

Effects of Castor Oil On Oestrous Cycles And Reproductive Hormone Levels In Letrozole-Induced Polycystic Ovary Syndrome In Female Wistar Rats

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Abstract

Background: Polycystic Ovarian Syndrome (PCOS) in young women is characterised by excess androgen and reduced oestrogen levels, whereas castor oil has been reported to have oestrogenic and anti-androgenic properties in animal model. The effects of castor oil on oestrous cycles and reproductive hormone levels in female Wistar rats in Letrozole-induced polycystic ovary syndrome were investigated. **Materials and method:** Forty-five female rats were divided into 4 groups, with group 1 consisting of 10 rats, group 2 of 15 rats and groups 3 and 4 both consisting of 10 rats each. 1 mg/kg p.o. Letrozole was dissolved in 1 % CMC and administered to induce PCOS in group 2 to 4 rats, while group 1 rats were given CMC (vehicle) for 21 days, after which 5 rats were removed from group 2, sacrificed and assayed for confirmation of PCOS induction. Thereafter, for the next 21 days, group 1 rats (negative control, NC) and group 2 rats (positive control, PC) were given distilled water, while groups 3 and 4 rats were given castor oil (500 mg/kg BW and 1000 mg/kg BW respectively). Oestrous cycles, reproductive hormone levels and histology of the ovaries were studied. Differences in means were considered significant at $p < 0.05$. **Results:** There was reduction in testosterone levels, increase in oestrogen levels, normalization of disrupted oestrous cycle phases and length as well ovarian histology. **Conclusion:** Castor oil (*Ricinus communis* seed oil) ameliorated oestrous changes associated with PCOS and caused alterations in the reproductive hormones of female Wistar rats.

Keywords: Polycystic Ovary Syndrome (PCOS); Castor Oil; Letrozole; Oestrous Cycles; Reproductive Hormones.

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Introduction

Polycystic ovary syndrome (PCOS) is a major endocrine disorder in young women affecting their health-related quality of life and mental well-being (1, 2). It is diagnostically characterized by chronic persistent anovulation, polycystic ovaries, biochemical and clinical manifestations of hyperandrogenism, infertility and metabolic disorders (3, 4). It is detected in approximately 4 to 10 % of women of

reproductive age (4, 5) with a tremendous negative impact on the physiology and metabolism of the body as it may evolve into a metabolic syndrome with insulin resistance, hyperinsulinemia, abdominal obesity, hypertension, dyslipidemia and cardiovascular disease (6).

The clinical management of PCOS is multifaceted but often unsatisfactory (7). The best-known treatment for PCOS at

present is by the use of allopathic medicines such as clomiphene citrate, metformin, tamoxifen and troglitazone. These medicines have mild to severe side effects such as hot flushes, arthritis, joint or muscle pain and psychological effects such as irritability, mood swings, depression, and bloating (8). Due to the adverse effects caused by these allopathic medicines, alternative medicines such as acupuncture, naturopathy and herbal medicines are gaining importance.

From time immemorial, several medicinal plants form the main source of health care due to better acceptability and many of these plants have been used to correct reproductive disorders (9, 10). *Ricinus communis* plant commonly called castor oil plant is one of the herbal plants used in treating reproductive disorders (11). It is a flowering plant that belongs to the family Euphorbiaceae. Different parts of the plants have been reported to possess several medicinal values (11). Among the parts normally used is the seed from which castor oil is extracted. Castor oil seed is similar to other vegetable oils due to its high concentration of monounsaturated fatty acid, triricinoleic acid and triricinolein (12). The presence of bioactive compounds including polyphenols, phytosterols, and tocopherols present in castor oil seed enhance its anti-inflammatory and antioxidant properties against oxidation, hence prolonging the shelf life and promoting the stability of the oil (12).

Polycystic ovarian syndrome, which is characterised by excess androgens and reduced oestrogen, may be treated by an agent that brings about a reduction in androgen level and elevation of oestrogen level in the system. Castor oil has been reported to have oestrogenic properties in female rats (12) and anti-androgenic properties in male rats (14), thus the current study was designed to determine the effect of castor oil on letrozole-induced polycystic ovary syndrome in Wistar rats.

Materials and method

Preparation of castor oil: Fresh seeds of castor oil plant were air-dried for 30 days until a constant weight was obtained. The dried seeds were blended and transferred into a desiccator. N-hexane (5L) was then added and allowed to stay for 72 hours for proper extraction of the oil i.e. via cold extraction. The mixture was filtered and concentrated later using a rotary evaporator at 40 degrees centigrade (15). This was to separate the oil from the solvent. The oil was later concentrated using a vacuum oven set at 30 degrees centigrade with a pressure of 500 mmHg for 5 hours and was weighed to be 130 ml. The desired oil sample had a yellow colour and an agreeable smell. It was liquid at room temperature.

Experimental Design: Forty-five adult female rats (150-180g) were used for this study. They were housed in breeding cages and fed with a standard pelleted rat diet (Ladokun feeds limited, Ibadan) and water *ad libitum*.

PCOS induction: 1 mg/kg p.o. Letrozole was dissolved in 1 % CMC. The 1 % CMC (Vehicle of administration) was prepared by dissolving 1 g of powdered carboxymethylcellulose (CMC) in 100 ml of distilled water, while stirring to produce a uniform dispersion. The stirring continued until a clear solution was produced. 2 ml/kg BW of this was administered to the rats to induce PCOS (16).

Experimental design: Forty-five female Wistar rats were used for this experiment. The rats were divided into 4 groups, with group 1 consisting of 10 rats, group 2 of 15 rats and groups 3 and 4 both consisting of 10 rats each. Group 1 served as the negative control (NC), group 2 was the positive control (PC), while groups 3 and 4 served as the test groups. Prior to administrations, an assessment of the oestrous cycle regularity was done on all the rats by vaginal smear test for 2 consecutive oestrous cycles (16, 17). Thereafter, the rats in group 1 (NC) were orally administered 2 ml/kg BW CMC

(vehicle) once daily for 21 days while groups 2 to 4 rats were orally treated with 1 mg/kg Letrozole p.o dissolved in 1 % CMC (2 ml/kg) once daily for 21 days. Vaginal smears were collected daily and evaluated microscopically by wet preparation (x 40 magnification) and using Papanicolaou staining technique (x 100 magnification). To confirm PCOS induction, at the proestrus phase, five rats were removed from group 2 (PC), sacrificed, blood was collected for hormonal assay and ovarian specimens were processed for histology. Thereafter for the next 21 days, NC and PC rats were administered distilled water orally, while group 3 and 4, on the other hand received oral administrations of 500 mg/kg and 1000 mg/kg castor oil respectively (18). This was followed by the sacrifice of all the rats at proestrus phase for hormonal assay and processing of ovaries for histology using haematoxylin and eosin staining technique.

Specimen collection: The technique described by Marcondes (18) was used. Using a Pasteur pipette, 0.1 ml of saline was used to flush the vagina of the rat and then the vaginal fluid (cell suspension) was collected. A drop of the cell suspension was spread evenly on a slide and viewed under the microscope and viewed. Thereafter the slides were fixed in 95% alcohol for at least 30 minutes so that Papanicolaou staining technique could be used to stain the smears for clearer viewing.

Collection of blood samples: Blood samples were collected during the proestrus phase from the orbital sinus of each rat. The blood was collected into plain sterile sample bottles, allowed to clot and thereafter centrifuged at 3000 rpm for 10 minutes. The serum was collected from the centrifuged blood samples into a separate tube and stored at -20⁰ C for further use. The serum levels of reproductive hormones were assayed at the Laboratory for Reproductive

Physiology and Developmental Programming, Department of Physiology, Faculty of Basic Medical Sciences, University of Ibadan, Ibadan. Determination of the serum concentration for follicle-stimulating hormone, oestrogen and testosterone was done using of Enzyme-Linked-Immunosorbent Assay ELISA kits (Fortress Diagnostics, UK).

Statistical analysis: The data from each group were summarised as mean $\pm$ standard error of mean (SEM). Analysis of data was done using Student's t-test and one-way ANOVA where applicable. The differences between the means were considered statistically significant $p < 0.05$ (20)

Results

The results of the effects of castor oil on oestrous cycles and reproductive hormone levels in letrozole-induced polycystic ovary syndrome in female rats showed changes in the oestrous phases when the negative control and positive control groups were compared (Fig. 1). Treatment with 1000 mg/kg BW castor oil showed significant differences when compared with the positive control whereas, there no statistically significant difference with 500 mg/kg BW when comparison was made between the means of the oestrous phases. There were significant increases ($p < 0.05$) in the means of the oestrous cycle length when the treatment groups were compared with the negative control (Fig. 2 to 4).

Serum testosterone and oestrogen levels were also significantly different ($p < 0.05$) when compared with the control (Fig. 5 and 7).

The results of the histology (plates 1 to 4) showed ovarian stroma with varying degrees of luteinization within the granular cells. There were visible degenerating follicles with cystic spaces.

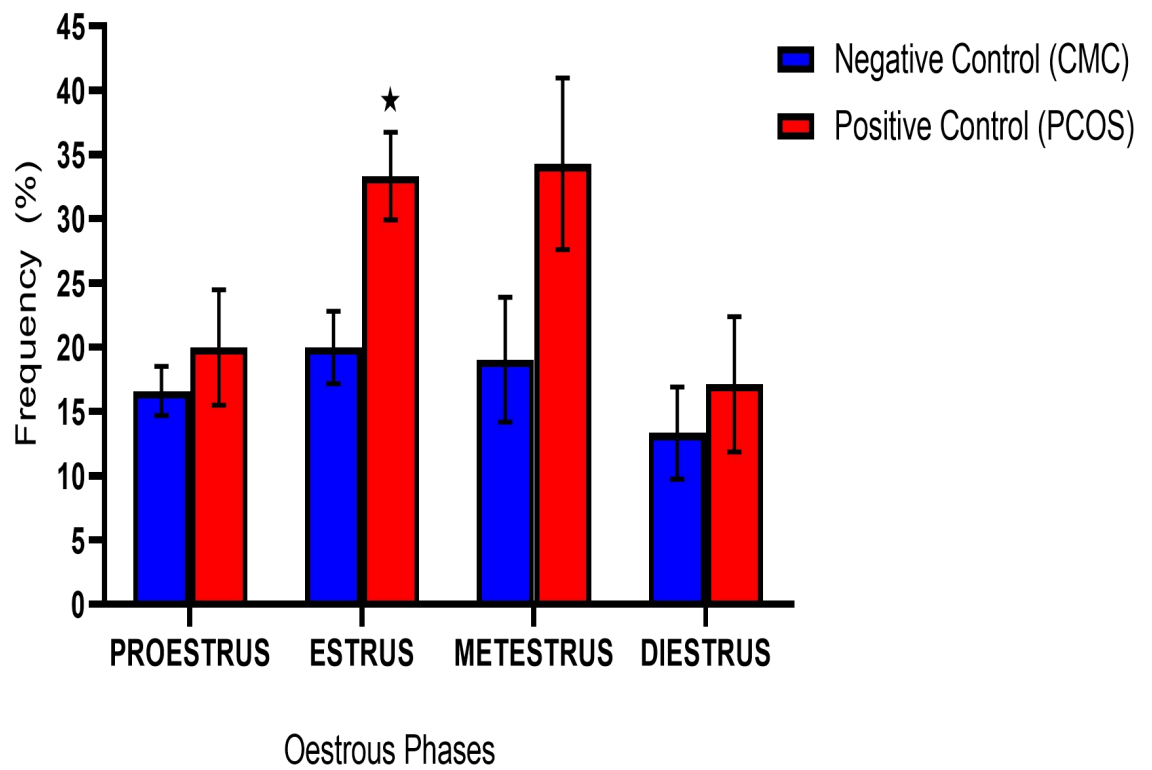


Fig.1: Frequency (%) of the oestrous phases in the negative control (CMC) and positive control (PCOS) groups. * $p < 0.05$ = statistically significant, $n = 10$. Results are expressed as mean $\pm$ SEM

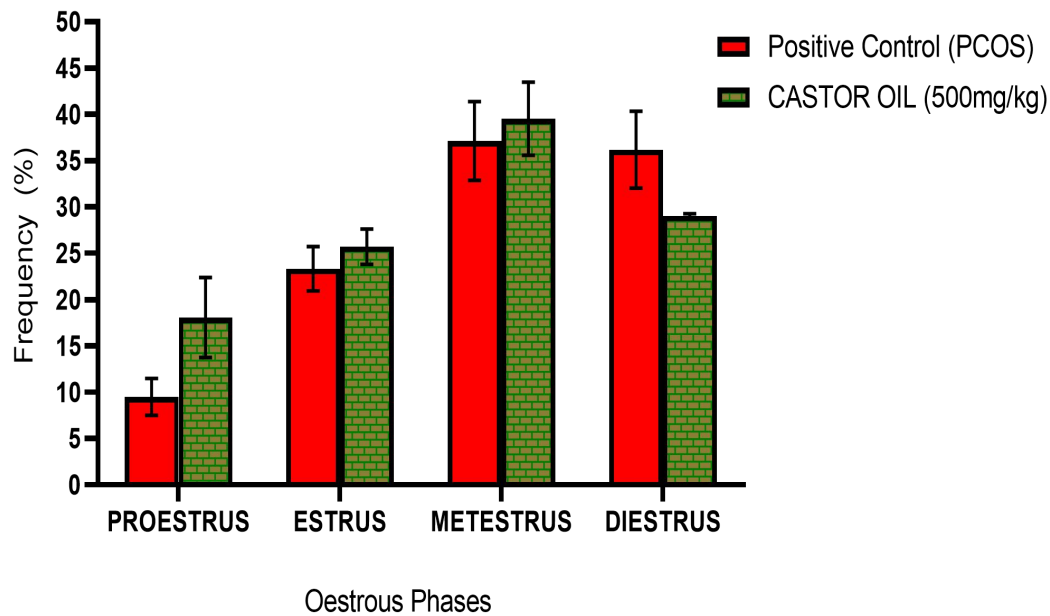


Fig.2: Frequency (%) of oestrous phase in the positive control (PCOS) and Castor oil (500 mg/kg) groups, $n = 10$. There was no statistically significant difference between the means. Results are expressed as mean $\pm$ SEM

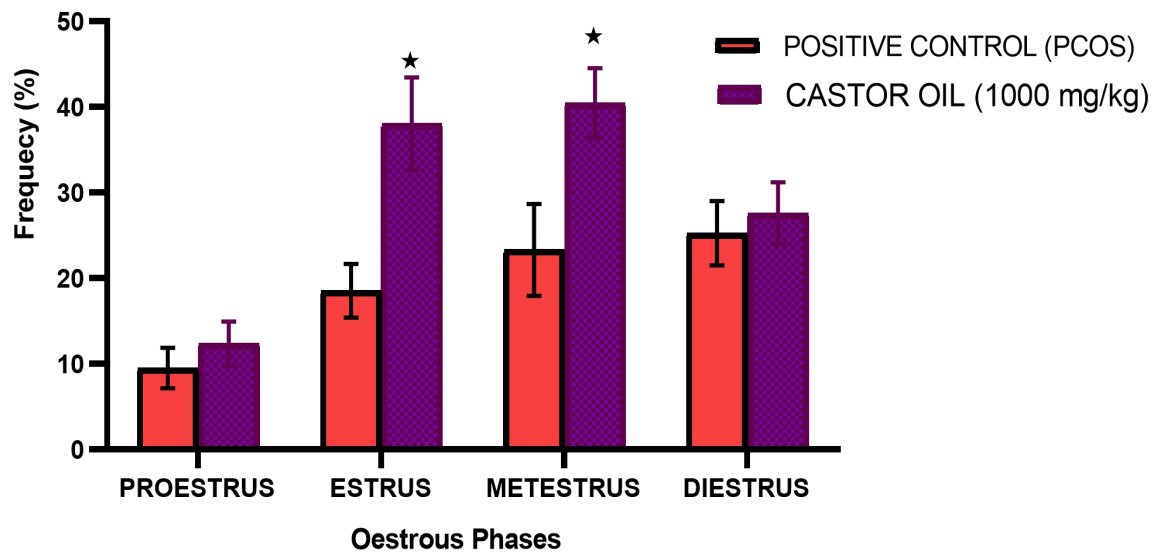


FIG. 3: Frequency (%) of the oestrous phases in the positive control (PCOS) and castor oil (1000 mg/kg) groups. * $p < 0.05$ = statistically significant, $n = 10$. Results are expressed as mean $\pm$ SEM

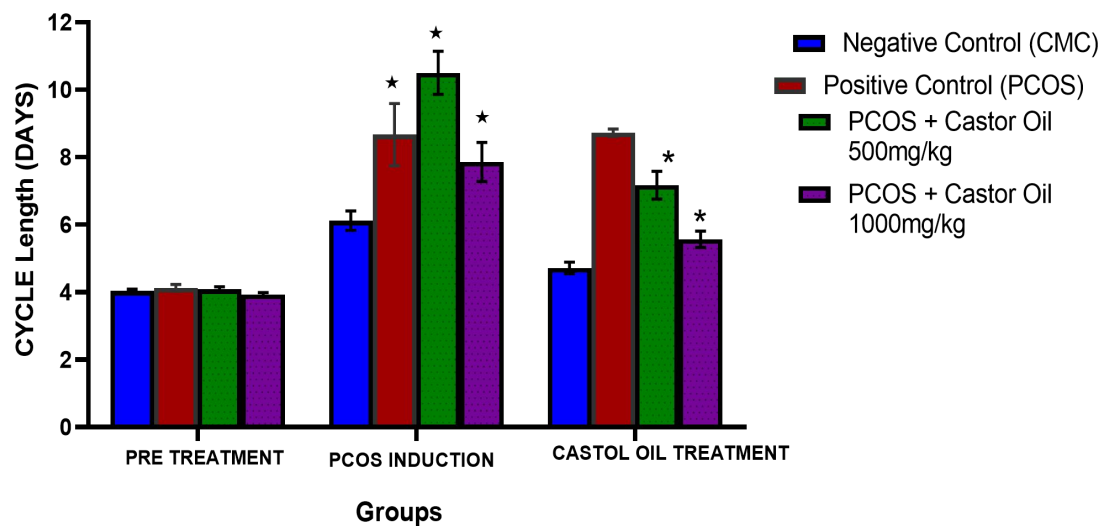


Fig. 4: The Letrozole treatment period: there were significant increases ($p < 0.05$) in the means of the oestrous cycle length when compared with the negative control. A significant increase ($p < 0.05$) was also observed in the cycle length of the positive control (PCOS) and castor oil 500 mg/kg treatment groups when compared with the negative control group. However, the length of the cycle significantly decreased ($p < 0.05$) when the Castor oil 1000 mg/kg group was compared with the positive control (PCOS) group. Results are expressed as mean $\pm$ SEM. * $p < 0.05$ statistically significant, $n = 10$.

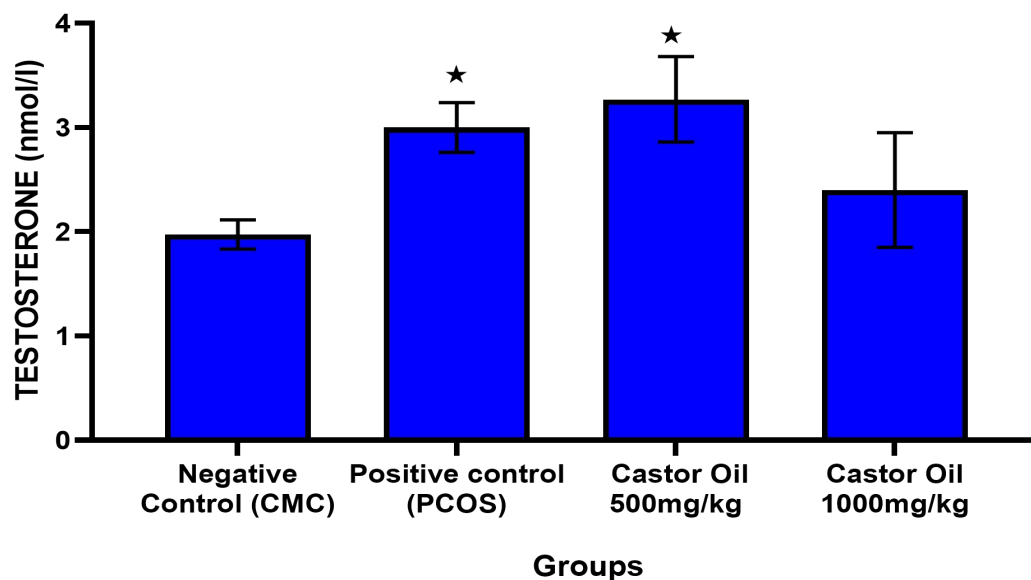


Fig. 5: Effects of castor oil on serum testosterone level in female Wistar rats, $n = 10$. Data presented as Mean $\pm$ SEM. The means of each group were compared with the control and considered significantly different ($p < 0.05$)

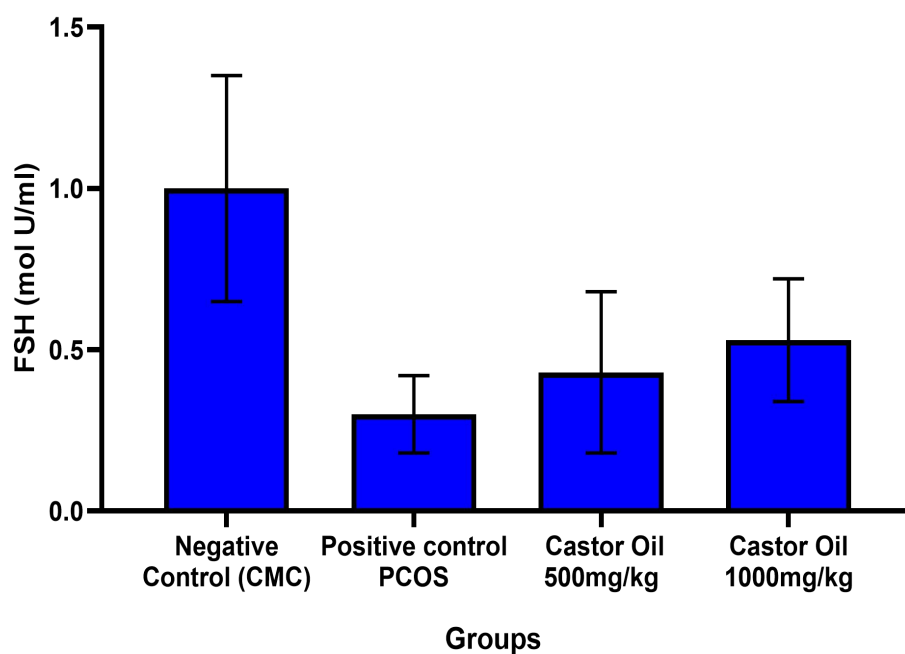


Fig. 6: Effects of letrozole-induced PCOS and treatment with castor oil on serum FSH level in female Wistar rats $n = 10$. Results were not significant. Results are expressed as mean $\pm$ SEM.

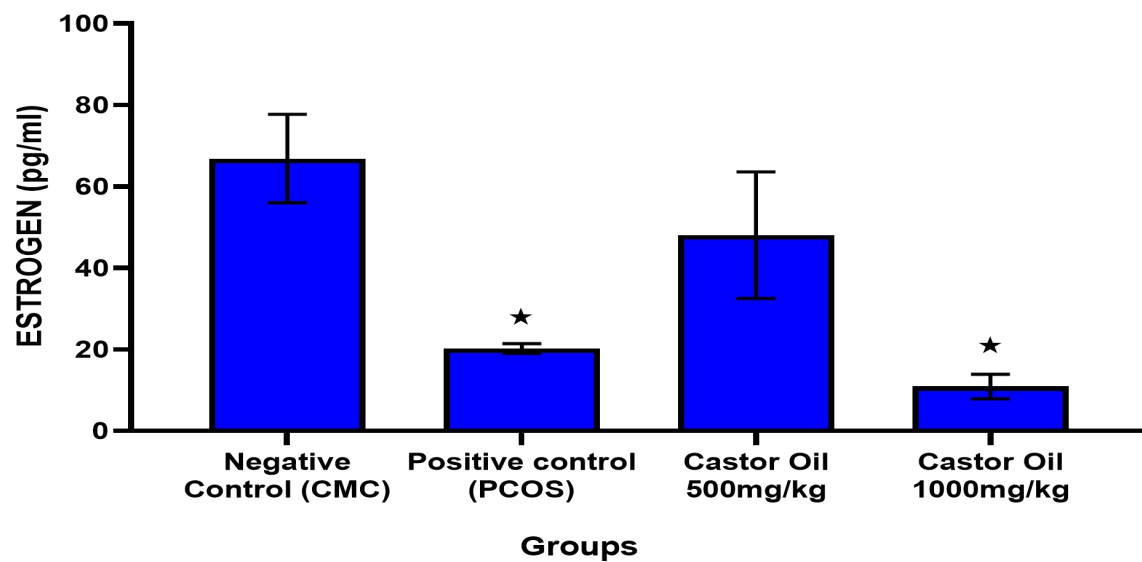


Fig.7: Effects of treatment with castor oil on serum oestrogen level in female Wistar rats. * $p < 0.05$ = statistically significant, $n = 10$. Results are expressed as mean $\pm$ SEM

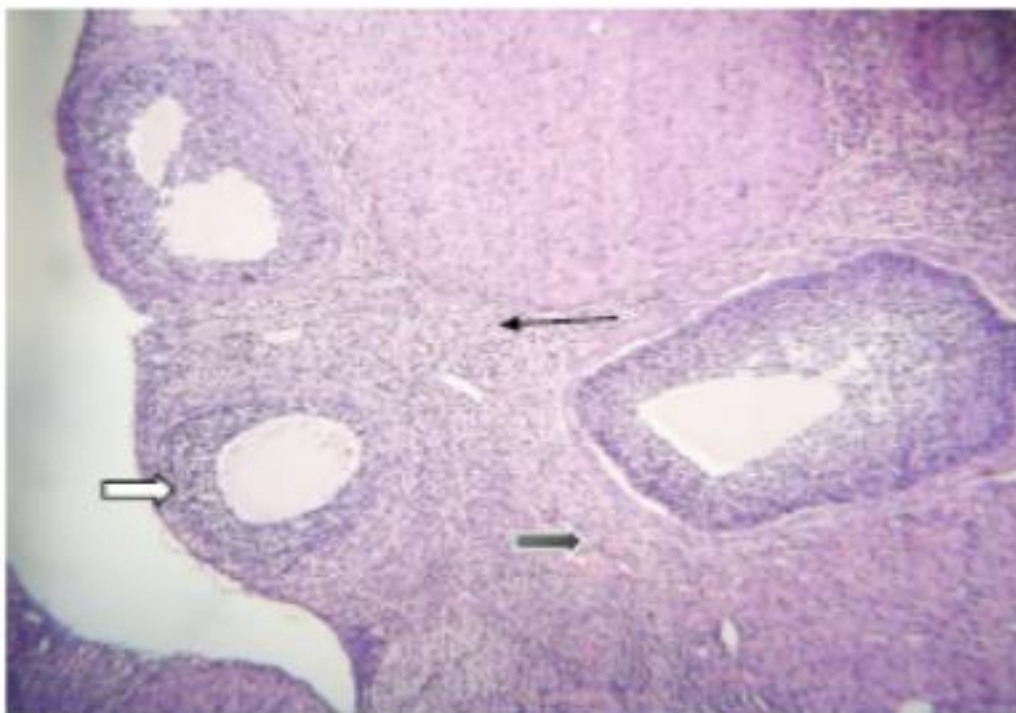


Plate 1: Photomicrograph of ovarian section of the negative control (CMC only) showing normal ovary tissue with follicles showing different stages of maturation (white arrow) Ovarian stroma appear normal (slender arrow), there are prominent thecal cells seen (black stumpy arrow) no cyst seen (H&E, mag. X 100).

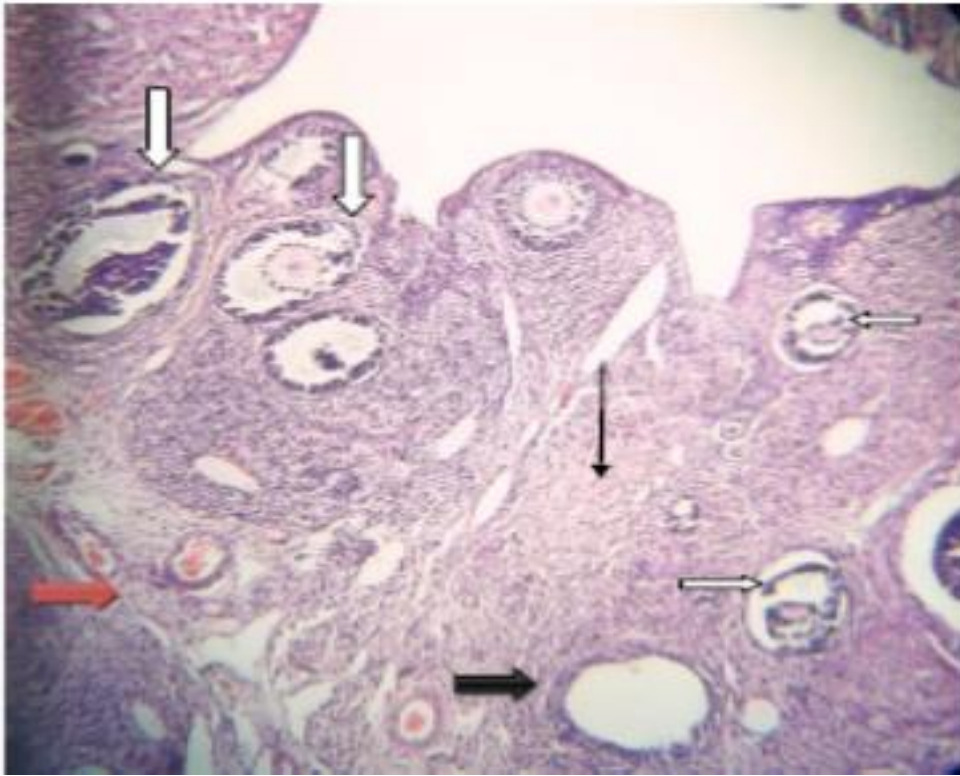


Plate 2: Photomicrograph of the positive control PCOS + distilled water. It shows an ovarian section with numerous follicles at different stages of maturation, containing abundant subcapsular cystic spaces (white arrows). Ovarian stroma shows incomplete luteinisation within the granular cells and prominent thecal cells (slender arrow). Areas of haemorrhage and slight vascular congestion are seen -red arrow (H&E, mag. X 100).

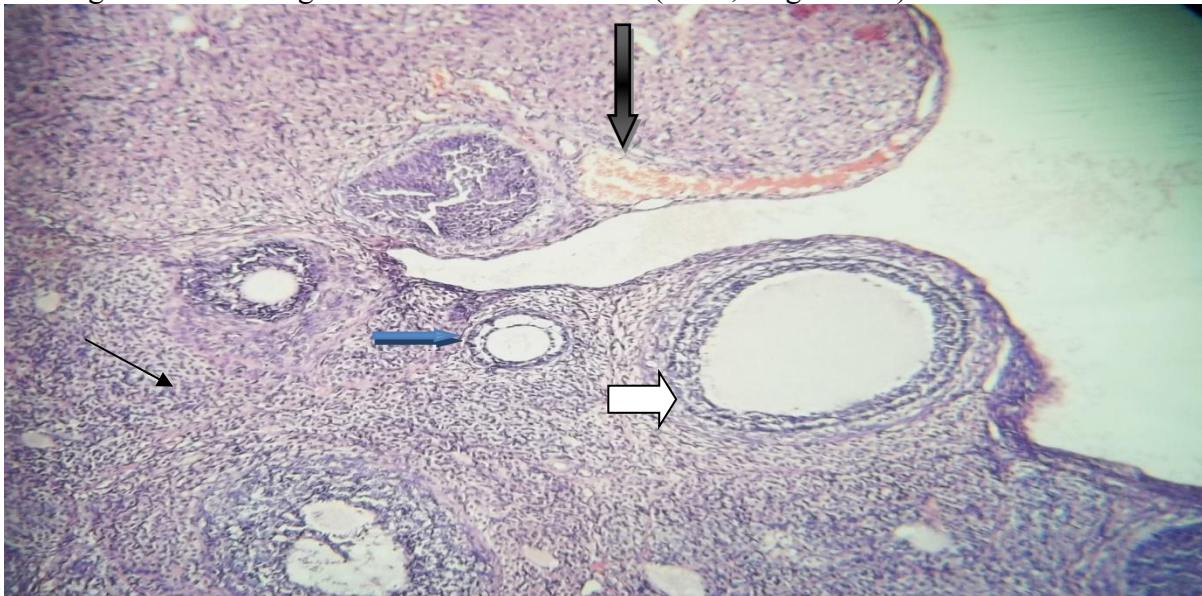


Plate 3. This is the photomicrograph of the confirmation of PCOS. It shows an ovarian section with different stages of follicular maturation containing primordial follicle with cystic space (blue arrow), prominent Graafian follicles (white arrow). Ovarian stroma shows luteinization within the granular cells and prominent thecal cells (slender arrow). Areas of haemorrhage and slight vascular congestion are seen (black arrow) X 100

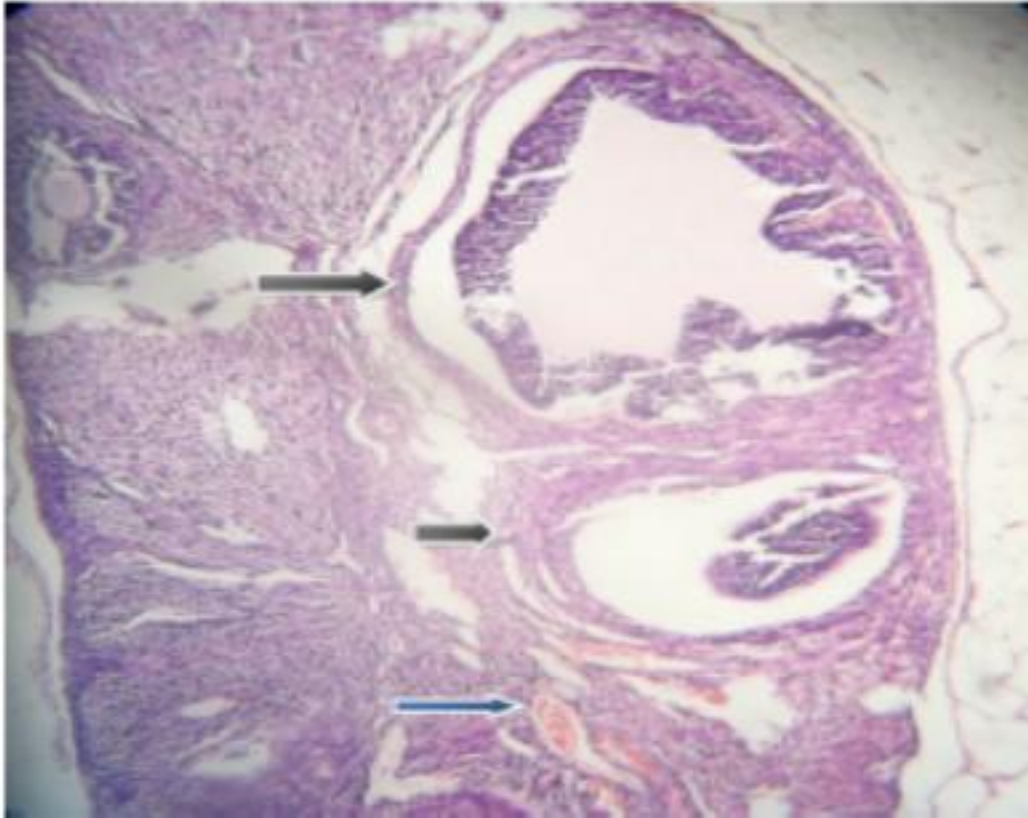


Plate 4: This is the photomicrograph of the letrozole-induced PCOS + castor oil 500 mg/kg BW group. It shows a section of an ovary section showing ovarian stroma with incomplete luteinization within the granular cells. There are few degenerating follicles with cystic spaces (black arrow), also seen is mild vascular congestion-blue arrow, (H&E, mag. X 100).

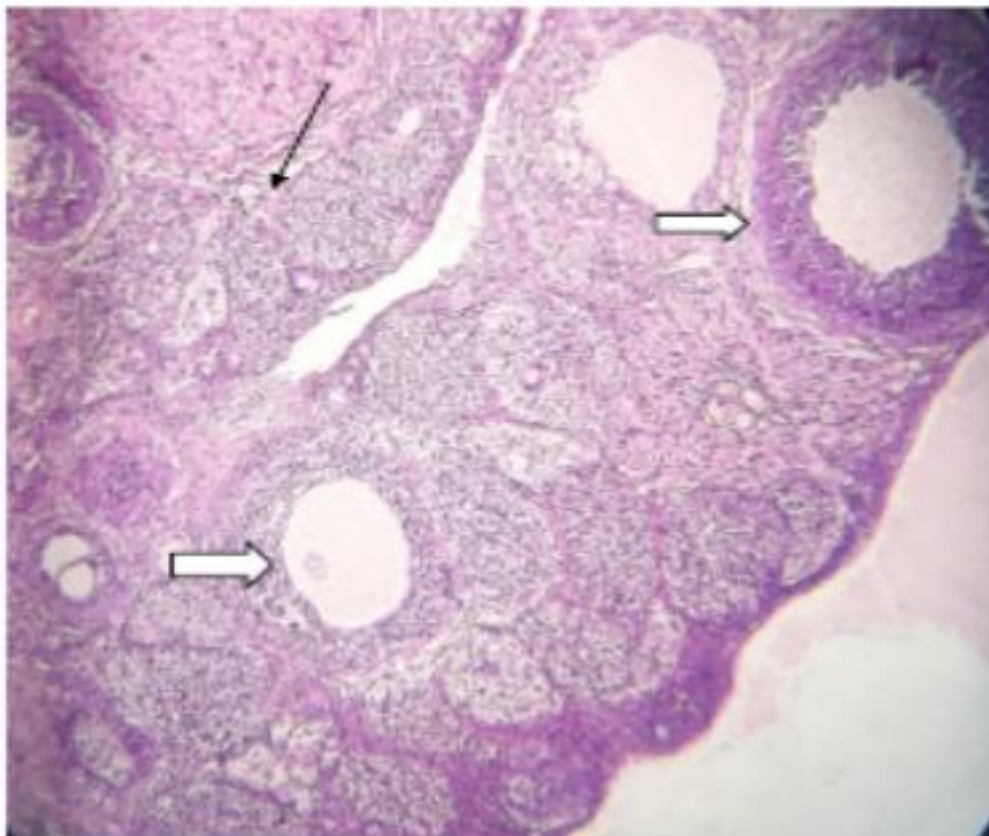


Plate 5: Photomicrograph of the letrozole-induced PCOS + castor oil 1000 mg/kg BW treated group. It shows a section of an ovary with normal ovarian stroma. Complete luteinization within the granular cells is seen (slender arrow). There are several follicles with differential stages of maturation consisting majorly the graafian follicles (white arrow) at the cortical region of the ovary (H&E mag. X 100).

Discussion

Polycystic Ovarian Syndrome (PCOS) has been reported as hormonal disorder affecting women presenting with reproductive, metabolic, and cardiovascular comorbidities (21). In the present study, a rat model was used to establish PCOS after treatment with letrozole, an aromatase inhibitor which blocks the conversion of testosterone and androstenedione to estradiol and estrone respectively and stimulates PCOS-like conditions (16) by causing hormonal imbalance, circulating hyperandrogenism and intraovarian androgen excess leading to the appearance of a polycystic ovary, hyperglycaemic condition, and metabolic disturbances. Follicular atresia and abnormal follicular development are also observed due to the induced elevation of androgen levels inside the ovary (21).

In a dose-dependent manner, castor oil decreased diestrus phase length and oestrous cycle irregularity (fig. 3 and 4) indicating a capacity to normalize the irregular cycle in the clinical setting and it may be a better treatment for menstrual irregularities associated with PCOS. The length of the cycle significantly decreased in the group administered castor oil (1000 mg/kg BW) in contrast to the prolonged cycle length recorded in the positive control group thereby suggesting an ameliorative role of castor oil on PCOS-induced reproductive cycle irregularities (fig. 4). This report is in agreement with a previous author (12).

The hormonal assay showed that at 1000 mg/kg BW of castor oil treatment, there was a reduction in plasma testosterone level when compared with the negative control

group (fig. 5), there was also an increase in oestrogen level with the group administered 500 mg/kg BW when compared with negative control (fig. 7). These observations are consistent with previous findings (14, 19). The decreased level of oestrogen and a significant increase in the serum testosterone level (fig. 5) of the positive control and castor oil treatment was consistent with reproductive hormonal derangement associated with PCOS which is one of the pieces of evidence in addition to the ovarian cysts that confirms the induction of PCOS in this group. This suggests that the induced PCOS was sustained until the 42nd day. The alleviation of the hormonal derangement by castor oil was shown by a decrease in all treatment groups when compared with the negative control (Fig. 4) which is consistent with previous findings (7) but the decrease was however not statistically significant.

The histology of the ovarian stroma with varying complete luteinization within the granular cells is seen (plate 4). Several follicles with differential stages of maturation consisting majorly the graafian follicles at the cortical region of the ovary demonstrated the ameliorative effects of castor oil.

In conclusion, Castor oil treatment resulted in a reduction in testosterone levels, and normalization of disrupted oestrous cycle phases and length following PCOS induction. The findings of the current study indicate that Castor oil may have therapeutic benefits in the management of PCOS and other related challenges.

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