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RESEARCH ARTICLE

Evaluation of leaf extracts of *Moringa oleifera* Lam. On testosterone enanthate-induced polycystic ovary syndrome (PCOS) in mice

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Abstract

Polycystic ovary syndrome (PCOS) is a multifactorial endocrinological and metabolic disorder that affects women of reproductive age. It is often associated with hyperandrogenism, obesity, anovulation, infertility and insulin resistance. In this study, the imminent efficacy of MoLP (*Moringa oleifera* leaf powder) and MoLE (*M. oleifera* leaf extract) in treating

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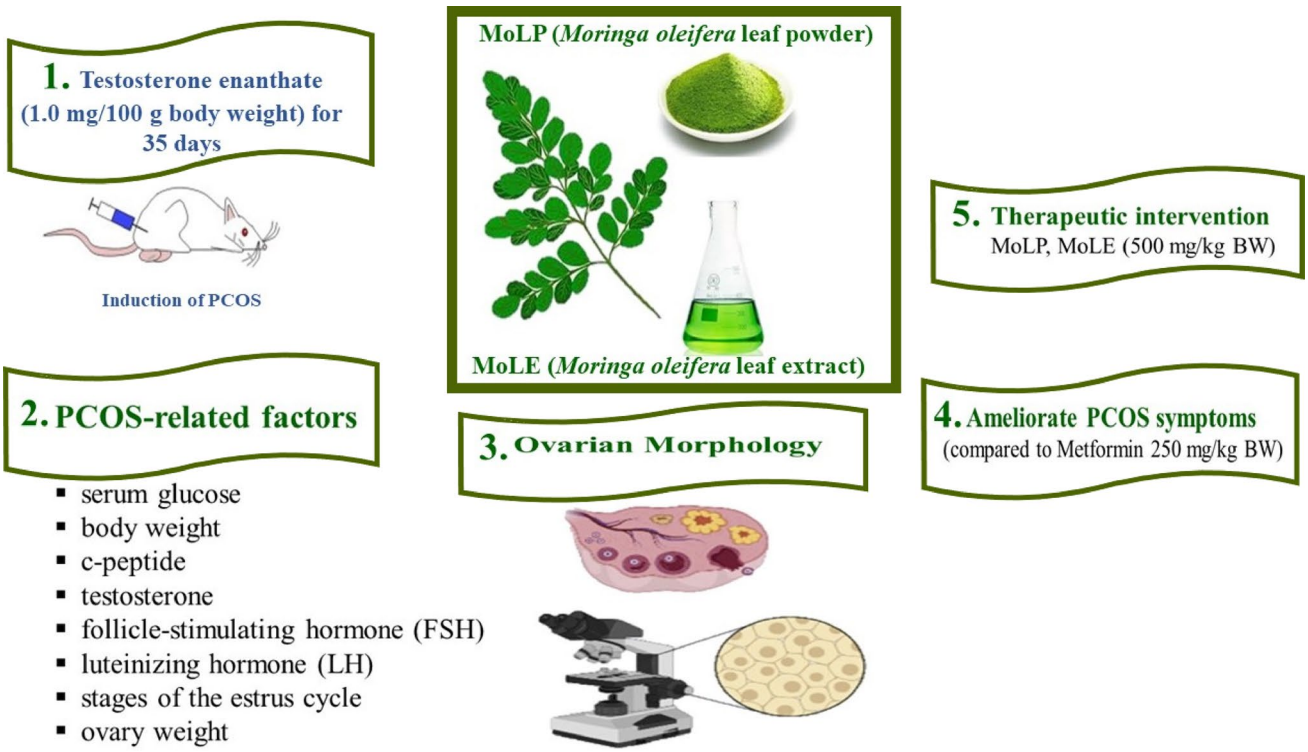
PCOS in female albino mice was explored. Female albino mice were injected with testosterone enanthate [1.0 mg/100 g body weight (b.w.)] for 35-days to induce PCOS. For the treatment, mice were administered with *M. oleifera* leaf powder (250 and 500 mg/kg b.w.), *M. oleifera* leaf extract (250 and 500 mg/kg b.w.), and metformin (250 mg/kg b.w.) for 14 days. Following the intervention, body weight, blood glucose, c-peptide, testosterone, follicle-stimulating hormone (FSH) and luteinizing hormone (LH) levels and stages of the estrus cycle were measured at 0, 7 and 14 days. The ovaries were examined stereologically to determine the number and diameter of follicles. Results indicated that MoLP, MoLE (500 mg/kg b.w.) and metformin (250 mg/kg b.w.) significantly decreased body weight, blood glucose, c-peptide, testosterone and LH levels while increasing FSH levels and ovary weight over time compared to PCOS-induced mice. Additionally, regulation of the estrus cycle and folliculogenesis in PCOS-induced mice was observed. Overall, the present study revealed that *M. oleifera* may have the potential as a therapeutic intervention for PCOS.

Graphical Abstract



Highlights

Polycystic ovary syndrome (PCOS): an ovulation related disorder escorted by hyperandrogenism.
Moringa oleifera Lam. leaf powder and extract suggested anti-infertility, anti-obesity and anti-diabetic role for anovulation related disorders.
Moringa oleifera Lam. leaf powder and extract regulate estrus cycle and folliculogenesis in PCOS-induced mice.
Moringa oleifera Lam. leaf powder and extract have the potential as a therapeutic intervention for PCOS.

Keyword Polycystic ovary syndrome (PCOS) · Testosterone enanthate · *Moringa oleifera* · Metformin · *Mus musculus*

Introduction

Infertility is a reproductive disorder affecting one in every six couples worldwide (Langarizadeh et al. 2023). It can result from multiple factors such as polycystic ovaries, ovulatory failure, fallopian tubes blockage, benign cervical tumors, uterine fibroids, age, hyperprolactinemia, hypothalamic dysfunction, ovarian endometriosis and pelvic inflammatory disease disease (Akbaribazm et al. 2021; Ozioko et al. 2022). Polycystic ovary syndrome (PCOS) is a common hormonal disorder and is a significant cause of infertility in nearly 5–21% of reproductive-age women (Zhang et al. 2020; Shrivastava 2022). The etiology of PCOS is ambiguous due to its multifaceted nature. Various environmental

and genetic factors, such as hormonal imbalance, obesity, poor dietary choices, stress and a sedentary lifestyle, contribute to the pathogenesis of this disease (Khaled et al. 2019; El-Eshmawy et al. 2022). The pathogenic mechanism of this syndrome can be clinical, biochemical, hormonal and metabolic. It is often associated with menstrual disorders, persistent anovulation, a high serum level of luteinizing hormone (LH) compared to follicle-stimulating hormone (FSH), increased androgen levels, ovarian polycystic changes, oxidative stress, chronic inflammation, obesity, diabetes and insulin resistance (Abasian et al. 2018; Zhou et al. 2022). Women with PCOS may also experience anxiety, depression, low self-esteem, social distance, psychosexual dysfunction and other psychological disorders (Zhang et al. 2020). PCOS cannot be diagnosed with any single test rather two out of three specific criteria, including polycystic ovaries, irregular ovulation and hyperandrogenism must be used to diagnose it (Divyashree et al. 2019).

The treatment of PCOS is quite complex due to its obscure pathogenesis and interindividual differences in symptomatology. A healthy lifestyle, a well-balanced diet, less caffeine consumption, weight loss, stress relief and regular exercise are the initial therapies to improve insulin sensitivity and restore ovulation (Ionescu et al. 2018; Hussain et al. 2022). Currently, pharmacological interventions such as oral contraceptives (including metformin), ovulatory stimulants (such as clomiphene citrate and letrozole) and antilipidemics have been used for the treatment of PCOS (Khaled et al. 2019; Hussain et al. 2022). However, these medications have shown limited effectiveness and can cause various side effects such as headache, nausea, dizziness, weight gain, lower abdominal pain, backache, lactic acidosis and dysmenorrhea (Domecq et al. 2013; Younas et al. 2022). As a result, it is necessary to identify and develop alternate broad-spectrum, readily available, natural-source medicines with minimal side effects.

Moringa oleifera has been used in herbal medication for centuries, often regarded as the "miracle tree" due to its numerous health benefits (Padayachee et al. 2020). Moringa is esteemed for its high level of bioactive compounds, particularly rich in phenolic compounds (Benkiran et al. 2024) and has lately been examined as a potential adjunctive treatment for several diseases, including malnutrition, anemia, blindness, arthritis, headaches, fever, inflammation, asthma, heart, kidney and various digestive disorders (Padayachee and Baijnath 2020). Moringa possesses antidiabetic, anti-obesity, anti-inflammatory, antioxidant, antimicrobial, antitumor, antihypertensive, antiepileptic activities and anticancer properties (Bhattacharya et al. 2018; Padayachee and Baijnath 2020). Given its miraculous properties, *M. oleifera* may have beneficial effects in the treatment of some reproductive disorders and, thus, could be applied in treating testosterone-induced PCOS.

Albino mice (*Mus musculus*) are commonly used to study PCOS due to their short lifespan, small body size, consistent genetic background, high reproductive success and long-term metabolic effects (Paixao et al. 2017). Researchers have used different methods to induce PCOS in mice, together with exposing them to estrogens and androgens (Shi and Vine 2012). PCOS can also be administered through daily injections of testosterone enanthate (Moshfegh et al. 2022).

The current study aims at exploring the potential therapeutic effects of MoLP *(M. oleifera* Lam. leaf powder) and MoLE (70% ethanolic extract) on PCOS in albino mice (*Mus musculus*), and at checking if the administration of MoLP and MoLE to the mice can ameliorate various PCOS-related factors such as serum glucose, body weight (b.w.), c-peptide, testosterone, follicle-stimulating hormone (FSH), luteinizing hormone (LH), ovary weight and histological parameters of ovaries in PCOS-induced albino mice. We anticipate that the potential of *M. oleifera* Lam. leaf powder and 70% ethanol extract will be related to that of metformin and could serve as an effective therapeutic intervention for reversing PCOS, insulin resistance and other contributory factors in humans.

Materials and methods

Chemicals and reagents

Formalin 10%, Hematoxylin, Eosin and Giemsa stain were purchased from Anamol Laboratories Private Limited (Kolgaon, Maharashtra, India). Ethanol (AnalaR (BDH Laboratory supplies Poole, Bh15 LTD, Poole, England) and distilled water were used for extract preparation. Microscopes (laboMed CXR2, Martin Microscope, Los Angeles, California, United states) and required apparatus were available in the laboratory of the department.

Mice were reared in animal houses in the Department of Zoology, University of Sargodha (Sargodha, Pakistan). ELISA kits, FSH (cat=Y-84232-48), LH (cat=Y-84344-48), Testosterone (cat=Y-88610-48) and C-Peptide (cat=Y-88621-48) were purchased from Wuhan Zokeyo Biotechnology Co. Ltd (Wuhan, China) and Testosterone enanthate injections were bought from Testoviron Depot, Bayer Pakistan Private Limited Korangi, Karachi, Pakistan).

Moringa leaves collection

Moringa oleifera leaves were harvested from the botanical garden of the University of Sargodha, washed, air-dried and

powdered (Sieve no 30 M), in high speed grinder (Silver Crest; Model SC 2880, Dongguan, China).

Preparation of aqueous and ethanolic extracts

For aqueous extraction, 300 g of *M. oleifera* leaf powder (MOLP) was infused in 5000 mL of distilled water for 72 h in a shaking incubator (Model: SKIR-601, SHIN/ SAENG Scientific Co Ltd., Germany). The resulting aqueous filtrate of MoLP was concentrated by applying limited pressure, using a Rotary Evaporator (Model: R-300, BUCHI Labortechnik AG, Switzerlandand then kept at 4 °C. Ethanol Extract (MoLE) was prepared by macerating 300 g leaf powder in 5000 mL of 70% ethanol. After filtration (Whatman, Filter paper, Whatman Limited, Maidstone England) and evaporation the extract was kept at 4 °C (Khalid et al. 2023).

Preparation of feed

Standard feed (Feed number 9, Karachi feeds Private Limited, Karachi, Pakistan) containing 2% fats, 16% carbohydrates and 18% proteins was used. Moreover, proximate analysis and high-performance liquid chromatography (HPLC) analyses of *M. oleifera* leaf powder and extracts were previously performed (Khalid et al. 2023).

Table 1 Treatments and interventions

Treatments	Mice status	Group	Intervention
T_o	PCOS induced	Placebo	Standard feed + 5% Tween 80
T_o^+	PCOS induced	Positive Control	Standard feed + Metformin @ 250 mg/kg b.w
T_o^-	Non-PCOS	Normal Control	Standard feed
T_1	PCOS induced	MoLP-250	Standard feed + MoLP @ 250 mg/kg b.w
T_2	PCOS induced	MoLP-500	Standard feed + MoLP @ 500 mg/kg b.w
T_3	PCOS induced	MoLE-250	Standard feed + Selected MoLE @ 250 mg/kg b.w
T_4	PCOS induced	MoLE-500	Standard feed + Selected MoLE @ 500 mg/kg b.w

MoLP = *M. oleifera* leaf powder

MoLE = *M.oleifera* leaf extract

b.w. = Body weight

T_O^- is non-PCOS induced while the others are PCOS induced

Animal study

One hundred female albino mice, aged 8–9 weeks and weighing 26 ± 2 g, were used. *Mus musculus* (albino mice) were bought from animal house, University of Sargodha, Sargodha, Pakistan. Standard housing conditions were maintained [12 h of light/dark cycles, relative humidity of (40 ± 5) % and temperature (21 ± 2) °C]. Acclimatization period of 10 days was provided for mice. During the acclimatization period, anatomically abnormal and pregnant mice were separated. Albino mice (10) were picked randomly to measure the body weight, serum glucose, LH, FSH, testosterone and c-peptide. The stages of the estrus cycle were studied by preparing vaginal smears, and the follicles were analyzed by excising the ovaries.

Induction of polycystic ovary syndrome (PCOS)

After acclimatization, PCOS was induced in the albino mice by using (1.0 mg/100 g b.w.) testosterone enanthate injection (Testoviron Depot, Bayer Pakistan-Private Limited, Korangi, Karachi) in the thigh muscles after diluting in sesame oil for consecutive 35 days (Kalhori et al. 2018).

Study plan

Non-PCOS induced mice was assigned T_0^- group. Diseased mice (PCOS induced) were randomly sorted into 6 groups, i.e. Placebo (T_0), Positive control (T_0^+), MoLP-250 (T_1), MoLP-500 (T_2), MoLE-250 (T_3), MoLE-500 (T_4). The treatments and interventions are summarized in Table 1.

Biochemical study

During acclimatization period, induction of disease, treatment period and at the accomplishment of the study various biomarkers of albino mice were analyzed.

Evaluation of serum glucose level

The fasting serum glucose (mg/dl) of mice was measured using Glucometer ACCU-CHEK Active (CE-0123, Roche, Germany). Albino mice having blood glucose > 250 mg/dl were considered hyperglycemic, whereas albino mice < 100 mg/dl were considered hypoglycemic.

Hormonal assays

Blood samples of mice were collected and serum was separated at 4500 rpm for 10 min using a centrifugation machine (MPW-352 R, MPW med. Instrument ul., Poland). The serum was analyzed in triplicate using an FSH kit with

0.1 mlU/mL (sensitivity), LH kit with 0.1 mlU/mL (sensitivity), Testosterone (T) kit with 0.1 ng/mL (sensitivity), and C-Peptide (C-P) kit with 0.1 ng/mL of sensitivity (Kalhori et al. 2018).

Assessment of estrus cycle by vaginal smears

Throughout the study period, vaginal smears were analyzed on daily basis to evaluate the stages of the estrus cycle in mice (Amelia et al. 2018).

Collection and processing of vaginal smears

To extract cells from the vaginal canal of mice, a dropper was used to draw up approximately 0.1 mL of saline. The dropper's tip was gently inserted 1–2 mm into the vaginal opening and the saline was put in and out to make a thin smear on a slide, which was left to air dry (Cora et al. 2015). Vaginal smears were collected once per day using this procedure. These samples were then preserved in methanol (AnalaR (BDH Laboratory supplies Poole, Bh15 LTD, Poole, England) and stained with Giemsa stain (Anamol Laboratories Private Limited Kolgaon, Maharashtra, India) before being analyzed using a light microscope with 10 and 40 × magnification (Hubscher et al. 2005).

Slide evaluation of vaginal cells

To identify vaginal cells, a 10 × objective lens was typically used, although in some cases, a higher magnification

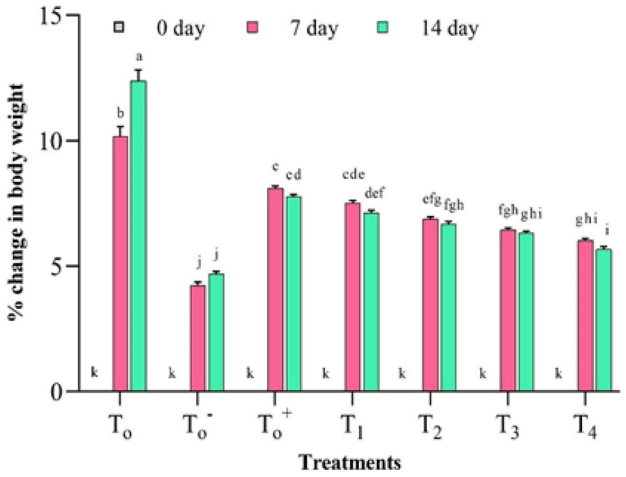


Fig. 1 Effect of days and treatments on % change in body weight (g) of *Mus musculus* (10 mice/week). Different symbols (a–k) indicate statistically significant differences among treatments at $P<0.05$. To = Standard Feed + 5% Tween 80; To^{-} = Standard feed; To^{+} = Standard feed + Metformin @ 250 mg/kg b.w.; T_1 = Standard feed + MoLP @ 250 mg/kg b.w.; T_2 = Standard feed + MoLP @ 500 mg/kg b.w.; T_3 = Standard feed + MoLE @ 250 mg/kg b.w.; T_4 = Standard feed + MoLE @ 500 mg/kg b.w

40 × objective lens was used to enhance the visualization of neutrophils. The estrus cycle length in mice normally ranged from 4 to 5 days but occasional cycles lasting 6 days were also observed in some cases (Cora et al. 2015).

Stereological analysis of ovarian tissues

The ovaries were excised from the placebo (T_o), positive control (T_o^+), normal control (T_o^-) and different treatment groups (T_1, T_2, T_3 and T_4) and promptly fixed in Cornoy's fixative, which contains absolute ethanol (85 mL), formaldehyde (10 mL) (Laboratory ULCW Chemicals, United Laboratory Chemical Works, Garden town, Lahore, Pakistan) and glacial acetic acid (5 mL) (Laboratory ULCW Chemicals, United Laboratory Chemical Works, Garden Town, Lahore, Pakistan). Then, the ovaries were dehydrated using increasing concentrations of alcohol, with each concentration ranging from 50 to 70%, 90% and, finally, 100%, and each step took 24 h to complete. For clearance, organs were immersed in xylene (Laboratory ULCW Chemicals, United Laboratory Chemical Works, Garden town, Lahore, Pakistan) for 5–6 h. For embedding, organs were placed in molten paraffin wax (56–58 °C) (Laboratory ULCW Chemicals, United Laboratory Chemical Works, Garden town, Lahore, Pakistan). Glass cavities (Laboratory ULCW Chemicals, United Laboratory Chemical Works, Garden town, Lahore, Pakistan) of various sizes were used to prepare appropriate blocks of paraffin-fixed organs. Serial sections of five microns were obtained by a rotary microtome (Laboid International, Kasauli, Himachal Pradesh, India). These sections were then mounted on albumenized glass slides. Sections were stained by using eosin (Anamol Laboratories Private Limited Kolgaon, Maharashtra, India) and hematoxylin (Anamol Laboratories Private Limited Kolgaon, Maharashtra, India) dyes, which were then fixed with Canada balsam (Anamol Laboratories Private Limited Kolgaaon, Maharashtra, India). Sections of ovaries were observed under a trinocular microscope (laboMed CXR2, Martin Microscope, Los Angeles, California, United states) at 40 and 100X magnifications (Kalhori et al. 2018). These histopathologic slides were utilized to measure folliculogenesis by observing under a microscope, counting and determining the primordial, primary, secondary and tertiary follicular diameter with de-Graff method (Amelia et al. 2018).

Digital photography and processing

Photomicrographs of histological sections from ovaries of randomly selected albino mice from all treatment groups were captured using a Camera (TECNO CG6j, 48 megapixels, Shenzhen, China) that was mechanically attached to a Trinocular Microscope (laboMed CXR2, Martin

Table 2 Results of general linear model indicating the significance of treatments and days on % change in body weight (g), serum glucose (mg/dl), c-peptide (ng/mL), testosterone (mg/dl), FSH (m1U/mL), LH (1U/l) and % (w/w) change in ovary weight

Source of variation		Body weight		Blood glucose		c-peptide		Testosterone		FSH		LH		Estrus cycle		Ovary weight	
	DF	F	*P*	F	*P*	F	*P*	F	*P*	F	*P*	F	*P*	F	*P*	F	*P*
Treatments (T)	6	283.08	0.00	722.78	0.00	1704.41	0.00	1166.70	0.00	267.49	0.00	134.65	0.00	0.00	1.00^{ns}	3670.62	0.00
Days (D)	2	5509.92	0.00	457.74	0.00	1486.78	0.00	503.17	0.00	368.30	0.00	53.35	0.00	280.31	0.00	1016.11	0.00
T×D	12	80.89	0.00	51.57	0.00	159.04	0.00	94.24	0.00	30.49	0.00	22.06	0.00	50.79	0.00	1013.15	0.00
Error	189																
Total	209																

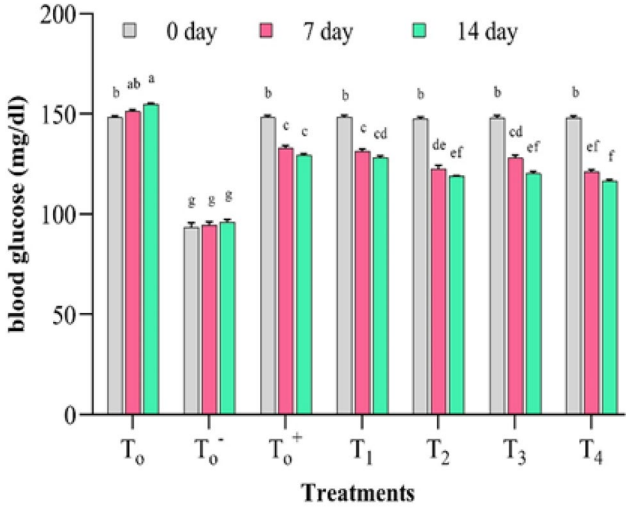


Fig. 2 Effect of days and treatments on serum glucose (mg/dl) of *Mus musculus* (10 mice/week). Different symbols (a–g) represent statistically significant variation among treatments at $P<0.05$

Microscope, Los Angeles, California, United states) at magnifications of 100 and 40X. The photomicrographs were processed in COREL DRAW 9 (Alludo (formerly Corel Cooperation, Ottawa, Ontario, Canada) to adjust color, sharpness and contrast, as well as for digital cropping, resizing, on-snap markings and background changes. These digitally improved photographs were then labeled and printed.

Micrometry

Micrometry was performed to estimate the count and diameter of primordial follicles, primary follicles, secondary follicles, tertiary follicles, corpus luteum and cysts. Two to four ovarian sections were examined under a light microscope (laboMed CXR2, Martin Microscope, Los Angeles, California, United states) at 40 × magnification equipped with an ocular micrometer. Follicular number was manually counted under the microscope and the diameter of each follicle was calculated by averaging two measurements taken at the broadest point of the follicle's cross-section (Myers et al. 2004; Griffin et al. 2006).

Data analysis

The experimental data were analyzed for normality of the distribution with Kolmogorov's test. All results were reported as mean ± standard error of the mean (SEM) with 10 replicates. General linear model (GLM), with 'days' and 'treatments' as independent variables was applied in order to compare the main effects and interactions. 1-way ANOVA (One-way analysis of variance) with multiple comparisons Tukey's test was selected to compare the significant differences among different treatment groups. Minitab software

18.1 (Minitab, LLC, State College, Pennsylvania, United States) was used to perform statistical analysis. Data were considered to be significant at $P<0.05$.

Institutional review board statement

All mice dealings were permitted by the Biosafety and Ethical Review Committee (BERC) (SU/ORIC/1802, 27 December 2022) at the University of Sargodha, Pakistan. Furthermore, all promising efforts were ensured to lessen the count of mice used and their distress.

Results and discussion

Percent change in body weight of albino mice (*Mus musculus*)

The percent change of body weight in *Mus musculus* was calculated to measure differences in body weight on days 0, 7 and 14 of exposure (Fig. 1). A significant change in body weight in *Mus musculus* was found due to days, treatments and their interaction, as shown in Table 2. The highest rise in body weight (12.38 ± 0.40%) of albino mice was found in T_0, while the lowest change in body weight (5.67 ± 1.04%) was observed in albino mice of T_4 on day 14 of exposure (Fig. 1). This shows that T_4 significantly ($P<0.05$) reduced body weight with time compared to T_0. T_0^+ showed a smaller decrease in body weight of albino mice (7.76 ± 0.09%) compared to T_4 (5.67 ± 0.10%) over time.

In the current research, a comparison was made between the body weight of normal control mice (T_0^-) and PCOS-induced (T_0) mice, which showed a highly significant change ($P<0.05$) due to days, treatments and their interaction. The highest increase in body weight (12.38 ± 0.40%) was observed in the albino mice of the T_0 group, while the lowest change in body weight (5.67 ± 1.04%) was found in the mice of the T_4 group (Fig. 1). The present study further concluded that the administration of MoLE (500 mg/kg b.w) in T_4 significantly reduced body weight compared to T_0 ($P<0.05$). The use of metformin at a dose of 250 mg/kg b.w. in T_0^+ gave rise to a decline in body weight of albino mice to a lesser extent (7.76 ± 0.09%) than T_4 (5.67 ± 0.10%) (Table 2).

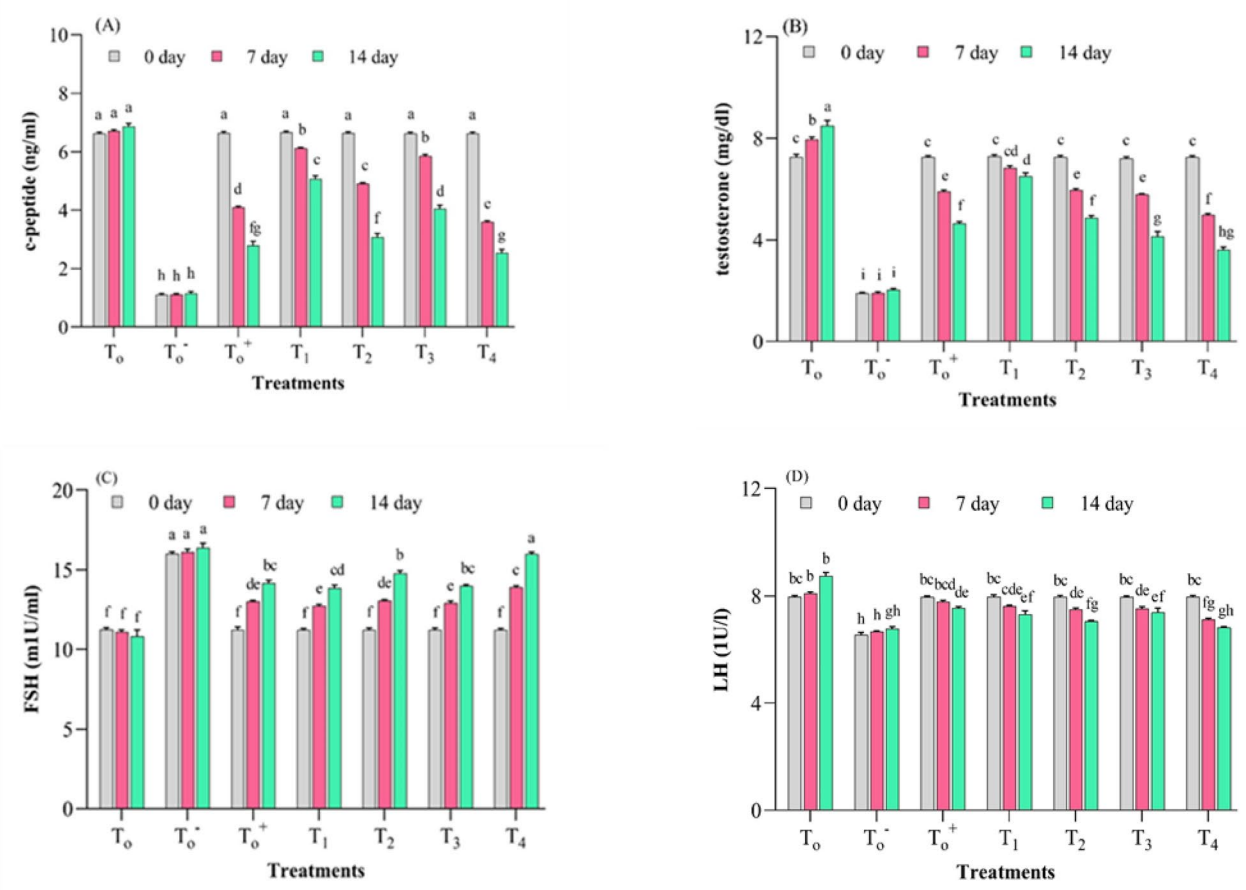


Fig. 3 Effect of days and treatments on (**A**) c-peptide (ng/mL), (**B**) testosterone (mg/dl), (**C**) FSH (mIU/mL) of albino mice, (**D**) LH (IU/l) of albino mice. Different symbols (a–h) indicate statistically significant variations among treatments at $P<0.05$

During this study, the mice were given daily injection of testosterone enanthate to induce PCOS. This treatment may lead to obesity and trigger insulin resistance. The same trend was found in PCOS model with insulin resistance, demonstrating significant weight gain in comparison to the control group. However, treatment with MoLE (500 mg/kg b.w.) leads to a significant decrease in weight gain when compared to the PCOS-insulin resistance control group (Amelia et al. 2018).

Previous studies have demonstrated that *M. oleifera* reduces obesity by redistributing and uptaking fat, likely due to the presence of quercetin. The findings of the current study showed that the use of *M. oleifera* leaf powder (MoLP), *M. oleifera* leaf extract (MoLE) and metformin (500 mg/kg b.w. for MoLP and MoLE, and 250 mg/kg b.w. for metformin), was more effective in curing PCOS in albino mice compared to other treatments. Unfortunately, patients using metformin report many side effects, such as epigastric pain and nausea (Kumar and Arora 2014). Therefore, there is a need for alternative treatment strategies for PCOS. Furthermore, metformin may not be effective in reducing body weight since it increases leptin hormone levels (Aguirre et al. 2011). Extracts rich in polyphenols, such as gallic and ferulic acids, are effective in anti-obesity activity (Boccellino and D'Angelo 2020). Supplementation with p-caumeric acid has shown potential in reducing mice's body weight to some degree (Xie et al. 2014). Moreover, this phenolic content may also have the ability to inhibit the onset of pathologies linked with hyperlipidemia. Moreover, this phenolic content has been demonstrated to possess antihypertensive activity (Fidelis et al. 2018).

Effect of treatments on serum glucose (mg/dl)

Albino mice in T_0 displayed a significant rise in serum glucose (mg/dl) over time compared to T_0^- (Table 2). The serum glucose of T_0^+ and all *M. oleifera* treated groups (T_1–T_4) were significantly lower than those of T_0 (Fig. 2). *Mus musculus* treated with T_4 and T_0^+ represented a significant decline in serum glucose over time as related to other treatments (Table 2 and Fig. 2). The highest serum glucose (154.7 ± 0.72 mg/dl) was found in T_0 on day 14, while the lowest was noted in T_4 (116.8 ± 0.57 mg/dl) compared to T_0^+ (129.6 ± 0.65 mg/dl).

Albino mice treated with testosterone enanthate (35 days) exhibited a significant rise in serum glucose. Additionally, albino mice in the T_0 exhibited a significant increase in serum glucose related to the T_0^- group ($P<0.05$). Hyperinsulinemia may perhaps enhance the chances of the pathogenesis of PCOS in human females by affecting hormone secretion, disrupting follicle formation and menstrual cycle, and may lead to more risk of high blood pressure and type-2 *diabetes mellitus* (T2DM) (Chitra and Dhivya 2017).

Mice T_4 and T_0^+ groups exhibited a definite fall in blood glucose levels (Fig. 2). The highest serum glucose level (154.7 ± 0.72 mg/dl) was found in T_0, while the lowest level was noted in T_4 (116.8 ± 0.57 mg/dl) compared to T_0^+ (129.6 ± 0.65 mg/dl). This might be due to the bulk of flavonoids and phenols present in moringa. The extracts of *M. oleifera* leaves are involved in improving insulin sensitivity in diabetic mice (Tuorkey 2016). According to the current research effort, in animals treated with moringa, glucose levels decreased by 1.28-fold likened to the diabetic group. The efficacy of moringa leaf extract to improve insulin sensitivity in mice may be linked to its antihyperglycemic and antioxidant properties.

In a previous study, *M. oleifera* leaves extract and metformin significantly reduced insulin resistance seems paralleled to the PCOS-induced mice ($P<0.05$), which is consistent with the current study's results (Amelia et al. 2018).

Plants can modulate hepatic genes that regulate insulin signaling and modify insulin resistance, thereby maintaining glucose levels (Mohamed et al. 2019). Studies have reported that tablet forms or aqueous extracts of *M. oleifera* leaves have significant antidiabetic potential in rats and humans with diabetes (Zade et al. 2013; Yassa and Tohamy 2014). Additionally, its ethanol extract has been shown to reduce serum glucose in diabetic rats (Tende et al. 2011). In the previous study, *M. oleifera* leaves extract and metformin significantly reduced insulin resistance seems paralleled to the PCOS-induced mice ($P<0.05$), which is consistent with the current study's results (Amelia et al. 2018).

Effect of treatments on hormonal assays

Serum c-peptide (ng/mL)

The PCOS-induced mice fed with metformin and *Moringa* clearly revealed a significant decline in serum c-peptide (ng/mL), as depicted in Table 2 and Fig. 3A. The highest c-peptide was recorded in T_0 (6.86 ± 0.11 ng/mL) whereas T_4 exhibited the least serum c-peptide (2.54 ± 0.11 ng/mL), and T_0^+ reduced serum c-peptide (2.78 ± 0.16 ng/mL) compared to the control, T_0^- (1.15 ± 0.05 ng/mL), as depicted in Fig. 3A. All tested factors (i.e., treatments and exposure time, individually and their interactions) were significantly different in all treatment groups (Table 2).

Serum testosterone (mg/dL)

Serum testosterone (mg/dL) rose significantly over time ($P<0.05$) in diseased mice compared to control (T_0^-) (Table 2 and Fig. 3B). Statistical data reported a mark

significant ($P<0.05$) decline in testosterone over time in mice fed with metformin and *Moringa,* as given in Table 2 and Fig. 3B. Serum testosterone was found to be highest in T_0 (8.49±0.19 mg/dl) and least in T_4 (3.60±0.11 mg/dl) as compared to the control T_0^- (2.03±0.04 mg/dl) and as shown in Fig. 3B.

Serum follicle stimulating hormone (m1U/mL)

Serum FSH (m1U/mL) varied significantly ($P=0.00$) in albino mice due to treatments, days and interaction (Table 2). The highest serum FSH was recorded in T_4 (15.99±0.11 mlU/mL), whereas T_0 exhibited the lowest serum FSH (10.82±0.39 mlU/mL). T_0^+, as a standard, showed a rise in serum FSH (14.16±0.21 mlU/mL) on day 14 as given in Fig. 3C.

Serum luteinizing hormone (IU/l)

The highly significant ($P<0.05$) reduction in serum LH was found in *Mus musculus* fed with *M. oleifera and* metformin due to days, treatments and interaction (Table 2). PCOS-induced mice T_0 showed a significant rise in serum LH over time (Fig. 3D). The highest serum LH, in the case of T_0 (8.73±0.14 lU/l), and lowest, in T_4 mice (6.81±0.03 lU/l), was recorded on day 14. Albino mice treated with T_0^+ and T_2 represent a low level of LH with time (7.55±0.06 lU/l) and (7.04±0.041 U/l), respectively.

Data indicated that T_4 treated albino mice represented a sharp ($P<0.05$) rise in serum FSH levels and a decrease in c-peptide, testosterone and LH levels. PCOS is a prevalent hormonal malady in gynecology, categorized by persistent anovulation, high levels of androgens, and polycystic changes in the ovaries. It frequently co-occurs with insulin resistance and obesity and affects 15–20% of women worldwide (Cassar et al. 2016). Generally, FSH leads to the maturation of oocytes in follicles and LH stimulates ovulation. However, in PCOS, a neuroendocrine abnormality can cause an increase in gonadotropin-releasing hormones, resulting in an imbalance in LH and FSH levels. This imbalance leads to a rise in LH/FSH ratio in circulation (Banaszewska et al. 2003).

Studies on mice models have revealed that hormonal changes linked with PCOS in different species are characterized by elevated levels of LH, testosterone and reduced levels of FSH (De Roux et al. 2003). Further, numerous studies demonstrated that controlling insulin sensitivity can lead to a decrease in androgen secretion (Cataldo et al. 2006). The present study unfolded that the treatment with T_4 revealed a significant rise in serum FSH ($P<0.05$) and a sharp decline in testosterone, c-peptide and LH levels in albino mice (Fig. 3).

In the current research, *Mus musculus* were used as a model to develop the PCOS phenotype. Polycystic ovarian syndrome is one of the major causes of infertility. Human females with PCOS have more chances of getting metabolic disorders, type-2 *diabetes mellitus*, obstetric abnormalities, ovarian cancers, oxidative stress, and mood swings. These women are also at risk of cardiovascular diseases. Important to emphasize that studies showed that there is no genetic involvement exists between obesity and PCOS (Zhao et al. 2014; Daan et al. 2014).

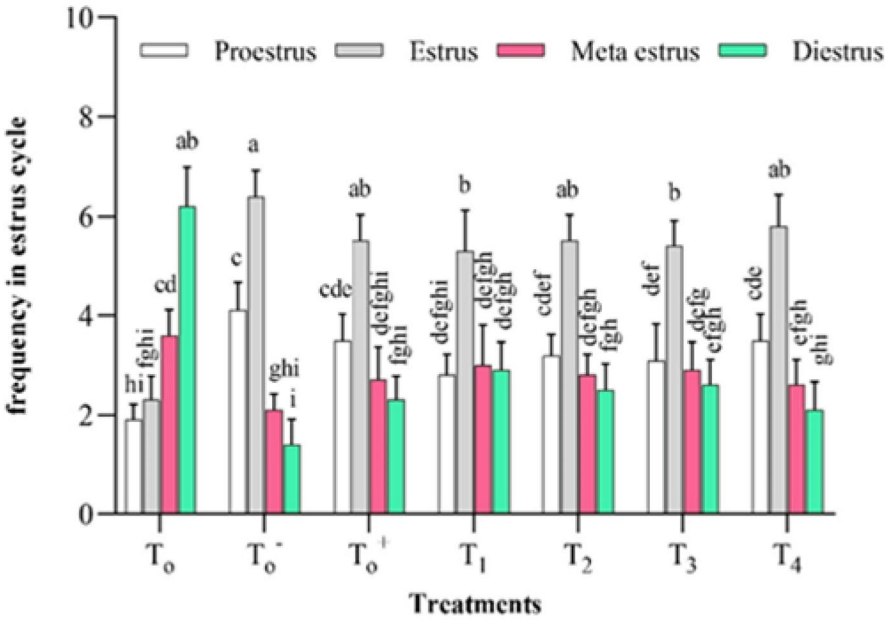


Fig. 4 Effect of stages and treatments on the estrus cycle of *Mus musculus*. Different symbols (a–i) indicate statistically significant differences among treatments at $P<0.05$

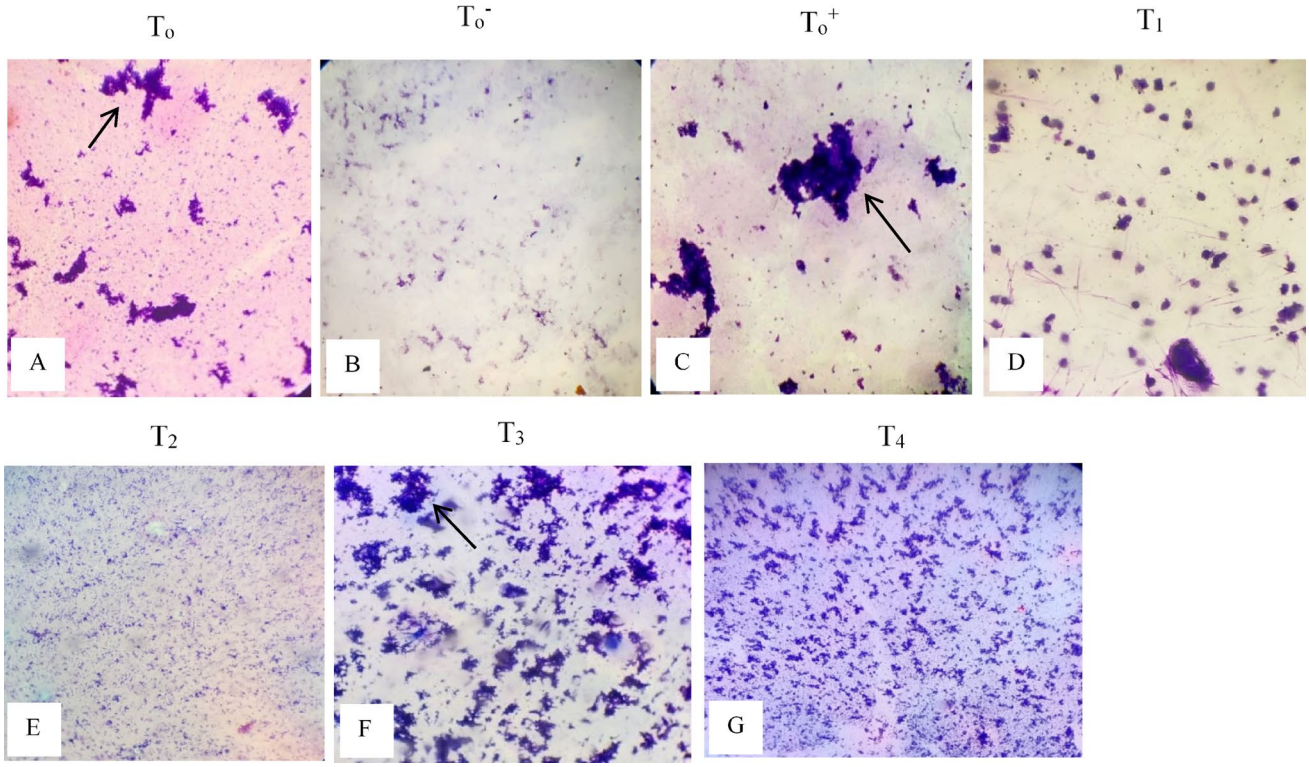


Fig. 5 Vaginal smears of proestrus from albino mice (*Mus musculus*), categorized by massive count of minute nucleated epithelial cells in control (**A**), treatment groups (**E**, **G**) showing epithelial strand (arrow), *i.e*. typical of proestrus. PCOS induced mice (**B**) showing cells resemble to diestrus stage. Giemsa staining at 40 x

The levels of C-peptide and glucose in the body have a positive correlation. C-peptide is released alongside insulin in equivalent amounts and is not eliminated by the liver (Cobelli et al. 2007). It is metabolically cleared at a constant rate and can be found in a wide range of serum concentrations. The highest serum c-peptide level was recorded in T_0 (6.86±0.11 ng/mL), while T_4 exhibited comparatively lower serum c-peptide level (2.54±0.11 ng/mL). T_0^+ also showed reduced serum c-peptide levels (2.78±0.16 ng/mL) compared to the control, T_0^- (96±1.25 ng/mL) (Fig. 3).

Previous studies have indicated that *M. oleifera* may reduce blood insulin and androgen levels, resulting in increased folliculogenesis in women with PCOS (Amelia et al. 2018). C-peptide is linked to the type and duration of diabetes, and levels below 0.2 nmol/L are linked with type-1 *diabetes mellitus* (T1DM) and other complications related to glycemic control. It is considered as a biologically inactive peptide and is primarily used as a surrogate marker for insulin (Amelia et al. 2018). On the other hand, another study showed no significant differences in C-peptide levels between healthy women and those with PCOS. Furthermore, no noteworthy associations were observed with FSH and LH levels. However, this study did report a negative correlation between C-peptide and glucose levels, and a positive correlation was observed in the overweight PCOS group, highlighting a multifaceted relationship (Leighton et al. 2017).

Several studies have suggested that the leading cause of PCOS is high androgen secretion in the ovary as well as in the adrenal glands (Azziz et al. 2006; Wehr et al. 2009). Elevated levels of testosterone have been linked to several metabolic instabilities, such as glucose intolerance, insulin resistance, obesity, and abdominal fat deposition. Moreover, hyperandrogenism is a significant factor used to promote PCOS and ovulatory dysfunction in both rodent models and women with PCOS (Li et al. 2020). In this study, serum testosterone were found to be higher in T_0 (8.49±0.19 mg/dl) and lower in T_4 (3.60±0.11 mg/dl) as compared to the control group T_0^- (2.03±0.04 mg/dl) mentioned in Fig. 3.

In the current study, T_0 represented least FSH (10.82±0.39 mIU/mL) after 14 days of PCOS induction treatment. A significant ($P<0.05$) rise in serum FSH was observed in albino mice due to days, treatments, and interactions. All medicinal treatment groups showed an increased FSH level, but the most significant rise was observed in the case of T_4 mice (15.99±0.11 mIU/mL), while T_0 exhibited the least serum FSH (10.82±0.39 mIU/mL). T_0^+ as a standard showed a rise in serum FSH (14.16±0.21 mIU/mL). A study was conducted on the LH/FSH ratio in PCOS women, and it was found that in non-PCOS women, the ratio LH/

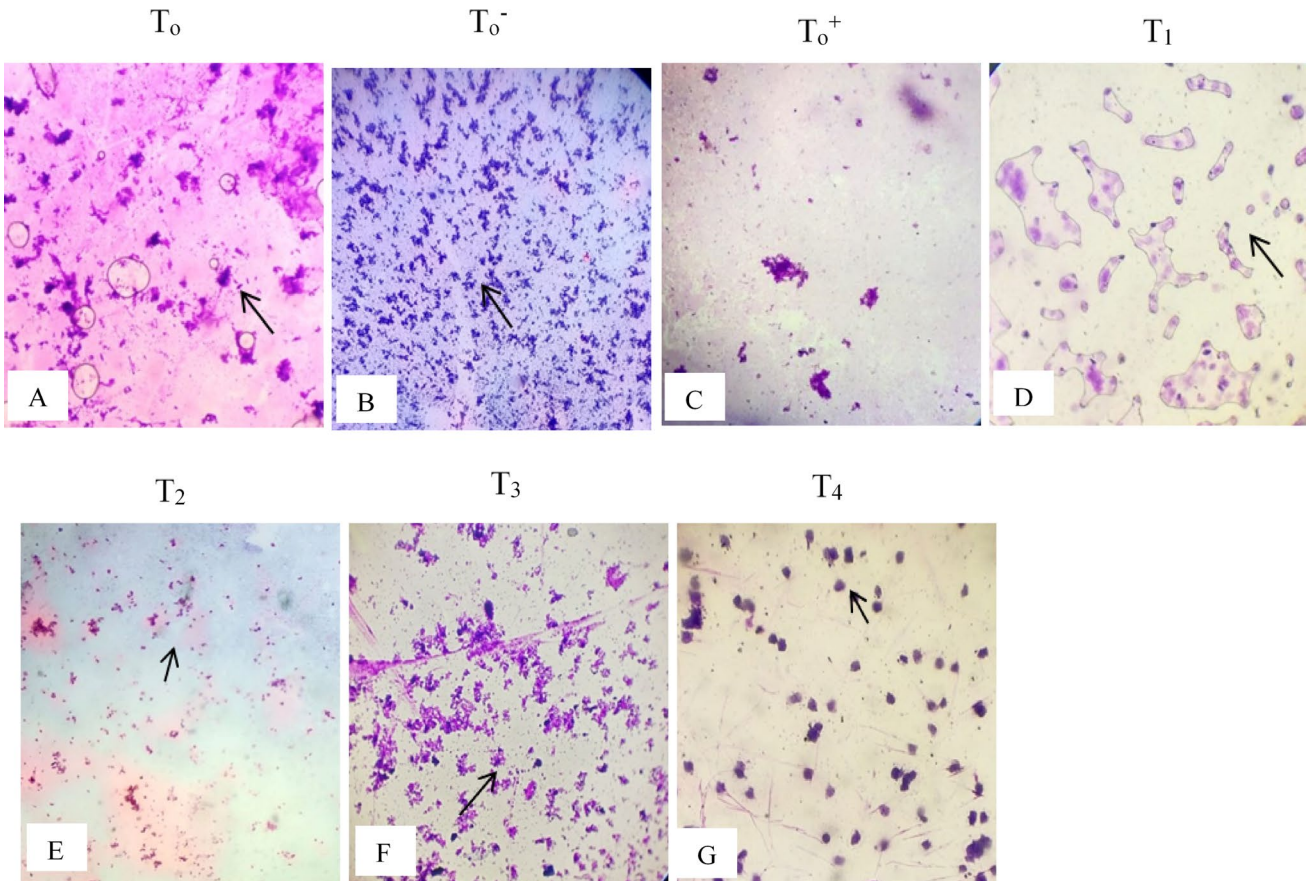


Fig. 6 Illustrative photomicrographs of first two phases of estrus cycle of albino mice (*Mus musculus*). Control (**A**) and treatment groups (**C**–**G**) are characterized by small anucleated epithelial cells (arrow) that may arrange in loose clusters reminiscent of proestrus. PCOS induced mice (**B**) showed irregular estrus most resembled to diestrus phase. Giemsa staining at 40 x

FSH typically falls between 1 and 2. However, in cases of PCOS, the ratio is often reversed and can increase as high as 2 or 3. This may be due to ovarian estrogen levels that cause an abnormal feedback mechanism, leading to elevated LH levels (Saadia 2020).

The current study showed a highly significant reduction in serum LH in albino mice due to days, treatments, and their interactions ($P<0.05$). The results indicate that T_0 mice had the highest serum LH levels (8.73 ± 0.14 IU/l), while the lowest levels were observed in T_4 mice (6.81 ± 0.03 IU/l) (Fig. 3, Table 2). A previous study has also shown that phytonutrient-rich herbal extracts can regulate hormone levels in women with PCOS by affecting the hypothalamus-pituitary axis, leading to the regulation of hormone levels, decreased levels of LH, testosterone and the LH/FSH ratio (Darabi et al. 2020). Another study found that the prevalence of LH/FSH ranged from 35 to 77% in PCOS patients. BMI, hyperandrogenism, and infertility showed a strong correlation with LH levels, but no correlation was observed between LH and insulin resistance or PCOS (Kadhim 2017; Lal et al. 2017).

Vaginal cytology

The examination of vaginal swabs from all groups was conducted and a significant difference ($P<0.05$) in the stages of the estrous cycle was found, as depicted in Table 2. In T_0 albino mice, the cycle had stopped in the diestrus phase, whereas the T_0^- group exhibited a regular estrus cycle. The T_2 and T_4 treated albino mice showed a regular estrus cycle compared to T_0^+, as shown in Fig. 4.

Furthermore, Fig. 5 shows vaginal smears from albino mice in proestrus, categorized by massive count of minute nucleus containing epithelial cells and (images A, E and G from Fig. 5), showing typical epithelial strands of proestrus.

Figure 6 shows the vaginal smears of albino mice treated with moringa and metformin, representing the first and second phases of estrus, while the PCOS-induced mice (B) show cells, representing the diestrus stage. Small anucleated epithelial cells scattered in slack masses indicates a significant proestrus. Metestrus comprises neutrophils intermingled or clunked along the anucleated surface cells (images A and C-G of Fig. 7) and irregular nucleated surface cells may also be visible at some stages. A rise in neutrophil

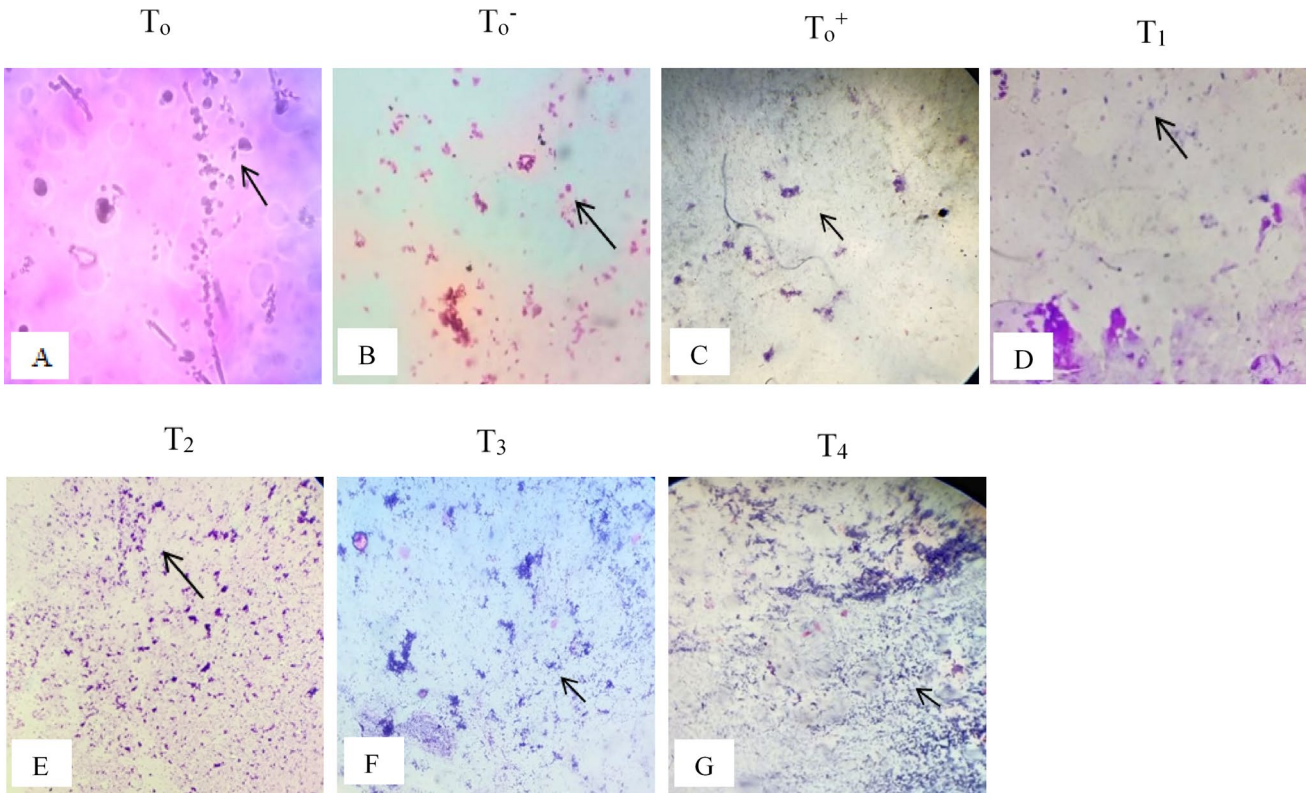


Fig. 7 Metestrus smears from control, PCOS and several treatments in albino mice (*Mus musculus*). Metestrus comprise appearance of neutrophils (arrow) interspersed or clumped among the anucleated epithelial cells (**A**, **C**, **D**, **E**, **F**, **G**), irregular nucleated epithelial cells observed at some stage, neutrophil number rise subsequently in massive cellular smears (**C**, **G**). PCOS induced mice (**B**) showed irregular metestrus most resembled to diestrus phase. Giemsa staining at 40 x

count appear in smears (images C and G of Fig. 7), whereas PCOS-induced mice (image B of Fig. 7) showed irregular metestrus, which most resembles the diestrus phase (Fig. 8). Diestrus is considered with a medium to few cells with chiefly arranged neutrophils together with a less count of epithelial cells (images A and C-G of Fig. 8), however, few clusters of round epithelial cells are visible in (images A-G of Fig. 8), as presented in Fig. 8.

The vaginal swab examined before treatment indicated that all albino mice were in the estrus phase and following the normal estrus cycle. After injecting testosterone enanthate (1 mg/100 g b.w.) intramuscularly (35 days), all mice were found to be arrested in the diestrus phase. This result indicates that the injection of testosterone could arrest the estrus cycle in the diestrus phase (Kalhori et al. 2018). After 14 days of treatment, vaginal swabs were taken from all groups and examined. T_0 albino mice showed a significant change in their estrus cycle, transitioning from the diestrus phase (6.2±0.24 days) to the estrus phase (2.3±0.15 days). In contrast, the normal control mice T_0^- maintained a regular estrus cycle, with a diestrus phase of 1.4±0.22 days and an estrus phase of 6.4±0.16 days. The PCOS-induced mice treated with T_2 and T_4 exhibited a regular estrus cycle, with an estrus phase of 5.5±0.17 days and 5.8±0.2 days, respectively, compared to the Metformin T_0^+ group (5.5±0.17 days). It appears that the treatment with T_2 and T_4 Improved estrus cycle of PCOS-induced mice, as they exhibited a regular cycle similar to the non-PCOS (Figs. 4, 5, 6, 7 and 8).

The above-mentioned photomicrographs provide a clear visual representation of the histological changes that occur during the estrus cycle. Each stage illustrated the progression of estrus cycle.

Stereological analysis of ovarian tissues

Change (%) in ovary weight

A stereological examination of ovaries of T_0 (−8.35±0.14%) exhibited a significant size reduction compared to T_0^- (5.55±0.08%) as shown in Fig. 9. Microscopic analysis of the ovaries at T_0 revealed anovulation, none corpus luteum, more cysts and a massive count of primary and preantral follicles. Albino mice treated with T_0^+, T_2 and T_4 presented a highly significant ($P<0.05$) rise in ovarian size over time, as shown in Table 2. Treatment with T_4 and T_0^+ ensued with a significant rise in wet ovary weight (3.02±0.05% and 2.60±0.08%, respectively) on day 14 compared to

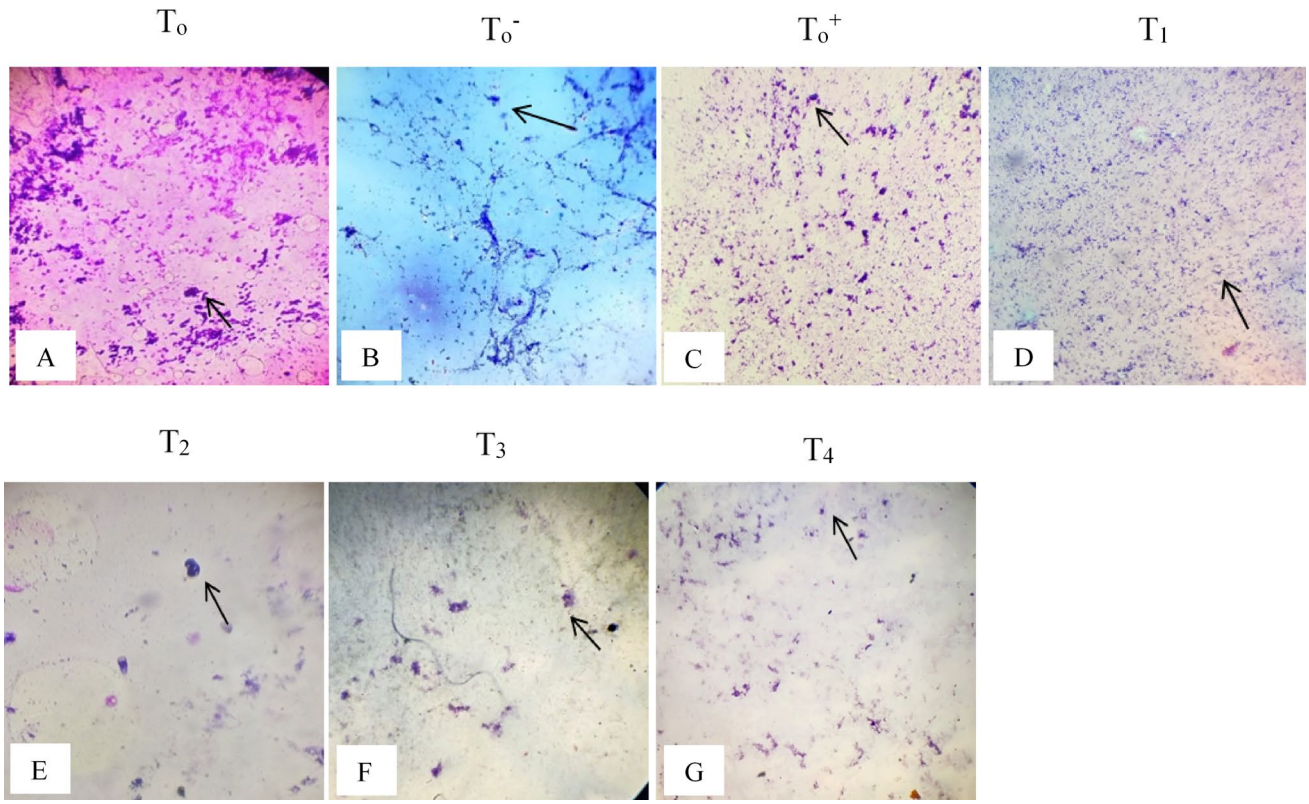


Fig. 8 Diestrus vaginal smears from control, PCOS and several treatments in albino mice (*Mus musculus*). Diestrus is characterized by a medium to less cellular smears with chiefly arranged neutrophils together with less count of epithelial cells (**A**, **C**–**G**), however a minute cluster of round surface cells (arrow) is visible in (**A**–**G**). PCOS induced mice (**B**) showed regular diestrus phase. Giemsa staining at 40 x

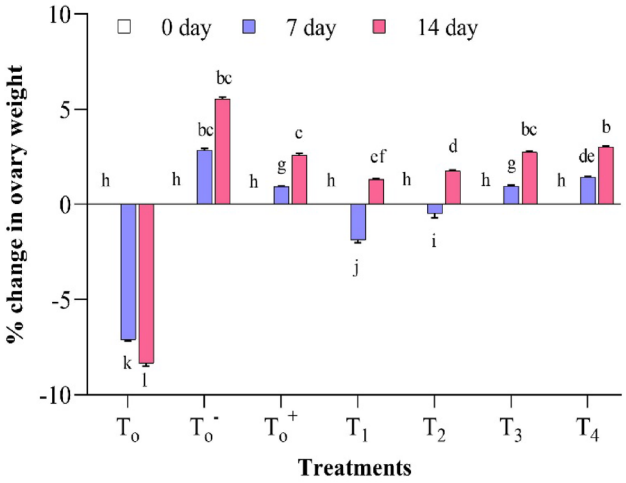


Fig. 9 Effect of days and treatments on % change in ovary weight (g) of albino mice. Different symbols (a–k) represented statistically significant variations among treatments at $P<0.05$

PCOS-induced mice in T_0 (−8.35±0.14%), as shown in Fig. 9.

Stereological study of the ovaries of T_0 (−8.35±0.14%) revealed a significant reduction in size compared to T_0^- (5.55±0.08%). The reduced ovaries in T_0 exhibited anovulation, having no *corpus luteum*, more cysts and more primary and preantral follicular count. *Mus musculus* fed with T_0^+, T_2 and T_4 presented a highly significant increase in size ($P<0.05$). T_4 and T_0^+ fed mice exhibited a significant increased wet ovary weight, measuring 3.02±0.05% and 2.60±0.08%, respectively.

Number of follicles

The analysis of variance of primordial, primary, secondary, tertiary, *corpus luteum* and cysts has been given in Table 3, which indicates a significant variation ($P<0.05$) in follicle count among different treatment groups. The means and SEM values of follicle counts are presented in Table 4. The microscopic study demonstrated that albino mice with PCOS (T_0) had the lowest *corpus luteum* (0.90±0.87), indicating anovulation. Furthermore, a low number of secondary follicles (3.50±1.17) and tertiary follicles (2.60±1.17) were found in T_0, with an increased number of cystic follicles (12.00±3.16) were observed compared to T_0^-. Diseased mice fed with T_2 and T_4 indicated a significant increase ($P<0.05$) tertiary follicular count given in Table 4.

Table 3 Analysis of variance indicating the significance of treatments on follicle count and diameter (µm) of primordial, primary, secondary, tertiary, *Corpus luteum* and cysts

Parameters	Follicle Count					Follicle diameter				
	Df	SS	MS	F	*P*	Df	SS	MS	F	*P*
Primordial	6	42.57	7.0952	10.28	0.00**	6	14,731,466	2,455,244	12.61	0.00**
Error	63	43.50	0.6905			497	96,734,940	194,638		
Total	69	86.07				503	111,466,405			
Primary	6	22.74	3.7905	4.33	0.00**	6	86,296,064	14,382,677	66.45	0.00**
Error	63	55.20	0.8762			497	107,569,228	216,437		
Total	69	77.94				503	193,865,293			
Secondary	6	29.17	4.8619	6.46	0.00**	6	5,294,116,802	882,352,800	858.89	0.00**
Error	63	47.40	0.7524			497	510,575,565	1,027,315		
Total	69	76.57				503	5,804,692,367			
Tertiary	6	229.77	38.295	28.72	0.00**	6	10,694,131,993	1,782,355,332	2237.28	0.00**
Error	63	84.00	1.333			497	395,941,050	796,662		
Total	69	313.77				503	11,090,073,044			
Corpus luteum	6	225.49	37.5810	66.51	0.00**	6	15,200,294,531	2,533,382,422	1149.59	0.00**
Error	63	35.60	0.5651			497	1,095,252,236	2,203,727		
Total	69	261.09				503	16,295,546,767			
Cyst	6	953.1	158.857	74.24	0.00**	6	2,973,776,657	495,629,443	5412.36	0.00**
Error	63	134.8	2.140			497	45,512,096	91,574		
Total	69	1087.9				503	3,019,288,752			

Table 4 Mean values indicating the effect of treatments on follicular count of *Mus musculus*

Treatments	Primordial follicles	Primary follicles	Secondary follicles	Tertiary follicles	*Corpus luteum*	Cysts
T_O	4.50 ± 1.08^{b}	5.20 ± 0.78^{b}	3.50 ± 1.17^{b}	2.60 ± 1.17^{d}	0.90 ± 0.87^{d}	12.00 ± 3.16^{a}
T_O^-	7.00 ± 1.05^{a}	7.00 ± 1.05^{a}	5.60 ± 0.96^{a}	8.50 ± 1.08^{a}	6.70 ± 0.94^{a}	0.00 ± 0.00^{e}
T_O^+	6.70 ± 0.94^{a}	6.00 ± 1.15^{ab}	4.20 ± 0.78^{b}	5.90 ± 1.44^{b}	5.00 ± 1.05^{c}	4.00 ± 0.94^{bc}
T_1	6.40 ± 0.51^{a}	5.60 ± 0.51^{b}	3.60 ± 0.69^{b}	4.10 ± 0.73^{cd}	5.50 ± 0.52^{bc}	5.00 ± 0.94^{b}
T_2	6.60 ± 0.69^{a}	5.80 ± 0.63^{ab}	3.90 ± 0.87^{b}	5.0 ± 1.05^{bc}	5.80 ± 0.63^{abc}	1.40 ± 1.17^{de}
T_3	6.50 ± 0.52^{a}	5.20 ± 1.03^{b}	4.00 ± 0.66^{b}	3.40 ± 0.96^{d}	5.60 ± 0.51^{bc}	3.00 ± 0.81^{cd}
T_4	6.80 ± 0.78^{a}	6.00 ± 1.15^{ab}	4.20 ± 0.78^{b}	5.90 ± 1.44^{b}	6.30 ± 0.48^{ab}	1.40 ± 1.07^{de}

Mean values carrying same letters in a row are not significantly different

T_O = Standard feed + 5% Tween 80

T_O^- = Standard feed

T_O^+ = Standard feed + metformin @ 250 mg/kg b.w

T_1 = Standard feed + MOLP @ 250 mg/kg b.w

T_2 = Standard l feed + MOLP @ 500 mg/kg b.w

T_3 = Standard feed + MOLE @ 250 mg/kg b.w

T_4 = Standard feed + MOLE @ 500 mg/kg b.w

MoLP = *M. oleifera* leaf powder

MoLE = *M. oleifera* leaf extract

T_O^- is non-PCOS induced while others are PCOS induced

ANOVA (analysis of variance) revealed a significant difference ($P < 0.05$) in the follicle count among different treatment groups, including primordial, primary, secondary, tertiary, corpus luteum, and cysts. The microscopic study of albino mice with PCOS (T_0) revealed that they had the least corpus luteum count (0.90 ± 0.87), indicating anovulation, and a lower number of secondary follicles (3.50 ± 1.17) and tertiary follicles (2.60 ± 1.17). In contrast, the cyst count was higher (12.00 ± 3.16) in T_0 than in T_0^- (Tables 3 and 4). The study also found that hyperandrogenism accelerated early follicular growth, resulting in a higher count of primary and preantral follicles matched with the results of previous studies (Diamanti-Kandarakis 2008; Diamanti-Kandarakis et al. 2010).

Many animal experiments investigating the effects of quercetin on PCOS have shown that it may reduce insulin, testosterone, LH, and circulating lipids, improving the development of regular folliculogenesis and corpus luteum (Diamanti-Kandarakis et al. 2010).

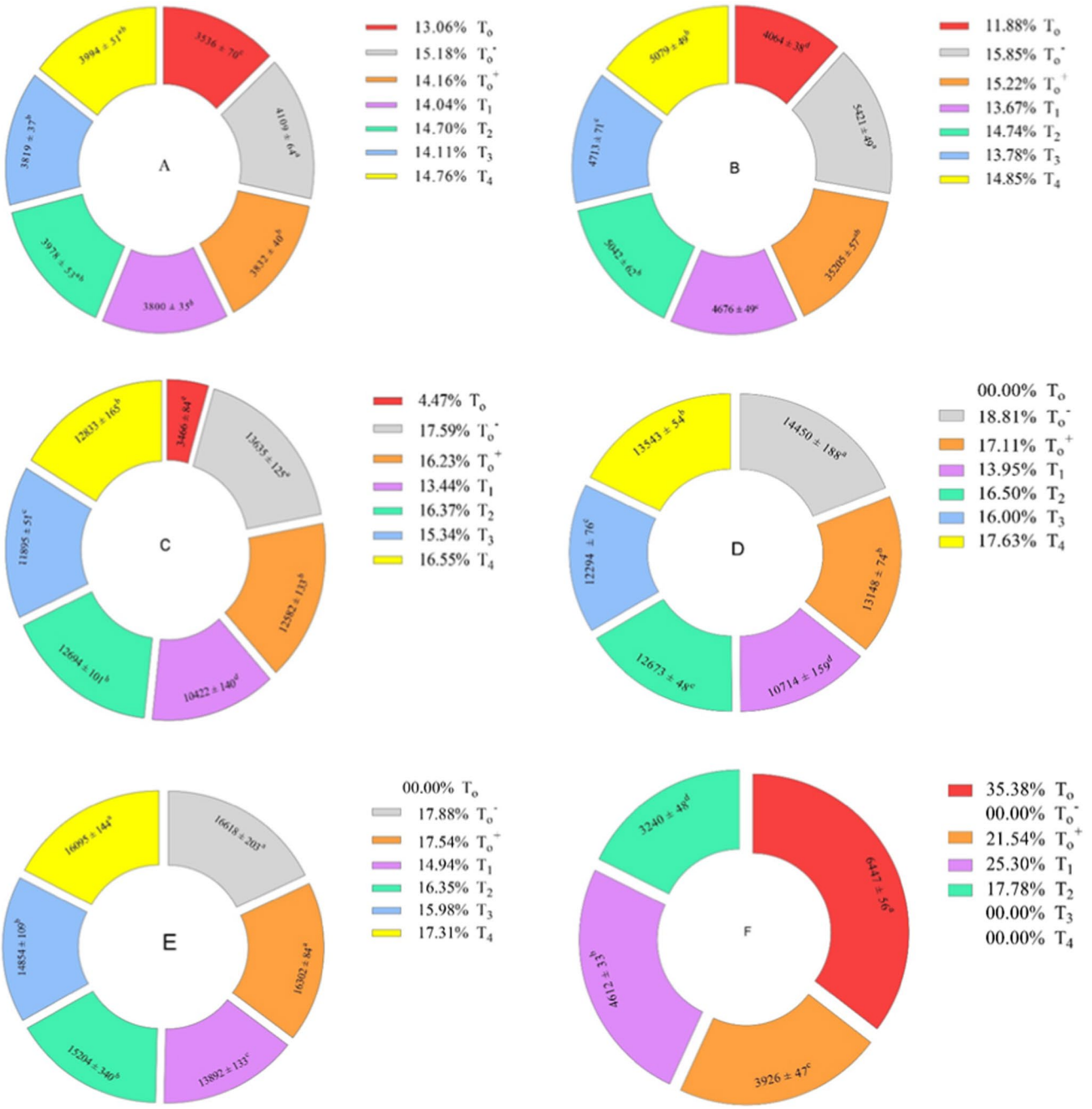


Fig. 10 Effect of treatments on diameter (µm) of (**A**) primordial follicles, (**B**) primary follicles, (**C**) secondary follicles, (**D**) tertiary follicles, (**E**) *Corpus luteum* and (**F**) cysts of albino mice. Different symbols (a–d) represented the statistically significant variations among treatments at $P<0.05$

Stereological examination of the ovaries of T_0 showed a significant reduction in size compared to T_0^-. This shrinkage was caused by decreased folliculogenesis in T_0 compared to T_0^-. In T_0, the weight and volume of the ovary decreased due to an increase in atretic follicle count, less antral follicle count, absence of corpus luteum, and ovary atrophy (Ikeda and Yamada 2014). However, an increase in androgen secretion can cause oxidative stress, ovary apoptosis, and follicular atresia, which may explain the reduction in ovary weight and volume (Rezvanfar et al. 2014). The current study supported that mice administered with T_4 led to an increase in wet ovary weight. Treatment with T_4 and T_0^+ resulted in an increased folliculogenesis and more ovary weight (Fig. 9).

In a study conducted on mice ovarian tissue, a significant reduction in the follicular count and diameter was observed in the PCOS group related to the control group. However, treatment with metformin and *M. oleifera* leaf extract (250 and 500 mg/kg b.w.) led to a significant increase in

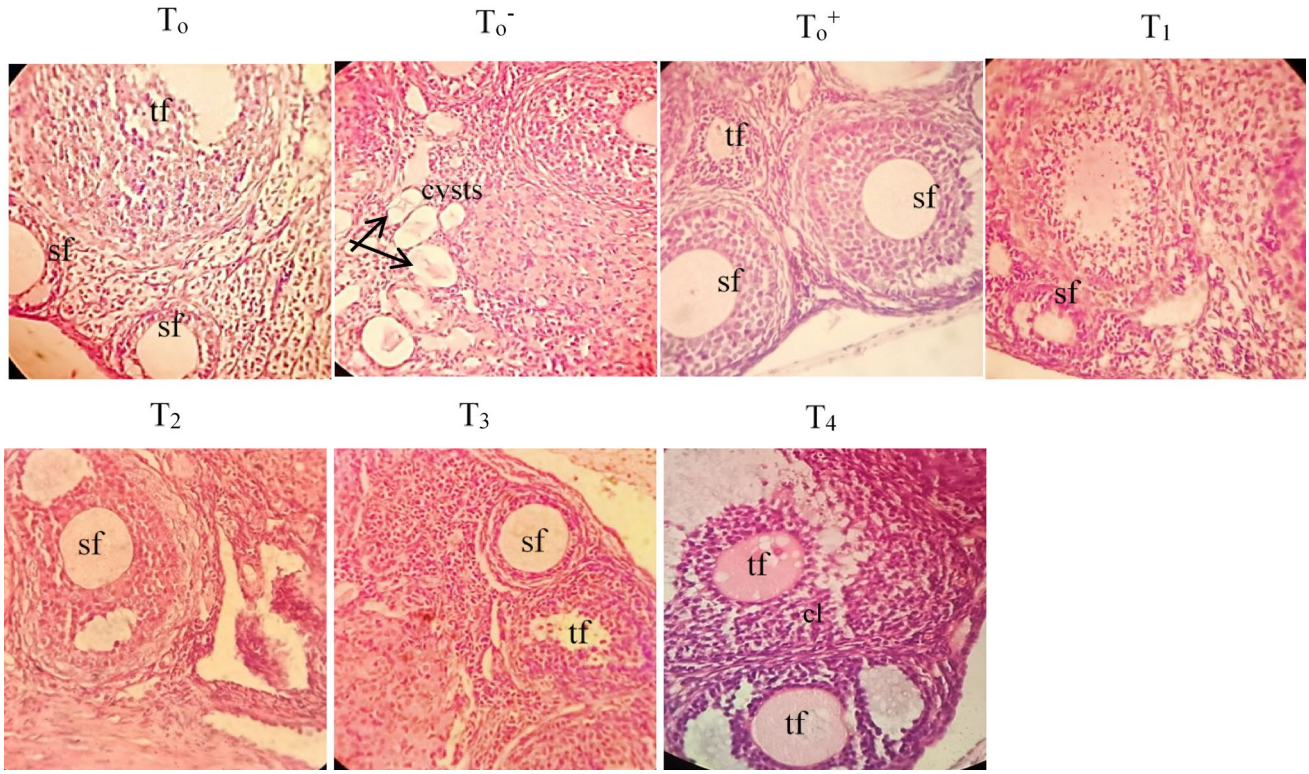


Fig. 11 Photomicrograph of ovary section of control, PCOS and treatment groups. Control (**A**) and treatment groups (**C–G**), Illustrates development of secondary follicles (SF), *Corpus luteum* (CL), and tertiary follicles (TF), showing recovery of PCOS in albino mice. PCOS group (**B**) however, showed appearance of cysts and reduction in follicle numbers

folliculogenesis compared to the PCOS group[21]. In the present study, albino mice with induced PCOS fed with T_2 and T_4 indicated a significant increase ($P<0.05$) in tertiary follicle count.

Diameter (μm) of follicles

Percent changes in the diameters of primordial, primary, secondary and tertiary follicles, *corpus luteum*, and cysts are presented in Fig. 10A–F. In the T_0 group, the diameter of primordial (3536.4±598.2 μm), primary (4064.7±324.6 μm) and secondary follicles (3466.3±715.2 μm) were lower than that of T_0^- group, which had larger primordial (4109.2±547.1 μm), primary (5421.1±416.2 μm), and secondary follicles (13,635±1061 μm). Tertiary follicles were absent in the T_0 (anovulation), whereas the T_0^- group had larger tertiary follicles (14,450±1594 μm). *Corpus luteum* were absent in the ovaries of the T_0 group, unlike the T_0^- (16,618±1719 μm). Finally, the T_0 mice had more cysts in ovaries (6447.8±480.8 μm) than the T_0^-, as shown in Fig. 10F. However, PCOS-induced mice treated with T_0^+, T_2, and T_4 showed a significant increase ($P=0.00$) in the diameters of tertiary follicles (Table 3). Photomicrographs of ovary sections (photographs A, C-G in Fig. 11) illustrate the development of primary follicles, secondary follicles, tertiary follicles and *Corpus luteum* (Fig. 11). The PCOS group (photograph B in Fig. 11) showed the presence of cysts and the absence of ovulatory follicles.

The diameter of primordial (3536.4±598.2 μm), primary (4064.7±324.6 μm) and secondary follicles (3466.3±715.2 μm) in the T_0 group was observed to be smaller than that of the T_0^- group's primordial (4109.2±547.1 μm), primary (5421.1±416.2 μm) and secondary follicles (13,635±1061 μm). Tertiary follicles were absent in T_0 (anovulation) compared to T_0^- (14,450±1594 μm). No *Corpus luteum* was reported in T_0 group, unlike T_0^- (16,618±1719 μm) (Figs. 10 and 11). T_0 mice ovaries exhibited more cysts (6447.8±480.8 μm) than T_0^-. However, T_0^+, T_2 and T_4 treatment groups showed a significant increase ($P<0.05$) in tertiary follicle diameter. A study conducted on rats treated with testosterone and found an increase count of cystic follicles, preantral and antral follicles, and decrease count of primary and tertiary follicles (Tehrani et al. 2014). As a matter of fact, the current study indicated that with an elevated level of testosterone, a significant reduction is found in the number of healthy follicles and *Corpus luteum*, while the number of atretic follicles increases (Manneras et al. 2009).

Conclusion

In conclusion, the data of our current research suggest that leaf powder and extract of *Moringa oleifera* has unmatched therapeutic efficacy for handling polycystic ovary syndrome and infertility related issues. *M. oleifera* was found to improve PCOS indicative biomarkers, such as body weight, blood glucose, c-peptide, testosterone, FSH, LH and ovary weight. The regular estrus cycle resumed and the number of cysts reduced while *Corpus luteum* increased. Furthermore, the study revealed that *M. oleifera* leaf extract (500 mg/kg b.w.) in female albino mice was more suitable in ameliorating PCOS symptoms compared to metformin (250 mg/kg b.w.). Overall, this study importantly supported the efficacy of *M. oleifera* leaf powder and extract for treating PCOS and infertility in human female.

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Data availability The data that support the findings of this study are available on request from the corresponding author.

Declarations

Ethical approval All experimental procedures involving mice were approved by the Biosafety and Ethical Review Committee (BERC) at the University of Sargodha, Pakistan (Approval No. SU/ORIC/1802, dated 27 December 2022). All efforts were made to minimize the number of animals used and to reduce their suffering in accordance with ethical guidelines.

Competing interest The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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