increase in serum progesterone levels (21.68±1.73, 25.15±2.31, and 30.4±1.8, respec vely). These outcomes are visually presented in Figure 8 of the research article.

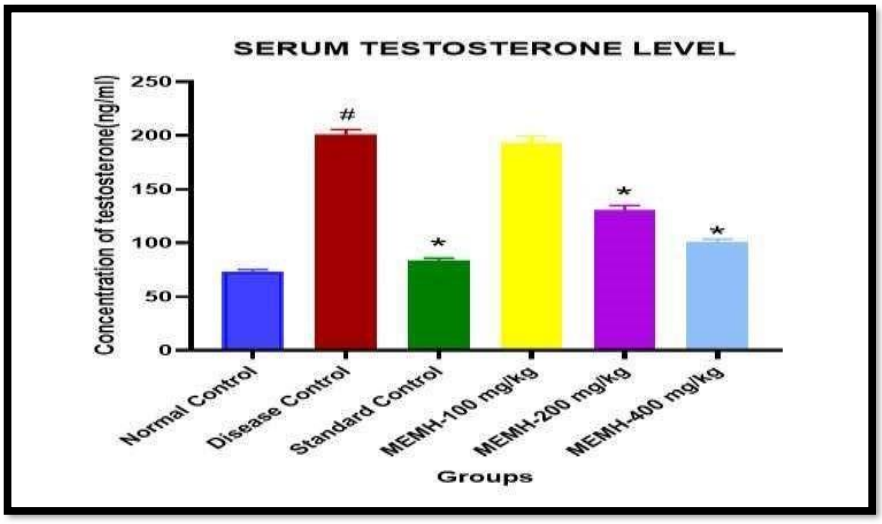



Figure 8 Effect of MEMH on serum testosterone level in female rats

All values represent Mean ± SEM; (n=6)
* - Significant different from disease control (P<0.05)

# - Significant different from normal control (P<0.05)

### 3.11. Effect of MEMH on serum FSH level in female rats

Following 21 days of letrozole administra on (1 mg/kg P.O.), a significant increase in serum FSH levels was observed in female rats of the disease control group (34.58±1.56) compared to the normal group (15.27±1.26). The rise in serum FSH levels is a ributed to letrozole administra on, impac ng GnRH levels and disrup ng the FSH and LH ra o in female rats. The standard control group, treated with Clomiphene citrate (1 mg/kg P.O.) for 45 days post-PCOS induc on, exhibited a decline in serum FSH levels (15.55±1.29) compared to the disease control group (34.58±1.56). Rela ve to the disease control group, treatment with low, intermediate, and high doses of MEMH (100 mg/kg P.O., 200 mg/kg P.O., and 400 mg/kg P.O., respec vely) resulted in a dosedependent decrease in serum FSH levels (26.46±1.56, 19.64±1.23, and 16.22±0.62, respec vely). These results are visually depicted in Figure 9 of the research article.

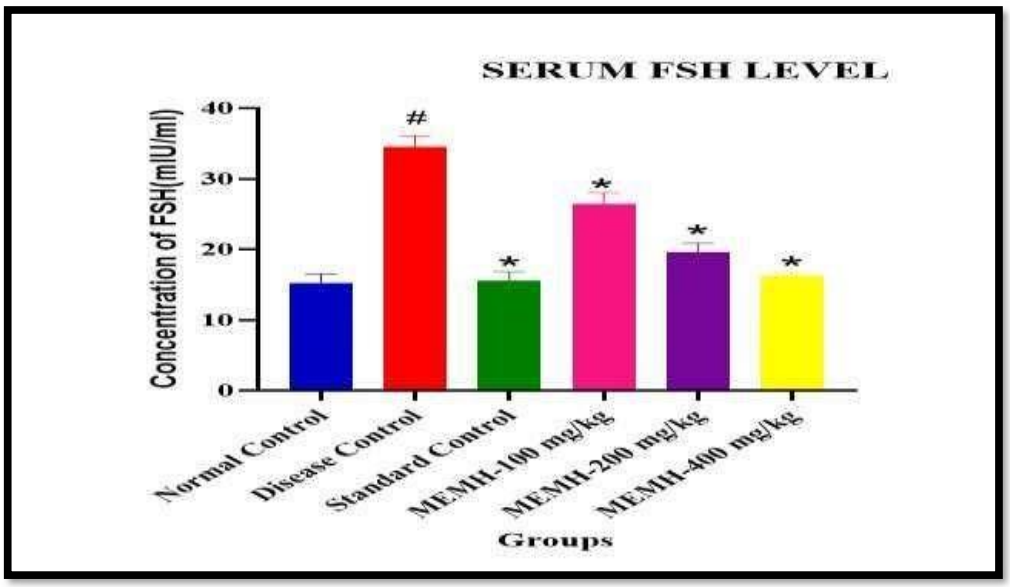



Figure 9 ' Effect of MEMF on serum FSH level in female ra s

All values represent Mean ± SEM; (n=6)
* - Significant different from disease control (P<0.05)
# - Significant different from normal control (P<0.05)

### 3.12. Effect of MEMH on serum LH level in female rats

Following 21 days of letrozole administra on (1 mg/kg P.O.), a significant increase in serum LH levels was observed in female rats of the disease control group (24.29±2.91) compared to the normal group (8.70±2.44). The eleva on in serum LH levels is a consequence of letrozole administra on, which disrupts GnRH levels and the FSH/LH ra o in female rats, leading to an increase in serum LH. In the standard control group, treated with Clomiphene citrate (1 mg/kg P.O.) for 45 days post-PCOS induc on, a decrease in serum LH levels (10.29±1.78) was noted compared to the disease control group (24.29±2.91). Rela ve to the disease control group, treatment with low, intermediate, and high doses of MEMH (100 mg/kg P.O., 200 mg/kg P.O., and 400 mg/kg P.O., respec vely) resulted in a dose-dependent decrease in serum LH levels (20.37±2.62, 18.29±1.39, and 14.61±3.54, respec vely). These findings visually depicted in Figure 10 of the research ar cle.

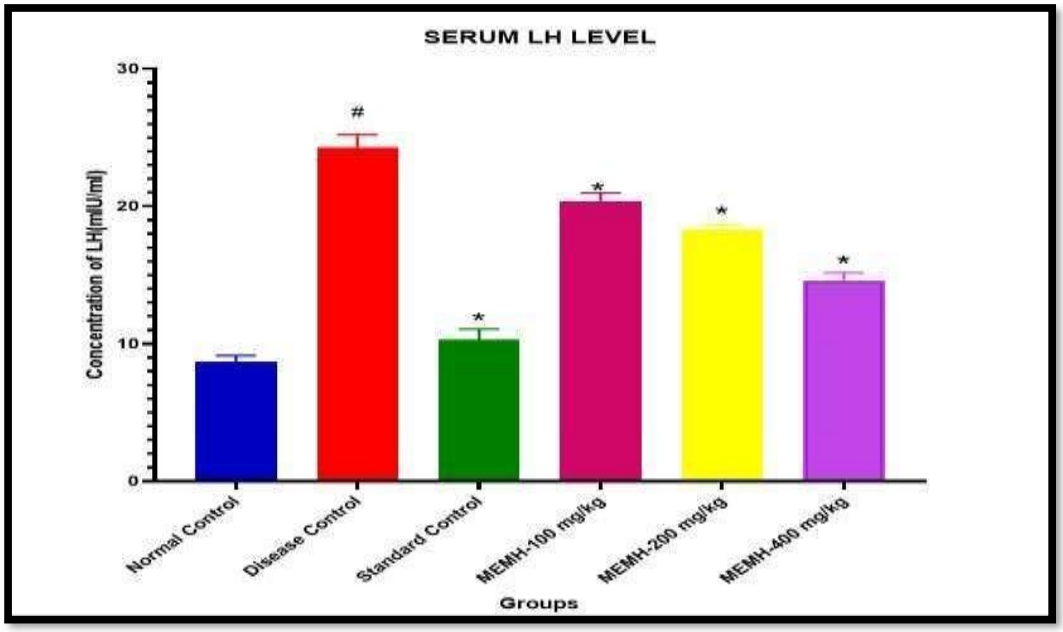



Figure 10 Effect of MEMH on serum LH level in female rats

All values represent Mean ± SEM; (n=6)
* - Significant different from disease control (P<0.05)
# - Significant different from normal control (P<0.05)

### 3.13. Effect of MEMH on lipid profile in female rats: Total Cholesterol (TC), Triglyceride (TGs), HDL, LDL, VLDL

At the study's conclusion, the disease control group of female rats exhibited a significant increase in mean total cholesterol, triglyceride, LDL, and VLDL levels, accompanied by a significant decrease in HDL levels compared to the normal control group. The standard control group, administered Clomiphene citrate (1 mg/kg P.O.) for 45 days post-PCOS induc on, demonstrated a decrease in serum total cholesterol, triglyceride, LDL, and VLDL levels, with a significant increase in HDL levels. In comparison to the disease control group, treatment with intermediate doses (200 mg/kg P.O.) and high doses (400 mg/kg P.O.) of MEMH resulted in a dose-dependent decrease in serum total cholesterol, triglyceride, LDL, and VLDL levels, while HDL levels increased. Conversely, treatment with low doses of MEMH (100 mg/kg P.O.) showed no significant difference in total cholesterol, triglyceride, HDL, LDL, and VLDL compared to the disease control group. These outcomes present a comprehensive view of the lipid profile altera ons induced by MEMH in the context of PCOS.

### 3.14. Effect of MEMH on liver profile in female rats

Upon comple on of the study, the disease control group of female rats exhibited a significant increase in mean SGOT (122.43±7.22), SGPT (95.91±4.86), and ALP (334.39±10.01) compared to the normal control group. The standard control group, treated with Clomiphene citrate (1 mg/kg P.O.) for 45 days post-PCOS induc on, demonstrated a decrease in serum SGOT (70.17±4.57), SGPT (70.85±4.30), and ALP (256.13±23.29) compared to the disease control group. In comparison to the disease
control group, treatment with intermediate doses (200 mg/kg P.O.) and high doses (400 mg/kg P.O.) of MEMH resulted in a decrease in serum SGOT levels in female rats. However, treatment with low doses of MEMH (100 mg/kg P.O.) showed no significant difference in serum SGOT levels. Similarly, regarding serum SGPT levels, treatment with low doses (100 mg/kg P.O.), intermediate doses (200 mg/kg P.O.), and high doses (400 mg/kg P.O.) of MEMH resulted in a decrease compared to the disease control group. While treatment with low, intermediate, and high doses of MEMH (100 mg/kg P.O., 200 mg/kg P.O., and 400 mg/kg P.O., respec vely) led to a decrease in serum ALP levels in female rats compared to the disease control group, sta s cal significance was not achieved. These results offer valuable insights into the hepatoprotec ve poten al of MEMH in the context of PCOS.

### 3.15. Effect of MEMH on ovarian weight in female rats

A er 21 days of Letrozole (1 mg/kg P.O.) administra on, a significant increase in the weight of the right (77.78±2.66) and le ovaries (74.33±2.79) was observed in the disease control group of female rats compared to the normal control group [Right ovary (44.53±2.96) and Le ovary (43.86±2.71)]. The standard control group, treated with Clomiphene citrate (1 mg/kg P.O.) for 45 days, exhibited a decrease in ovarian weight [Right ovary (46.56±3.46) and Le ovary (45.22±2.98)] compared to the disease control group [Right ovary (77.78±2.66) and

Le ovary (74.33±2.79)], as presented in Table 7-13. In comparison to the disease control group, treatment with intermediate doses of MEMH (200 mg/kg P.O.) and high doses of MEMH (400 mg/kg P.O.) resulted in a decrease in ovarian weight in female rats. However, treatment with low doses of MEMH (100 mg/kg P.O.) showed no significant difference in ovarian weight in female rats.

### 3.16. Effect of MEMH on uterine weight in female rats

A er 21 days of Letrozole (1 mg/kg P.O.) administra on, there was a significant increase in the length of the uterine (4.8±0.15) in female rats in the disease control group compared to the normal control group of uterine length (3.47±0.15). The standard control group, treated with Clomiphene citrate (1 mg/kg P.O.) for 45 days, exhibited a decrease in uterine length (3.13±0.20) when compared to the disease control group (4.8±0.15). MEMH contains different phytoestrogens that act through estrogen receptors to regulate hormone levels, normalize ovarian and uterine func on, and increase uterine weight in female rats in a dose-dependent manner. In comparison to the disease control group, treatment with low doses of MEMH (100 mg/kg P.O.), intermediate doses of MEMH (200 mg/kg P.O.), and high doses of MEMH (400 mg/kg P.O.) all led to a decrease in uterine length in female rats.

### 3.17. Histopathology of ovary of female rats

Histopathology of normal female rat ovaries displayed primordial, primary, secondary, ter ary follicles, and corpus luteum (Figure A). In contrast, Letrozole-induced PCOS ovaries in the disease control group exhibited sub-capsular cysts, resembling human polycys c ovaries. However, treatment with MEMH (100 mg/kg, 200 mg/kg, 400 mg/kg P.O.) resulted in a reduc on in cyst numbers and an increase in mature follicles compared to the disease control group (Figure C, D, E).

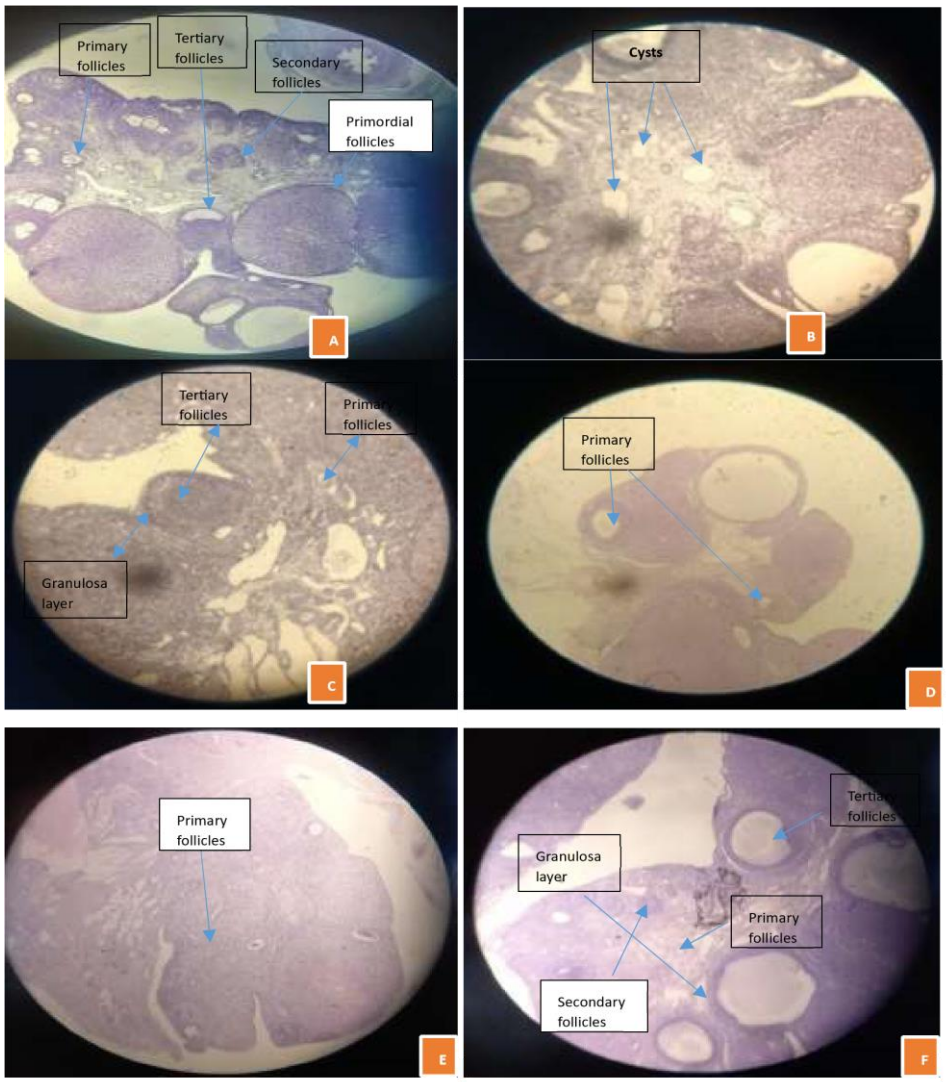
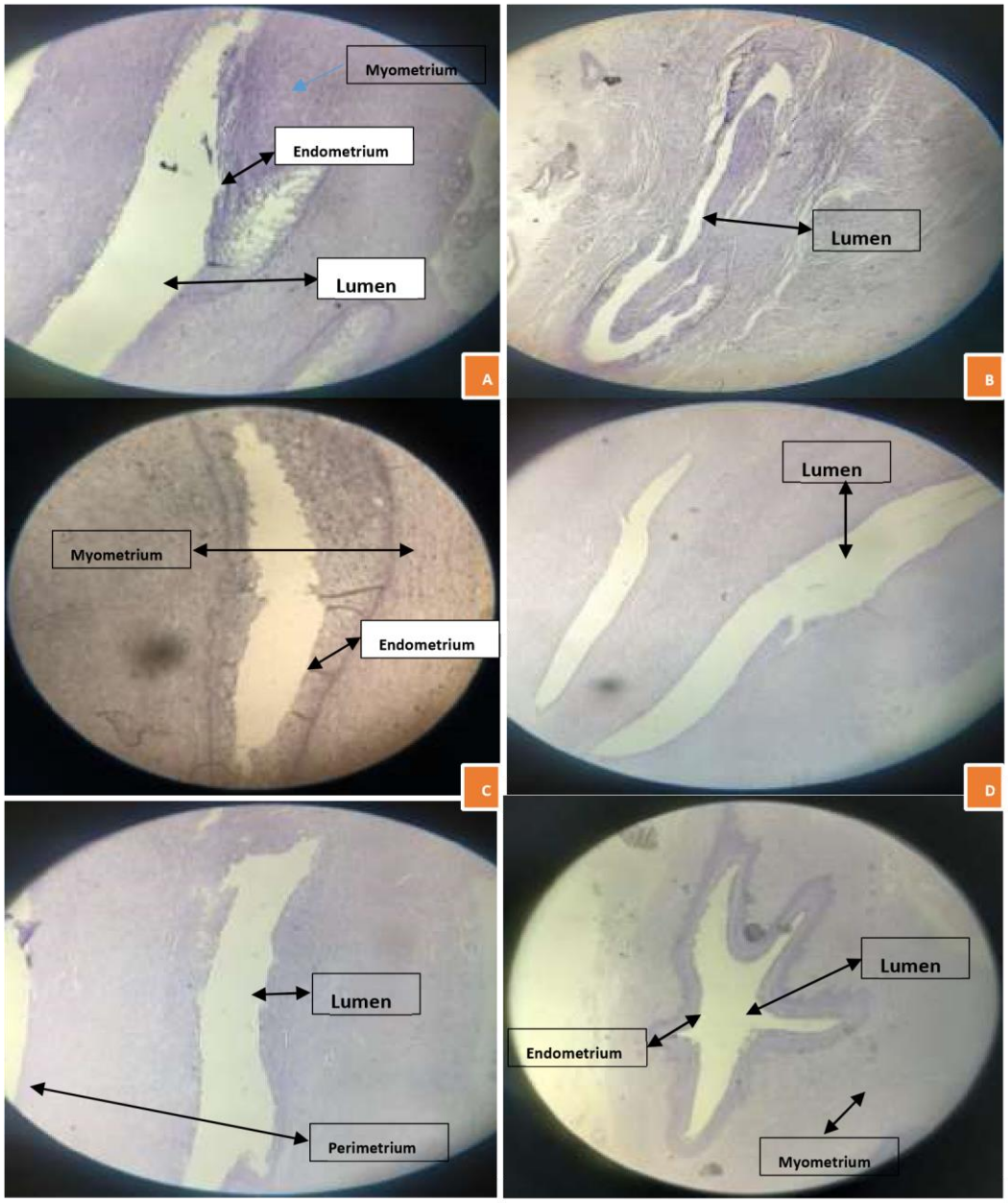



Figure 11 Histopathology of ovary of female rats A) Normal control B) Disease control C) standard control D) 100 mg/kg MEMH E) 200 mg/kg MEMH F) 400 mg/kg MEMH

### 3.18. Histopathology of uterus of female rats

Histopathology of the uterus in normal rats reveals the presence of endometrium, myometrium, perimetrium, and lumen, as depicted in Figure A. Conversely, the histopathology of PCOS-induced rats demonstrates endometrium hyperplasia and a reduc on in the lumen propor on of the uterus, as shown in Figure B. In comparison to the disease control group, treatment with low doses of MEMH (100 mg/kg P.O.), intermediate doses of MEMH (200 mg/kg P.O.), and high doses of MEMH (400 mg/kg P.O.) all resulted in an increased lumen propor on and decreased endometrium hyperplasia.

*Figure 12 Histopathology of uterus of female rats*




A) Normal control B) Disease control C) standard control D) 100 mg/kg MEMH E) 200 mg/kg MEMH F) 400 mg/kg MEMH

## 4. Conclusion

Polycys c ovary syndrome includes menstrual disorders such as oligomenorrheaamenorrhea, anovula on with this hormonal imbalance and hyperandrogenism, which results in an increase in testosterone levels, which cause decrease in estrogen and progesterone level and also it affects the FSH/LH ra o. Moreover, PCOS increase the weight of ovaries and length of uterine. According to the current study, methanolic extract of Manilkara hexandra leaves had a posi ve effect in treatment of letrozole induced PCOS. It has a range of pharmacological effects, including estrogenic ac vity, progesterone ac vity, and decrease the level of androgen and also it has hepatoprotec ve ac vity, and hypoglycaemic ac vity, all of which are directly related to the allopathic therapy of PCOS. The methanolic extract of Manilkara hexandra leaves may have such

pharmacological effects due to presence of a variety of phytoestrogens, including querce n, myrice n and gallic acid. Based on the discovery that a methanolic extract of Manilkara hexandra leaves has a broadly beneficial impact on polycys c ovary syndrome (PCOS).

## Acknowledgement

We extend our sincere gra tude to B.K. Mody Government Pharmacy College Rajkot for providing invaluable support and resources throughout the course of this research. Special thanks to the faculty and staff whose guidance and exper se significantly contributed to the success of our study. We also appreciate the ins tu on's commitment to fostering a conducive research environment. This work is a testament to the collabora ve spirit fostered by B.K. Mody Government Pharmacy College, Rajkot.

## References

1. Sawant A, Pa l S and Shah S, “Review on PCOD/PCOS & its Treatment in different Medicinal Systems-Allopathy, Ayurveda, Homeopathy.” Sci Journo. 2017.
2. Homburg R, “Polycys c ovary syndrome.” Clinical Obstetrics and Gynaecology. 2008, 22, 261–274.
3. Farquhar C, “Introduc on and history of polycys c ovary syndrome.” Polycys c Ovary Syndrome. 2007, 4–24.
4. Whitaker KN, “Polycys c ovary syndrome: An overview.” J Pharm Pract. 2011, vol 24, 94–101.
5. Soumya V, “Polycys c Ovary Disease (PCOD)- An Insight into Rodent Models, Diagnosis and Treatments.” J Clin Med Img. 2021, 5, 1–13.
6. Kafali H, Iriadam M, Ozardali I and Demir N, “Letrozole-induced polycys c ovaries in the rat: A new model for cys c ovarian disease.” Arch Med Res. 2004, 35, 103–108.
7. Shi D and Vine DF, “Animal models of polycys c ovary syndrome: A focused review of rodent models in rela onship to clinical phenotypes and cardiometabolic risk.” Fer l Steril. 2012, 98, 185-193.
8. Sadgat Ayurvaidmatand Sankar Shastri. Aryabhishak; 6th edi on; Sastu Sahitya Vardhak Karyalaya, Ahmedabad and Mumbai, 1994, pp 325.
9. Jahan S, “Therapeu c poten als of Querce n in management of polycys c ovarian syndrome using Letrozole induced rat model: A histological and a biochemical study.” J Ovarian Res. 2018, 11.
10. Hu T, Yuan X, Wei G, Luo H, Lee HJ and Jin W, “Myrice n-induced brown adipose ssue ac va on prevents obesity and insulin resistance in db/db mice.” Eur J Nutr. 2018, 57, 391-403.
11. Monisha SI, Leela GD, Immaculate AA, Vimala JR. Compara ve studies on yield and the phytochemical appraisal (Quality and Quan ty) of Manilkara hexandra (Roxb) Dubarb using leaf, stem, and bark. Journal of Pharmacognosy and Phytochemistry. 2017;6(4):2052-8.
12. Shanmugapriya, K., Saravana, P. S., Payal, H. A. R. S. H. A., Mohammed, S. P., & Binnie, W. I. L. L. I. A. M. S. (2011). An oxidant ac vity, total phenolic and flavonoid contents of Artocarpus heterophyllus and Manilkara zapota seeds and its reduc on poten al. Interna onal Journal of Pharmacy and Pharmaceu cal Sciences, 3(5), 256-260.
13. Kakadia N, Patel P, Deshpande S, Shah G. Effect of Vitex negundo L. seeds in letrozole induced polycys c ovarian syndrome. Journal of Tradi onal and Complementary Medicine. 2019 Oct 1;9(4):336-45.
14. Padmanabhan V and Lopez AV, “Animal models of the polycys c ovary syndrome phenotype.” Steroids. 2013, 78, 734–740.
15. Cora MC, Kooistra L and Travlos G, “Vaginal Cytology of the Laboratory Rat and Mouse:Review and Criteria for the Staging of the Estrous Cycle Using Stained Vaginal Smears.” Toxicol Pathol, 2015, 43, 776–793.
16. Mishra N, “Tradi onal Uses, Phytochemistry and Pharmacology of Mimusops hexandra Roxbs.” Advances in Pharmaceu cal and Ethnomedicines. 2014, 2, 32–35.
17. Singh S and Jha MK, “A Comprehensive Pharmacognos c Review: Manilkara hexandra (Roxb.) Dubard.” Int J Pharm Sci Res. 2020, 11, 3645.
18. Mahesh Chand Meena and Vidya Patni, “Isola on and Iden fica on of Flavonoid "Querce n" from Citrullus colocynthis (Linn.) Schrad.” Asian J. Exp. Sci. 2008, 9.
19. Mihma M, Gangooly S and Mu ukrishna S, “The normal menstrual cycle in women.” Animal Reproduc on Science. 2011, 124, 229–236.
20. Livadas S and Kandarakis ED, “Polycys c Ovary Syndrome: Defini ons, Phenotypes and Diagnos c Approach Novel Insights into Causes and Therapy.” Horm Res. 2013, 40, 1–21.
21. Garabadu D, Singh S and Gautam T, “ Manilkara hexandra (Roxb.) Dubard Ameliorates Ace c Acid-induced Rat Gastric Ulcer.” JOURNAL OF DIETARY SUPPLEMENTS. 2020.
22. Chanda S and Parekh J, “Assessment of An microbial Poten al of Manilkara hexandra Leaf.” PHCOG J, 2020.