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Mormodica charantia L. Leaf Alleviates Menstrual Cycle Disruption and Hormonal Fluctuations In Letrozole-Induced Polycystic Ovarian Syndrome Rat

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ABSTRACT

Polycystic ovarian syndrome affects 7 to 10% of women. To manage PCOS in women without lowering the likelihood of becoming pregnant, researchers are looking for botanicals with antioxidant potential, optimum bioavailability, efficacy, and safety. Therefore, this research investigated the effect of *Mormodica charantia* leaves on PCOS in rats. The toxicity of the crude extract was evaluated using Brine shrimp larva. The total phenolic and flavonoid contents (TPC and TFC), as well as the antioxidant activity of the extract and solvent fractions (n-hexane, DCM, and ethyl acetate), were evaluated using established methods. Methanol extract and clomiphene citrate were administered to the letrozole-induced PCOS albino rat. The methanol extract's LC_{50} value was 1818.79±0.22 µg/mL. The hexane fraction had a significant antioxidant activity (IC_{50} = 37.06±0.11 µg/mL). The methanol extract reduced the levels of luteinizing hormone and increased follicle-stimulating hormone, as well as estradiol levels in PCOS rats. The ovary of *M. charantia*-treated rat had normal thecal cells and granular cell hyperplasia. Thus, *M. charantia* leaf has a beneficial impact on menstrual cycle disruption as well as hormonal fluctuations associated with PCOS. Therefore, *Mormodica charantia* leaves may have the potential to reduce the risk of PCOS in women.

Keywords: *Mormodica charantia*, Polycystic ovary syndrome, Luteinizing hormone, Follicle stimulating hormone.

Introduction

Polycystic ovarian syndrome (PCOS) affects 7–10% of women globally. An ultrasound used for diagnosis showed oversized ovaries in 22% of women during their period of reproduction, leading to infertility.[1,2] National Institutes of Health (NIH) and the 2003 Rotterdam measures are two reliable and superior benchmarks for the diagnosis of this health condition in women. Before biochemical markers like elevated testosterone levels in the blood and luteinizing hormone (LH), follicle-stimulating hormone (FSH), and weight gain were introduced, the science-based criteria for PCOS examination encompassed irregular menstrual cycles, obesity, sterility, as well as the detection of polycystic ovaries.[1,2] Women who have PCOS frequently experience irregular menstrual cycles, miscarriages, and a range of pregnancy difficulties.[1] Due to oxidative stress, which is caused by an increase in free radical generation and a decrease in endogenous antioxidant concentrations, a variety of biological components in the body may suffer chemical damage. Numerous diseases associated with inflammation have oxidative damage as a root cause. Recent research has connected the formation of reactive oxygen species to PCOS.[3,4] There are currently many medications available to control PCOS and stimulate ovulation.

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One of these therapies claims that PCOS women who use an oral contraceptive have a decreased risk of getting cancer. Therefore, oral contraceptives are usually recommended for PCOS-afflicted women. Each of these treatment methods has been linked to serious side effects, including arthritis, joint and muscle pain, arthritic mood abnormalities, and arthritis. Their use has also been restricted because of their potential health effects, poor solubility, and limited antioxidant capacity.[5] A helpful step toward improving women's reproductive health would be to give them access to a safe and effective infertility treatment option. In nations where medical interventions would be difficult to access or prohibitively costly, especially in remote places, the concerned women delayed until their medical situation was critical before seeking treatment. Because of the abundance of flora in these tropical nations, women prefer using traditional medicines to treat their health problems, including menstrual disorders, family planning, and fertility issues.[6-8] To cure or control PCOS in women without lowering the likelihood of becoming pregnant, researchers are looking for remedies, including botanicals, that have antioxidant chemicals with optimum bioavailability, efficacy, and safety. [5] In humid areas of East Africa and Asia, *Momordica charantia* L. (Cucurbitaceae), popularly known as "karela" or bitter melon, is grown for food and medicine. The fruit on this perennial climber is shaped like a gourd. When underripe and ripe, fruits are both green and yellowish, while ripe fruits possess a more overtly bitter flavor. *Momordica charantia* serves as an antioxidant in the treatment of illnesses like cancer, diabetes, inflammation, as well as bacterial infections. Numerous research has examined the usefulness of *M. charantia* extracts (mainly the fruit) in treating conditions like cancer, bacterial infections, diabetes, as well as dyslipidemia. Studies in this category include Pahlavaniet al.[9], Peter et al.[10], Liu et al.[11], and Zhang et al.[12], to name just a few. This study examined the effectiveness of *Momordica charantia* , which was recently mentioned for the treatment of PCOS in an ethnobotanical survey[13], in albino rats with letrozole-induced PCOS.

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Materials and Methods

Materials and reagents
Among the solvents bought from BDH Ltd. in England for the investigation were methanol, n-hexane, chloroform, dichloromethane, as well as ethyl acetate. Folin-Ciocaltaeu, $AlCl_3$, 2,2-diphenyl-1-picrylhydrazyl (DPPH), $FeCl_3$, Vitamin C (ascorbic acid), gallic acid, PCA, sodium bicarbonate, rutin, as well as CH_3COOK are among the reagents provided by Merck in Germany. As *Artemia salina* cysts, brine shrimp eggs were bought from a pet shop in the USA.

Plants collection and identification
In Oluponna, Osun state, Nigeria, leaves of the *Momordica charantia* plant were collected in Oluponna, Osun state (7° 35′ 34.7″ N 4° 11′ 27.5″ E) on December 8, 2022. The plant was identified and authenticated by Dr Patrick Agwu in Department of Pharmacognosy Herbarium Ibadan (DPHUI), Nigeria where voucher specimen (DPHUI No: 94) was also deposited.

Plant extraction
After a maceration of 1 kg of the pulverized plant sample in methanol (10 L) for 72 h at room temperature with intermittent stirring and shaking, the sample was filtered using a fresh cotton plug and Whatman (Number l) filter paper. The filtrate was concentrated *in vacuo* using a rotary evaporator (Bucchi, Germany). The crude extract's weight was established, and the yield percentage was computed.

Solvent-solvent partitioning
Methanol extract of *M. charantia* (200 g) was reconstituted in methanol-water (3:l). The reconstituted methanol extract was partitioned in separating funnel, with n-hexane, dichloromethane (DCM), and ethyl acetate successively in aliquots (50 mL per aliquot). The aliquots of n-hexane, DCM, as well as ethyl acetate fractions were collected differently and concentrated *in vacuo*. The yield of n-hexane, DCM, and ethyl acetate fractions were preserved in airtight containers for subsequent bioassay. The yield for each fraction was recorded. The crude extract and all fractions were kept in air-tight container.

Brine shrimp lethality assay
To test the samples for toxicity, a dried methanol extract (30 mg) and standard (cyclophosphamide) were reconstituted in MeOH (3 mL) to produce a stock solution with a concentration of 10 mg/mL. Concentrations of 1000, 500, 100, l0, and l µg/mL were obtained by serially diluting a portion of the stock solution. A standard technique was used to determine the toxicity effect.[14] The LD_{50} of this plant extract and the standard were calculated using Finney's Probit technique at 95 percent confidence intervals.

Diphenyl picrylhydrazyl (DPPH) radical scavenging assay
The methanol extract and fractions' capacity to scavenge free radicals was evaluated using the technique described by Bursal and Gülçin[15], with a couple of minor modifications. To conduct the DPPH antioxidant test, 3 mL (0.004%) of 2, 2-diphenyl-1-picryl-hydrazyl-hydrate and 2 mL of the crude extract of *M. charantia* , solvent fractions, and standards (ascorbic acid and rutin) were separately added. Methanol in the amount of two milliliters was added to the control as the test sample. After being shaken ferociously, the resultant solution was incubated in the dark for 30 minutes at 27 °C. The Spectrumlab 752S UV-VIS spectrophotometer was used to measure the absorbance of each vial at 557 nm and to calculate the percentage of inhibition.

Measurement of total phenolic content (TPC)
Using the Folin-Ciocalteu spectrophotometric technique, the total phenolic content of the methanol extract and its fractions were determined.[16] After 30 minutes of incubation, the mixture's 765 nm absorbance using a UV-VIS spectrophotometer (Spectrumlab 752S) was measured. The total phenolic content was determined utilizing a linear dose-response regression curve derived from gallic acid absorbance. The findings of the TPC were expressed in milligrams (mg) of gallic acid equivalent per gram of dry weight of the extract of fractions.

Measurement of total flavonoid content (TFC)
A modified version of the aluminum chloride colourimetry method previously described by Woisky and Salatino[17] was used in this investigation. The calibration curve was made using quercetin which has been diluted in ethanol (l00-6.25 g/mL) as the standard. The reaction's absorbance at 455 nm was measured using a UV-VIS spectrophotometer (Spectrumlab 752S) after 30 minutes of incubation at 27 °C. Each test sample's total flavonoid content was determined using an equation generated from the quercetin calibration curve, and this value was then expressed as mg of quercetin equivalent per gram of extract and fractions.

In vivo PCOS study
Dosage for plant extract
According to an ethnobotanical survey, the recommended human therapeutic dose of *M. charantia* leaf powder for treating irregular menstruation and related gynecological problems ranges from 1 to 9 g.[18] Based on body surface area, a conversion factor from humans to albino rats (conversion factor = 0.0l8) was used to determine the dosage for rats. This was accomplished by multiplying it by a number that accounted for the animal's body surface area and dividing it by the 60 kg weight of an adult male human.[19,20] Therefore, a dosage level of 1000 mg/kg body weight was used in this study. This study was approved by the University of Ibadan's Animal Care and Use Research Ethics Committee (UI-ACUREC/19/0051).

Selection and grouping of animals for assay
Twenty female albino rats were randomly separated into four groups of five, each consisting of five animals. The female albino rats ranged in weight from 150 to 200 g and were not pregnant. 15 of these albino rats received letrozole oral treatments for 21 days at a dose of 1 mg/kg per day utilizing 0.5% carboxymethyl cellulose (CMC) as the delivery system to develop PCOS. The groups were distributed thus: Group one received 1 mg of clomiphene citrate (Colid, Pfizer Pharmaceuticals, USA) for 15 days, whereas Group Two received 100 mg of *M. charantia* extracts per kilogram of body weight. Two milliliters of 5% w/v CMC in distilled water were given to groups three (disease control without treatment) and four, respectively.[18,21]

Determination of oestrous cycle pattern
Vaginal cytology was used to determine the stages of the estrous cycle.[18,22] A Pasteur pipette was gently inserted into the rat's vagina and filled with 0.1 mL of normal saline (0.9% NaCl) to acquire vaginal lavage. The withdrawn vaginal fluid was placed on a glass slide and immediately examined under a microscope with an x l0 objective to check how the cells were distributed. Throughout the study, this was done every day.

Blood and organ collection
After 15 days of therapy with 2% sodium pentobarbital (30 mg/kg), the animals were euthanized for laparotomy (24 hours after the last dosage) and their ovaries were examined. Estradiol, luteinizing hormone (LH), as well as follicle-stimulating hormone (FSH) concentrations in each group, were determined using the blood taken.

Hormonal analysis
Serum samples were tested for estradiol levels using the ELISA technique (Fortress Diagnostics Limited, Unit 2C Antrim Technology Park, Antrim, BR4l lQS, United Kingdom). Dialab's ELISA was used to measure the hormones luteinizing hormone (LH) and follicle-stimulating hormone (FSH) in serum samples. Before the test, room temperature equilibration of the test reagents and sample was performed. Following the pipetting of 0.05 mL of calibrators and rat samples into each well—aside from the blank well—was the addition of 0.1 mL of diluted enzyme conjugate. For 60 minutes, these combinations were incubated at room temperature. The microwell mixes were thrown away, and 0.2 mL of distilled water was used to keep the wells clean. Drain the well of its water, this was done repeatedly. After adding a blank well and pipetting the substrate solution (0.1 mL) into each microwell in the same manner as the enzyme conjugate, the microwells were incubated at room temperature in the dark for 20 min. Each microwell received a 0.1 mL

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addition of stop solution at the same time and in the same manner as the substrate solution reaction. Each microwell's absorbance was assessed at 450 nm in contrast to control using a microplate reader. Before reading the optical densities, the produced colour was stabilized for at least 30 minutes. After use, all unused samples and reagents were promptly put back in the refrigerator. The necessary microwell strips were carefully taken out of the pouch and put back in to avoid condensation in the unused wells. I put the pouch back in the fridge right away.

Ovarian histology
The ovaries were examined using a standard procedure.[23] The sections were removed with clean, labeled slides from a water bath that Raymond Lamb had heated to 55 °C, dehydrated on a hotplate for an hour at 60 °C, and then inspected under a light microscope with x 100 and x 400 objectives.

Statistical analysis
Values were displayed as Mean ± Standard Error of Mean (SEM). Data were analyzed using one-way analysis of variance (ANOVA) and group means were compared using Dunnett's Multiple Comparison and Bonferroni tests using GraphPad Prism version 5.01 for Windows, GraphPad Software, San Diego, California, USA. P values that were less than 0.05 were deemed significant.

Results and Discussion

The yields of the hexane, DCM, as well as ethyl acetate fractions, were 8.80, 7.14, and 57.68%, respectively, whereas the yield of the crude extract was 25.12% (Table 1). The brine shrimp experiment is a standard method for determining the toxic potential of botanicals.[24] Cyclophosphamide (standard) and the methanol extract of *M. charantia* had LC_{50} values of 8.79±0.22 **µ**g/mL and 224.74±0.35 **µ**g/mL, respectively (Table 2). Per Meyer's toxicity index [25], the *Mormodica charantia* methanol extract had an LC_{50}> l000 **µ**g/mL (non-toxic) value. This plant extract's toxicity levels were further categorized as non-toxic using Clarkson's criteria for exploratory examination of harmfulness.[26] The bulk of toxic bioactive ingredients have been found to be infrequently biosynthesized in botanical remedies alongside other essential ingredients.[27] The recent emphasis on assessing the toxic effects of plant-based medicines gives us comfort that phytotoxicity is not to be regarded as an impediment but rather as a helpful tool in the design of novel pharmaceuticals.

Table 1: The yield, DPPH (IC_{50}), TPC, and TFC values of the extract and solvent fractions

Extracts	Solvents (weight obtained)	DPPH (IC_{50}; µg/mL)	TPC (µg GAE/g)	TFC (mg QE/g)
M. charantia	Crude (251.20 g)	3l4.43 ± 5.57 $^{b/b}$	70.5 ± 5.54	96.70 ± l3.33 ^{b}
	Hexane (22.01 g*)	37.06 ± 0.11$^{a/NS}$	247.50 ± 80.03	l.69 ± 0.0l
	DCM (17.85 g*)	323.55 ± 4.76 $^{b/b}$	4700.33 ± 2l.5 ^{b}	0.54 ± 0.09
	Ethyl acetate (144.20 g*)	290.62 ± 2.88 $^{b/b}$	49l2.l7 ± l0.6 ^{b}	34. 90 ± 0.0l ^{b}
Ascorbic acid		2.76 ± 0.0l		
Rutin		20.6 ± 9.26		

Data represented as mean ± (SEM) (n = 3). One-way ANOVA followed by Dunnett's Multiple Comparison Test at $P < 0.05$. Degree of significance is represented by ^{a} (moderately significance), ^{b} (highly significance) at $P < 0.05$. *NS* indicates no significant difference with Ascorbic acid/Rutin. *200 g of methanol extract was partitioned, which yielded all the fractions.

Table 2: The LC_{50} (µg/mL) of *Mormodica charantia* using BSLA

Extracts	LC_{50} (µg/mL)
Mormodica charantia	l8l8.79 ± 0.22***
Cyclophosphamide	224.74 ± 0.35

Data are presented as means ± (SEM) (n = 3). One-way ANOVA followed by Dunnett's Multiple Comparison Test at $P<0.05$. Each extract was compared with standard (cyclophosphamide) and level of significant difference presented with ^{b}.

Various human diseases, including PCOS, are made worse by oxidative damage. Synthetic antioxidants have a restricted utility due to their high antioxidant activity, potential health risks, and poor solubility.[28,29] This necessitates the development of safer, more soluble organic antioxidants as a substitute. To discover novel antioxidants, various plant species have recently been studied. The reactive oxygen species (ROS) are vulnerable to damage from readily accessible and less damaging phyto-antioxidants, which are significant therapeutic food ingredients.[30] The extract of *M. charantia* crude extract, hexane, DCM, and ethyl acetate fractions had IC_{50} values of 314.43±5.57, 37.06±0.11, 323.55 ± 4.76, and 290.62±2.88 µg/mL, respectively (Table 1). The presence of -OH groups with functional properties makes phenols a crucial component of herbal remedies that are used for a range of medicinal applications, including the scavenging of free radicals. Numerous investigations have demonstrated a comparison between phenolics and antioxidant capacity.[31] The remarkable scavenging ability of the extract as well as solvent fractions examined in this study may be due to the hydroxyl functional groups that are found in the framework of their phenolic component architecture. The IC_{50} values for the crude extract of *M. charantia* , the hexane, DCM, and ethyl acetate fractions were 314.43±5.57, 37.06±0.11, 323.55±4.76, and 290.62±2.88 µg/mL, respectively. Owing to the existence of -OH functional groups, phenols are an essential part of medicinal plants that are employed for a variety of therapeutic uses, among which is the scavenging of free radicals.[31] The hydroxyl functional groups in the phenolic compound structure's skeleton may be responsible for the extracts and solvent fractions' outstanding scavenging performance. Due to the polarity of -OH functional groups in phenolics, the quantity of total phenolics in ethyl acetate fraction was the highest (4912.l7 ± 10.6 g GAE/g), while methanol extract contained the highest total flavonoids (96.70 ± l3.33 mg QE/g), as shown in Table 1. The findings support previous research outcomes, which show a similar correlation between the quantity of phenolic compounds and antioxidant capabilities in medicinal plants.[32,33]

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Letrozole, a drug that blocks the actions of aromatase inhibitors, produces a PCOS model with traits that, in some ways, mimic the characteristics of the PCOS syndrome in humans. By producing hyperandrogenism, hormonal imbalance, and intra-ovarian androgen excess, which causes a polycystic ovary, it mimics a syndrome comparable to PCOS. It stops estrone and estradiol from being produced from androstenedione and testosterone, as well as from androstenedione and testosterone. Atypical follicular development and follicular atresia are brought on by a persistent rise in levels of androgen in the ovary. In addition, letrozole promotes hyperglycemia, which can result in insulin resistance, hyperlipidemia, and other metabolic disturbances.[34]The effects of herbal remedies on hormonal imbalance and polycystic ovarian syndrome are poorly understood, even though they can assist PCOS patients in re-establishing the ability to regulate their menstrual cycles, as well as treat endocrine abnormalities. Letrozole alters albino rats' abnormal reproductive cycles when consumed orally for 21 days (Figure 1).

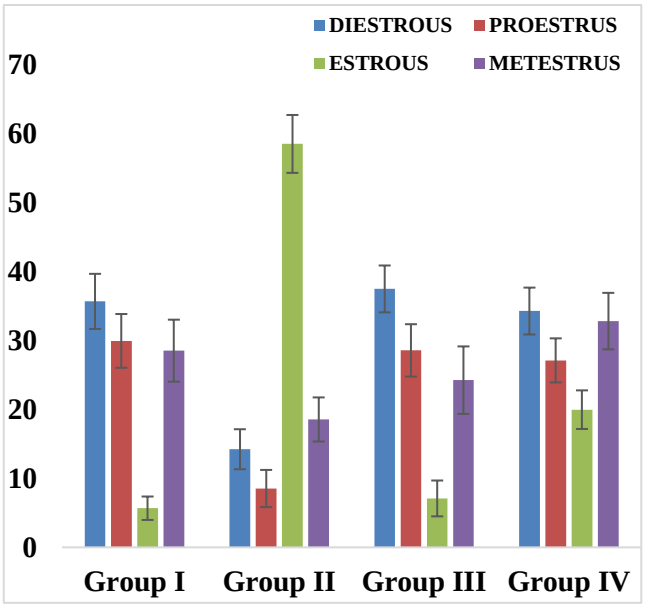

Figure 1: Estrous cycle phase index of albino rats during l5 days of treatment

Estrous phase index (%) = Number of days with clear phase smear × l00/total duration of treatment (l5 days).The data represent the mean ± SEM animals, n = 5. Data represented as mean ± (SEM) (n = 5). Group I - l00 mg/kg body weight of *M. charantia* , Group II- Clomiphene citrate (l mg/kg bw, p.o.), Group III - disease control group, Group IV - normal control group.

Rats given letrozole did not display any proestrus or estrous phase signs. Endogenous testosterone levels have been primarily implicated in PCOS. The disparities seen in the rat menstruation phase may be due to variations in the amounts of reproductive hormones, as well as gonadotropins. These sex hormones regulate ovarian features, hormonal dysregulation, and follicle development, which can lead to an unpredictable estrous cycle and ovarian dysfunction.[35] Extract from *M. charantia* support the existence of a diestrous phase in letrozole-induced PCOS rats. The estrous cycle was altered by this plant in ways that were similar to those observed in regular albino rats, including lengthening the diestrous phase and delaying the onset of the estrous phase. Figure 2 displays the histology of the animal's ovaries. The ovary of the group I animal had an ovarian cortex with typical Graffian follicles as well as follicles with mildly degenerate theca cells. *M. charantia* leaf extract increased the likelihood that the diestrous phase would exist. The stroma has granular cells with proper leutinization. There is slight vascular congestion. The ovarian cortex of the clomiphene citrate group (Group II) had several Graffian follicles, healthy theca cells, and a moderate to high level of vascular obstruction with thickly walled capillaries. The ovaries of the untreated PCOS rats (Group III) included several typical Graffian follicles, healthy theca cells, a little degree of granular cell fibrosis and hyperplasia, and moderate levels of vascular congestion. Group IV (the healthy control group) showed an ovarian cortex featuring Graffian follicles displaying an antrum, a mildly fibrotic stroma with significant vascular congestion, and a mild infiltration of inflammatory cells.

The elevated levels of luteinizing hormone (LH) observed in the majority of PCOS patients are caused by mechanisms related to high levels of circulatory androgen, exposure of the ovarian theca and granulosa cells to LH, and elevated levels of cAMP.[36-39] Additionally, cholesterol is frequently converted into steroid hormones in PCOS women due to the activation of steroidogenic proteins (enzymes). Androgen production necessitates the enzymes 17α-hydroxylase/17,20-lyase (CY17A1), 3β hydroxysteroid dehydrogenase/Δ5-Δ4-isomerase type 2 (HSDB2), and DHEA. These enzymes control the transport of cholesterol and the conversion of steroids to androgen. PCOS patients have high levels of endogenous androgen since this hormone promotes the growth of steroidogenic enzymes in ovarian theca cells.[40] Table 3 shows the effects of *M. charantia* and clomiphene citrate on the levels of LH, FSH, as well as estradiol in the animals being tested. FSH levels were 0.69±0.01 mIU/mL in the *M. charantia* treatment group and 0.93±0.19 mIU/mL in the control group. LH levels in each group vary from 0.22±0.01 to 0.23±0.01 mIU/mL. LH variation has a significant negative influence on rats that are in the estrous stage.

Table 3: Effect of test samples on LH, FSH and estradiol

Parameters	Group I	Group II	Group III	Group IV
LH (mIU/mL)	0.22±0.0l*	0.23±0.0l*	0.23±0.0l*	0.22±0.0l
FSH (mIU/mL)	0.69±0.0l*	0.75±0.05*	0.73±0.03*	0.93±0.l9
Estradiol (pg/mL)	9.26±0.46*	7.63±0.89*	5.70±0.77*	9.84±l.44

Data is represented as mean ± (SEM) (n = 5). Evaluated by ANOVA followed by Bonferroni tests. * Indicate $P < 0.00l$ vs Group IV (Normal control). Group I - l00 mg/kg body weight of *M. charantia* , Group II - Clomiphene citrate (l mg/kg bw, p.o.), Group III - disease control group, Group IV - normal control group.

Due to the irregular LH hormone synthesis, PCOS patients have difficulty controlling their oestrus cycle. In PCOS rats, letrozole results in morphological changes that include the growth of many cysts, theca cell hyperplasia, as well as thicker ovarian capsules. Additionally, granulosa cells may enclose subcapsular cysts. The presence of therapeutic FSH levels increased LH, and a decrease in contact between granulosa and theca cells are the causes of these histological traits.[21] The physiological action of chemical constituents in the extract, which reasserts the steroidal prestige and allows fertility to be restored, may be the cause of the return of oestrus anomalies and follicular generation after treatment with *M. charantia* .[41,42] The active constituents in *M. charantia* were believed to be phenolics and flavonoids. In southwest Nigeria, this plant's leaf juice is taken orally to treat gynaecological disorders ranging from menstrual cramps to infertility issues.[19]This study presented the first scientific data, justifying the use of *M. charantia* leaves in normalizing the hormonal imbalance and menstrual cycle irregularity associated with PCOS in premenopausal women.

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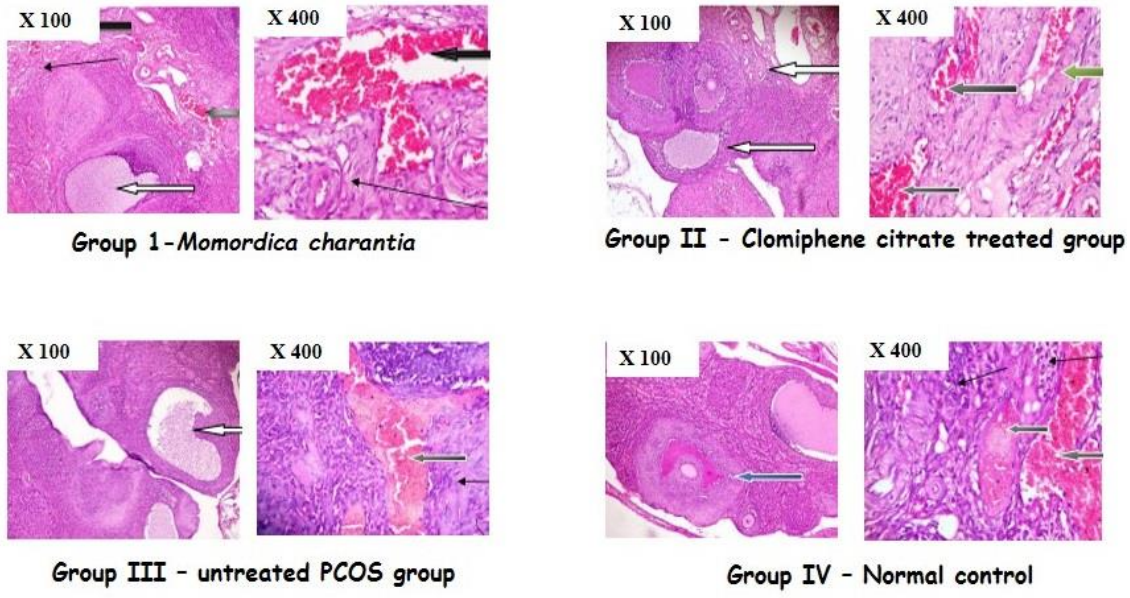

Figure 2: Photomicrographs of ovary of experimental animals

Group I - l00 mg/kg body weight of *M. charantia* , **Group II** - Clomiphene citrate (l mg/kg bw, p.o.), **Group III** - disease control group, **Group IV** - normal control group. Graffian follicles exhibiting antrum (blue arrow); several normal Graffian follicles with normal theca cells (white arrow); mild infiltration of inflammatory cells (slender arrow); moderate vascular congestion (black arrow); and vessels with thick wall (green arrow).

Conclusion

A useful tool for managing health challenges is indigenous herbal knowledge. Estradiol levels increased after receiving a dose of *Mormodica charantia* extract. Findings from the *in vivo* investigation indicate that *M. charantia* leaf extract has a beneficial impact on menstrual cycle irregularity as well as the hormonal fluctuations associated with PCOS. Therefore, *Mormodica charantia* leaves may have the potential to reduce the risk of PCOS in women.

Conflicts of interest

The authors declare that they have no conflicts of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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References

1. Teede HJ, Tay CT, Laven JJ, Dokras A, Moran LJ, Piltonen TT, Costello MF, Boivin J, Redman LM, Boyle JA, Norman RJ. Recommendations from the 2023 international evidence-based guideline for the assessment and management of polycystic ovary syndrome. Eur J Endocrinol. 2023; 189(2):G43-64.
2. Rotterdam ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group. Revised 2003 consensus on diagnostic criteria and long-term health risks related to polycystic ovary syndrome. Fertil Steril. 2004; 81(l):19-25.
3. Amini L, Tehranian N, Movahedin M, Tehrani FR, Ziaee S. Antioxidants and management of polycystic ovary syndrome in Iran: A systematic review of clinical trials. Iran J Reprod Med. 2015; 13(1):1-8.
4. Xu W, Zuo T, Zhu M. Roles of Oxidative Stress in Polycystic Ovary Syndrome and Cancers. Oxid Med Cell Longev. 20l6; 20l6(l): l-l4.
5. Badawy A and Elnashar A. Treatment options for polycystic ovary syndrome. Int J Womens Health. 20ll; 3(l): 25-35.
6. Bates GW and Legro RS. Longterm management of polycystic ovarian syndrome (PCOS). Mol Cell Endocrinol. 20l3; 373(l-2): 9l-97.
7. Sonibare MA and Ayoola IO. Medicinal plants used in the treatment of neurodegenerative disorders in some parts ofSouthwest Nigeria. Afr J Pharm Pharmacol. 20l5; 9(38): 956-965.
8. Ogunlakin AD, Sonibare MA, Yeye OE, Jabeen A, Shah SF, Ojo OA, Gyebi GA, Ayokunle DI. Design, Synthesis, and Characterization of Cinnamic Acid Derivatives with Two Novel Acrylohydrazones on HeLa and CHO-1 Cancer Cell Lines: The Experimental and Computational Perspective. Chem Afr. 2024; 7:583-604.
9. Pahlavani N, Roudi F, Zakerian M, Ferns GA, Navashenaq JG, Mashkouri A, Ghayour-Mobarhan M, Rahimi H. Possible molecular mechanisms of glucose-lowering activities of *Momordica charantia* (karela) in diabetes. J Cell Biochem. 2019; 120(7):10921-10929.
10. Peter EL, Kasali FM, Deyno S, Mtewa A, Nagendrappa PB, Tolo CU, Ogwang PE, Sesaazi D. *Momordica charantia* L. lowers elevated glycaemia in type 2 diabetes mellitus patients: Systematic review and meta-analysis. J Ethnopharmacol. 2019; 231:311-324.
11. Liu Z, Gong J, Huang W, Lu F, Dong H. The Effect of *Momordica charantia* in the Treatment of Diabetes Mellitus: A Review. eCAM. 2021; 16(2021):3796265.
12. Zhang F, Zhang X, Yu J, Tan Y, Guo P, Wu C. The gut microbiota confers the lipid-lowering effect of bitter melon (*Momordica charantia* L.) in high-fat diet (HFD)-induced hyperlipidemic mice. Biomed Pharmacother. 2020; 131:110667.
13. Ogunlakin AD and Sonibare MA. Ethnobotanical survey of medicinal plants used as remedy for female infertility and menstrual disorder in southwestern Nigeria. Niger J Pharm Res. 2019; 15(2):205-217.

14. McLaughlin JL, Rogers LL, Anderson JE. The use of biological assays to evaluate botanicals. Drug Inf J, l998; 32(l): 5l3-524.
15. Bursal E and Gülçin I. Polyphenol content and in vitro antioxidant activities of lyophilized aqueous extract of kiwifruit (*Actinidia deliciosa*). Food Res Int. 20ll; 44(5): l482–l489.
16. Miliauskas G, Venskutonis PR, Van BTA. Screening of radical scavenging activity of some medicinal and aromatic plant extracts. Food Chem. 2004; 85(2):23l–237.
17. Woisky R and Salatino A. Analysis of Propolis: some parameter and procedures for chemical quality control. J Apicult Sci, l988; 37(2): 99–l05.
18. Ogunlakin AD, Sonibare MA, Yeye OE, Gyebi GA, Ayokunle DI, Arigbede OE, Ibrahim IM, Odugbemi AI, Jabeen A, Shah SF, Shaheen F. Isolation and characterization of novel hydroxyflavone from *Kigelia africana* (Lam.) Benth. fruit ethyl acetate fraction against CHO 1 and HeLa cancer cell lines: In vitro and in silico studies. J Mol Struct. 2023; 1282:135180.
19. Nair AB and Jacob S. A simple practice guide for dose conversion between animals and human. J Basic Clin Pharm. 20l6; 7(2):27-3l.
20. Ojo OA, Grant S, Amanze JC, Oni AI, Ojo AB, Elebiyo TC, Obafemi TO, Ayokunle DI, Ogunlakin AD. *Annona muricata* L. peel extract inhibits carbohydrate metabolizing enzymes and reduces pancreatic β-cells, inflammation, and apoptosis via upregulation of PI3K/AKT genes. PLoS ONE, 2022; 17(10):e0276984.
21. Rana S, Hussain L, Saleem U, Asif M, Lodhi AH, Barkat MQ, Riaz M, Jamil A. Dose Dependent Effects of Aqueous Extract of *Garcinia cambogia* Desr. Against Letrozole Induced Polycystic Ovarian Syndrome in Female Adult Rats With Possible Mechanisms Exploration. Dose Response. 2023; 21(2):15593258231169381.
22. Shehab NG, Anbar HS, Alrouby NM, Abouelalamin A, Lutfi L, Allo IT, Elayoty SM. New alternative herbal remedies for treatment of letrozole-induced polycystic ovary syndrome in rats. J Appl Pharm Sci. 2023; 13(1):163-179.
23. Avwioro OG. Histochemistry and tissue pathology - Principles and Techniques. Textbook of Histochemistry and Tissue Pathology," 2nd Edition, Claverianun Press, Ibadan, Nigeria. 20l0.
24. Campbell DL, Lawton LA, Beattie KA, Codd GA. Comparative assessment of the specificity of the brine shrimp and microtox assays to hepatotoxic (microcystin-LR-containing) cyanobacteria. Environ Toxicol, l994; 9(l): 7l-77.
25. Alamgir AN. Therapeutic use of medicinal plants and their extracts: volume 1. Springer International Publishing AG.; 2017.
26. Niksic H, Becic F, Koric E, Gusic I, Omeragic E, Muratovic S, Miladinovic B, Duric K. Cytotoxicity screening of Thymus vulgaris L. essential oil in brine shrimp nauplii and cancer cell lines. Sci Rep. 2021; 11(1):13178.
27. Cock IE, Luwaca N, Van Vuuren SF. The traditional use of Southern African medicinal plants to alleviate fever and their antipyretic activities. J Ethnopharmacol. 2023; 303:115850.
28. El-Beltagi HS, Mohamed AA, Mohamed HI, Ramadan KM, Barqawi AA, Mansour AT. Phytochemical and potential properties of seaweeds and their recent applications: A review. Marine Drugs. 2022; 20(6):342.
29. Hassanpour SH and Doroudi A. Review of the antioxidant potential of flavonoids as a subgroup of polyphenols and partial substitute for synthetic antioxidants. Avicenna J Phytomed. 2023; 13(4):354.
30. Aryal S, Baniya MK, Danekhu K, Kunwar P, Gurung R, Koirala N. Total phenolic content, flavonoid content and antioxidant potential of wild vegetables from Western Nepal. Plants. 2019; 8(4):96.
31. Hussain T, Fatima I, Rafay M, Shabir S, Akram M, Bano S. Evaluation of antibacterial and antioxidant activity of leaves, fruit and bark of *Kigelia africana*. Pak J Bot, 20l6; 48(l): 277-283.
32. Zhang C, Feng S, Wang Q, Wang P, Xu J, Chen T. Flavonoids and phenolic compounds from *Smilax scobinicaulis*. Chem Nat Compd, 20l4; 50(2): 254-257.
33. Sonibare MA, Onifade TR, Ogunlakin AD, Akinmurele OJ, Adebodun SA. Microscopic Evaluation and Antioxidant Activity of *Glyphaea brevis* (Spreng.) Monach (Family Tiliaceae). Free Radic Antioxid. 2022; 12(1):27-32.
34. Choi JE, Kang SH, Lee SJ, Bae YK. Androgen receptor expression predicts decreased survival in early stage triple-negative breast cancer. Ann Surg Oncol, 20l5; 22(l): 82-89.
35. Walters KA, Bertoldo MJ, Handelsman DJ. Evidence from animal models on the pathogenesis of PCOS. Best Pract Res Clin Endocrinol Metab. 2018; 32(3):271-281.
36. Laganà AS, Garzon S, Casarin J, Franchi M, Ghezzi F. Inositol in polycystic ovary syndrome: restoring fertility through a pathophysiology-based approach. Trends Endocrinol Metab. 2018; 29(11):768-780.
37. Sanchez-Garrido MA, Tena-Sempere M. Metabolic dysfunction in polycystic ovary syndrome: Pathogenic role of androgen excess and potential therapeutic strategies. Mol Metab. 2020; 35:100937.
38. Ye W, Xie T, Song Y, Zhou L. The role of androgen and its related signals in PCOS. J Cell Mol Med. 2021; 25(4):1825-1837.
39. Kumariya S, Ubba V, Jha RK, Gayen JR. Autophagy in ovary and polycystic ovary syndrome: role, dispute and future perspective. Autophagy, 2021;17(10):2706-2733.
40. Jozkowiak M, Piotrowska-Kempisty H, Kobylarek D, Gorska N, Mozdziak P, Kempisty B, Rachon D, Spaczynski RZ. Endocrine disrupting chemicals in polycystic ovary syndrome: the relevant role of the theca and granulosa cells in the pathogenesis of the ovarian dysfunction. Cells. 2022; 12(1):174.
41. Ogunlakin AD and Sonibare MA. Phytochemistry and biological activities of *Tetracera* species. Trends Phytochem Res. 2022; 6(4): 339-352.
42. Ogunlakin AD, Sonibare MA, Ojo OA. Review on Effect of Medicinal Plants on Female Reproductive System. Trop J Nat Prod Res. 2023; 7(3): 2473-2483.