J Endocrinol Reprod 17 (2013) 2 : 99 - 112 **Original Article**

Effects of aqueous extract of *Cnestis ferruginea* (Vahl ex DC) root on the biochemical and clinical parameters of anastrozole-induced polycystic ovarian syndrome rat model

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Summary

Aqueous extract of *Cnestis ferruginea* root was investigated for its effects on biochemical and clinical parameters of rat model with polycystic ovarian syndrome (PCOS). Female animals (150.46 ± 2.31 g) assigned to group A were not induced into PCOS, while those in groups B, C, D, E and F were induced into PCOS by oral administration of 0.5 mg/kg body weight of anastrozole dissolved in 1% CMC (2 mL/kg) daily for 21 days. Animals in groups A and B both received 0.5 mL of distilled water while those in groups C, D, E and F received same volume corresponding to 25, 50, 100 mg/kg body weight (bw) of the extract and 7.14 mg/kg bw of metformin, respectively, once daily for thirty days. Weight of the animals, vaginal cytology and levels of some hormones in serum were determined. The extract contained alkaloids (26.80 mg/L), tannins (0.10 mg/L), saponins (4.60 mg/L), flavonoids (14.60 mg/L) and anthraquinones (0.30 mg/L). The irregular and lengthened estrous cycle, absence of follicles in the ovarian stroma, elevated ($P<0.05$) serum testosterone and reduced ($P<0.05$) serum progesterone, LH and FSH were reversed and/or attenuated by the extract treatment in a manner similar to metformin. The increase in body weight of the animals was not significantly different. The extract treatment reversed the hyperandrogenemia and attenuated the irregular estrous cycle in PCOS-induced rats. The saponins and flavonoids present in the plant are considered responsible for the clinical benefits of *Cnestis ferruginea* roots in the management of PCOS.

Keywords: Anastrozole, *Cnestis ferruginea*, estrous cycle, hyperandrogenemia, polycystic ovarian syndrome

Introduction

Polycystic Ovarian Syndrome (PCOS) is the most common female heterogeneous endocrine and metabolic disorder and one of the leading causes of female sub-fertility affecting 4-10% women of reproductive age (Goldenberg and Glueck, 2008; Strowitzki et al., 2010; Teede et al., 2010). The precise cause of polycystic ovarian syndrome is unknown; however, it is considered to be a complex multi-genetic disorder characterized by disordered gonadotropin release, dysregulation of steroidogenesis, insulin insensitivity, chronic annovulation, menstrual irregularities, clinical or biochemical hyperandrogenism, and ultrasound data of polycystic ovaries (Himelein and Thatcher, 2006; Pagan et al., 2006; Soares et al., 2009). The features of PCOS manifest at any age ranging from childhood (premature puberty), teenage years (hirsutism, menstrual abnormalities), early adulthood and middle life (infertility, glucose intolerance) to later life (diabetes and cardiovascular diseases) (Norman et al., 2004). The currently available modalities of treatment use insulin sensitizers such as metformin and letrozole, since the central core of PCOS etiologies is through insulin resistance, while others include clomiphene citrate, tamoxifen and troglitazone (Moghetti et al., 2000; Tang et al., 2012). These orthodox medicines are not without mild to severe side effects such as hot flushes, arthritis, joint or muscle pain, irritability, mood swings, depression and bloating (Choi et al., 2005). As a result of these undesirable adverse side effects, and in the light of insulin resistance as one of the aetiologies of PCOS, there is pertinent need to search for an alternative medicine with proven anti-diabetic activity in botanicals that are efficacious, cheaper, and readily available, with fewer side effects, to manage this infertility problem.

Cnestis ferruginea (Connaraceae), locally called "Fura amarya", "otito" (Hausa tribe of northern Nigeria); "Okpu nkita", "amunkita" (Igbo tribe of southeast Nigeria) and "Akara oje", "Bonyin bonyin" (Yoruba tribe of Southwest Nigeria), is a climber of deciduous forest widely dispersed in West Africa and other tropical parts of Africa. The plant is about 3.0-3.6 m tall with orange to red fruits and velvety hairs on the follicle (Burkill, 1985). The plant has an ornamental value, and different parts of the botanical have been claimed to be used across tropical Africa to manage diverse ailments such as constipation (leaf or root), dysentery and gonorrhoea (leaf), pains and inflammation (powdered bark), headache (root back paste), bronchitis (leaf decoction), eye troubles (leaf sap), dysmenorrhoea (leaf or root), migraine and sinusitis (root sap or powder), toothache (root), conjunctivitis (fruit juice),

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abortion, sexual dysfunction and ovarian troubles (root decoction), rheumatism, stroke and syphilis (Irvine, 1961; Burkill, 1985).

C. ferruginea have been scientifically investigated for some of its acclaimed pharmacological activities such as aphrodisiac activity in paroxetine-induced sexual dysfunction male rat model, hepatoprotective effect, laxative activity, analgesic and anti-inflammatory effects, hypoglycemic and anti-stress potential as well as its toxicological, haematological and reproductive effects (Ishola and Ashorobi, 2007; Olayemi et al., 2008; Atere and Ajao, 2009; Adisa et al., 2010; Olayemi et al., 2010; Ishola et al., 2011; Yakubu et al., 2011; Akharaiyi et al., 2012; Yakubu and Nurudeen, 2012).

Despite all these pharmacological and toxicological studies, there is dearth of scientific studies that evaluated the acclaimed use of the plant in managing gynaecological problems. Therefore, this study was carried out to substantiate the pharmacological effects of *C. ferruginea* roots against anastrazole-induced PCOS in female Wistar rat model using biochemical and clinical parameters as indices.

Materials and methods

Materials

Plant material and authentication

Cnestis ferruginea roots were purchased from herb sellers at a market (Baboko Market, Ilorin, Nigeria) and authenticated at the Department of Plant Biology, University of Ilorin, Ilorin, Nigeria. Voucher specimen (I.U.V. No. 007) was deposited in the Herbarium of the Department.

Experimental animals

Fifty-six female albino rats (*Rattus norvegicus*) weighing 150.46 ± 2.31 g were obtained from the Animal Holding Unit of the Department of Physiology, University of Ibadan, Ibadan, Nigeria. The animals were kept in well-ventilated house conditions (temperature: 22±3^{o}C; photoperiod: 12h/12h light/dark cycle; humidity: 45-50 %) and fed with rat pellets (Vital Feeds, Grand Cereals, Jos, Nigeria) and water *ad libitum*.

Assay kits and drugs

Progesterone, testosterone, FSH and LH assay kits were products of Diagnostics Laboratories, Freiburg, Germany, while anastrozole was from AstraZeneca Pharmaceuticals LP, Newark, Delaware, USA. Metformin hydrochloride was manufactured by Merck Sante Pharmaceuticals, Semoy, France.

Methods

Preparation of aqueous extract of Cnestis ferruginea root

A known weight (300 g) of *C. ferruginea* root was washed, cut into very thin slices, oven-dried at 40ºC and pulverized in a blender (Crownstar Blender CS- 242B, Trident (H.K) Ltd, China) after which 50 g of the powder was extracted in 500 ml of distilled water for 48 hr and filtered (Whatman No. 1 filter paper). The filtrate was lyophilized to give 10.82 g corresponding to a yield percent of 21.64%. The resulting powder was reconstituted in distilled water to give the required doses of 25, 50, 100 mg/kg body weight used in this study. The doses used in the present study were adopted from the previous study on the aphrodisiac activity of the same plant as described by Yakubu and Nurudeen (2012).

Phytochemical screening of the extract

Phytochemical screening of the *C. ferruginea* root was carried out as described for alkaloids, steroids, phenolics, flavonoids, saponins, glycosides, anthraquinones, and tannins (Wall et al., 1954; Harborne, 1973; Odebiyi and Sofowora, 1978; Trease and Evans, 1983; Sofowora, 1993; Awe and Sodipo, 2001). Quantitative analysis of the detected phytochemicals was carried out for flavonoids, alkaloids, saponins, phlobatannins, anthraquinones, and tannins (Van-Burden and Robinson, 1981; Brunner, 1984; El-Olemy et al., 1994; Obadoni and Ochuko, 2001; Edeoga et al., 2005).

Induction and confirmation of polycystic ovarian syndrome

Fifty-six rats were randomized into two groups (I and II). Group I – (control) consisted of twelve rats which received 0.5 ml of the vehicle only (1% aqueous solution of carboxmethlycellulose [CMC]) while animals in group II, which consisted of forty-four rats, received the same volume corresponding to 0.5 mg/kg body weight of anastrozole dissolved in 1% CMC (2 mL/kg) daily for 21 days (Kafali et al., 2004). The stage of estrous cyclicity in the rats was monitored on daily basis for 12 days for the presence of the predominant cell type in the vaginal smears using a light microscope (Olympus, Shinjuku, Tokyo, Japan). Twenty four hours after the last dose of anastrozole, four rats were randomly selected from each group, sacrificed and an aliquot of the blood sample obtained from the jugular veins. A known volume of the blood (5 ml) was used to prepare the serum as described by Yakubu et al. (2008). The concentrations of serum reproductive hormones were assayed as described for

testosterone, progesterone, LH and FSH (Uotila et al., 1981; Wang et al., 1993; Tietz, 1995).

Vaginal cytology

Vaginal smears were obtained on daily basis between 9:00 - 10.30 am. The rats were held at the thorax, ventral side upper, whilst providing lumbar support. Vaginal secretions were collected using cotton-tipped swabs softened with a drop of physiological saline. After about 1-2 inches of the swab was inserted into the vagina of the female rats and the end was rotated through 2-3 revolutions (which allowed the cotton tip to pick an adequate load of cells), the swab was then gently withdrawn and the tip of the cotton rolled along the length of a glass slide. The dried smear was fixed by dipping it in a container of 70% alcohol. The slides were then stained with 0.5% methylene blue solution, rinsed in tap water and examined under ×10 objective lens (without the condenser lens) of a light microscope as described by Marcondes et al. (2002). A digital camera (Sony Corporation Digital Camera, Minato, Tokyo, Japan) was used to obtain the photomicrographs.

Animal grouping and extract administration

The PCOS-positive female albino rats with evidence of irregularities in their estrous cycle were randomly assigned to five groups (B - F) of eight animals each. Animals in group A (control), which were not induced into PCOS, were administered 0.5 ml of distilled water while those in groups B, C, D, E, and F, which were induced into PCOS also received 0.5 mL of distilled water and same volume corresponding to 25, 50, 100 mg/kg bw of aqueous extract of *C. ferruginea* root and 7.14 mg/kg bw of metformin (reference drug), respectively. The extract, metformin and distilled water were administered daily for thirty days. All the animals in each of the groups were weighed at intervals of ten days. The study was conducted following the guidelines on the care and use of laboratory animals of the Ethical Committee of the Department of Biochemistry, University of Ilorin, as well as according to the guidelines of European Convention for the Protection of Vertebrate Animals and other scientific purposes (ETS, 2005).

Histopathological examination

The animals were quickly dissected and the right ovary was excised from each rat, cleaned of fatty layers and fixed in 10% formalin for at least 24 hr before the slides were prepared according to the procedure described by Drury and Wallington (1980). The thin sections (5 µm thick) were stained with hematoxylin and eosin (H & E) dyes and examined under the light microscope (Olympus, Model CX21FSI, Philippines) at x400.

Preparation of serum

Twenty-four hours after the exposure period (30 days of treatment), the rats in the oestrus phase were weighed individually and thereafter sacrificed under light ether anaesthesia. The neck area was cleared of fur and the skin was dissected to expose the jugular veins. The jugular veins were slightly displaced from the neck region (to prevent contamination of the blood with interstitial fluid) and then cut with a sharp sterile blade. The rats were held head down and 5 ml of the blood was allowed to bleed into clean, dry centrifuge tubes, which were left undisturbed at room temperature for 10 minutes to clot. The tubes were centrifuged at 103 xg for 15 minutes using Uniscope Laboratory Centrifuge (Model SM800B, Surgifriend Medicals, Essex, England). The sera were thereafter aspirated using Pasteur pipette into clean, dry sample bottles and frozen overnight before being used for the determination of serum testosterone, progesterone, FSH and LH levels as earlier described.

Statistical analysis

Statistical analysis of the data was performed using Statistical Package for Social Sciences, version 15.0 (SPSS Inc., Chicago, USA). Data were expressed as the mean ± SEM of eight determinations unless otherwise stated. Data which were analysed using One Way Analysis of Variance followed by Duncan Multiple Range Test for multiple comparison were complemented with unpaired Student's t-test. Differences were considered statistically significant at $P < 0.05$.

Results

Phytochemical analysis of aqueous extract of *C. ferruginea* root revealed the presence of alkaloids, tannins, saponins, flavonoids and anthraquinones while phlobatannins, phenolics, steroids and cardiac glycosides were not detected. Among the detected phytochemicals, alkaloids were the highest while tannins and anthraquinones were present in very small amounts (Table 1).

The body weight of the animals administered anastrozole increased significantly ($P<0.05$) during the induction of PCOS in a manner similar to the control (Table 2). At the end of the induction period, there was no significant difference ($P>0.05$) in the body weight of the PCOS-induced animals as well as the control (Table 2). However, the percentage weight gain was

Table 1 Phytochemical constituents of the aqueous extract of *Cnestis ferruginea* root

Phytochemicals	Concentration (mg/L)
Alkaloids	26.80±0.10
Tannins	0.10±0.00
Saponins	4.60±0.10
Flavonoids	14.60±0.70
Anthraquinones	0.30±0.01
Phlobatannins	Not detected
Phenolics	Not detected
Steroids	Not detected
Cardiac glycosides	Not detected

n = 3 ± SEM

Table 2 Weight of animals during the period of induction of PCOS

	Weight of animals (g)		
Groups	**Day 0**	**Day 21**	**Weight gain by the animals (%)**
Group I (CMC)	155.67± 6.67[a]	183.50± 9.34[b]	17.88
Group II (PCOS-induced)	150.21± 2.08[a]	182.38± 3.03[b]	21.42

Data are mean of eight determination ± SEM.

Test values carrying superscripts, different from the control for each parameter, are significantly different ($P<0.05$).

Day 0 represent weight of the animals before induction of PCOS

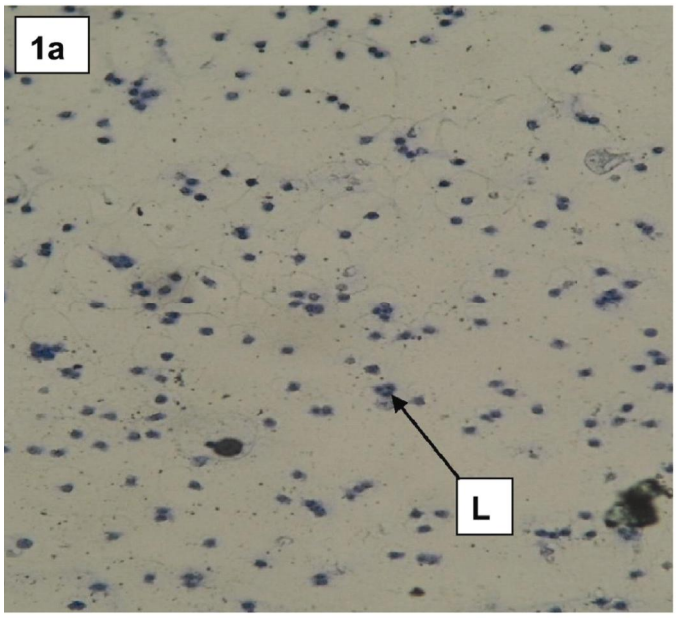
Day 21 represents weight of the animals 21 days after the induction of PCOS

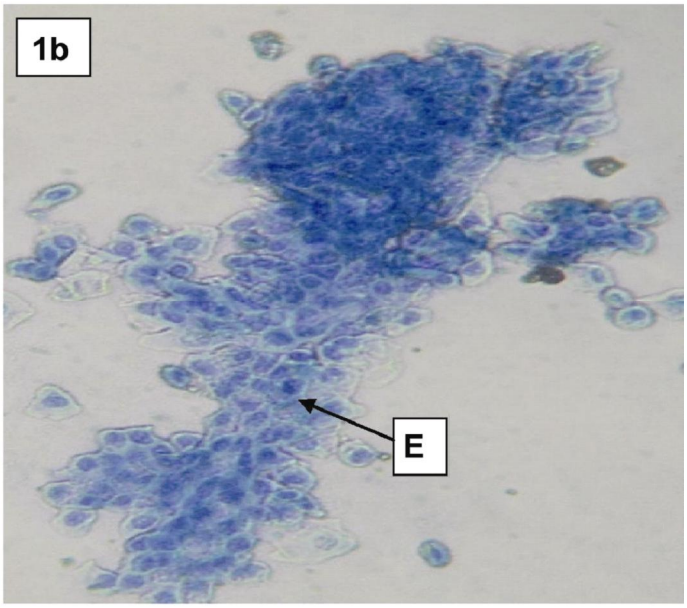
Table 3 Effect of aqueous extract of *Cnestis ferruginea* root on weight of PCOS rats

	Weight of animals (g)		
Groups	**Before extract administration**	**After extract administration**	**Weight gain by the animals (%)**
Non-PCOS + distilled water	182.50 ± 5.50[a]	193.75 ± 5.25[b]	6.16
PCOS + distilled water	187.25 ±13.70[a]	193.25 ± 10.96[b]	3.20
PCOS + 25 mg/kg bw of extract	180.50 ± 5.52[a]	191.25± 04.77[b]	5.96
PCOS + 50 mg/kg bw of extract	170.50 ± 3.01[a]	185.75 ± 03.15[b]	8.94
PCOS + 100 mg/kg bw of weight	183.50 ± 5.69[a]	186.75 ± 02.98[b]	1.77
PCOS + 7.14 mg/kg bw of Metformin	190.00 ± 7.44[a]	201.25 ± 08.59[b]	5.92

Data are mean of eight determinations ± SEM.

Test values carrying superscripts different from the control for each parameter are significantly different ($P<0.05$).





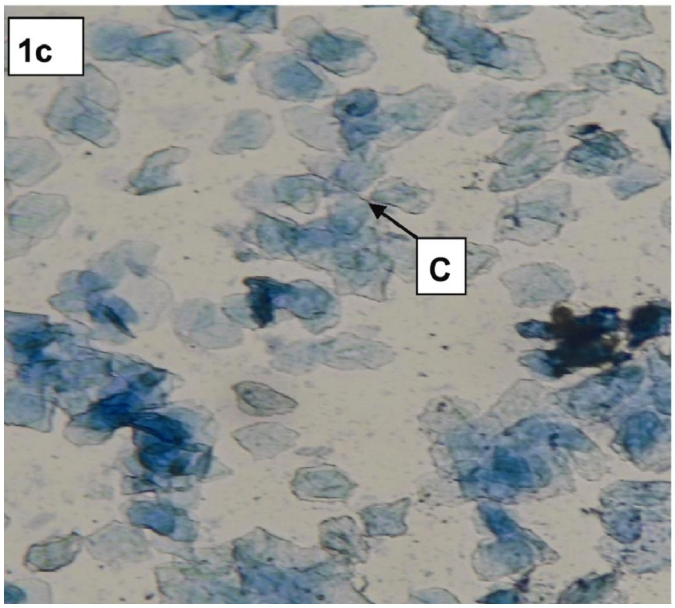


Plate 1a : Photomicrograph of vaginal smear of dioestrus phase of the oestrous cycle in female rats (× 400; Methylene blue)

Plate 1b: Photomicrograph of vaginal smear of pro-oestrus phase of the oestrous cycle of female rats (× 400; Methylene blue)

Plate 1c: Photomicrograph of vaginal smear of oestrus phase of the oestrous cycle of female rats (× 400; Methylene blue)

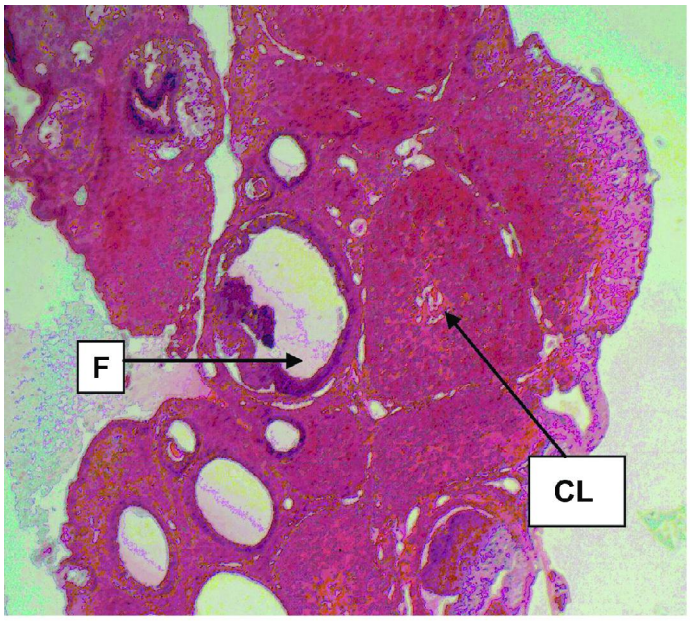


Plate 2a: Photomicrograph of cross section of right ovary of normal cycling rat administered carboxymethyl cellulose (×400; H & E) CL-corpus luteum, F-developing follicles

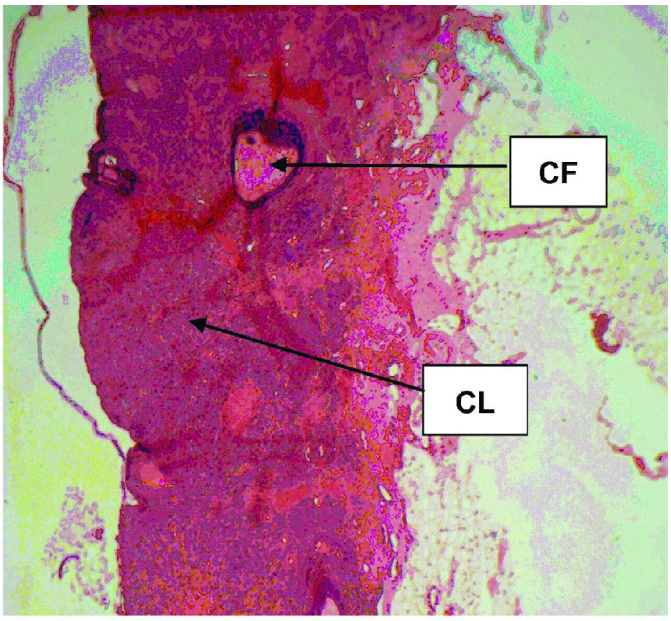


Plate 2b: Photomicrograph of cross section of the right ovary of a rat administered anastrozole (×400; H & E) CL-corpus luteum, CF-cystic follicle

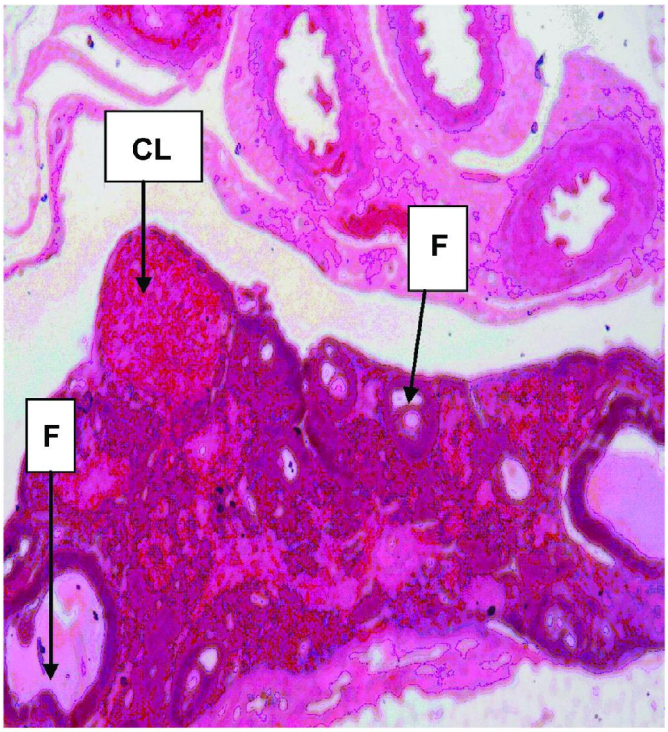


Plate 2c: Photomicrograph of cross section of right ovary of anastrozole-induced PCOS rats treated with aqueous extract of *Cnestis ferruginea* root (×400; H & E) CL-corpus luteum, F-developing follicles

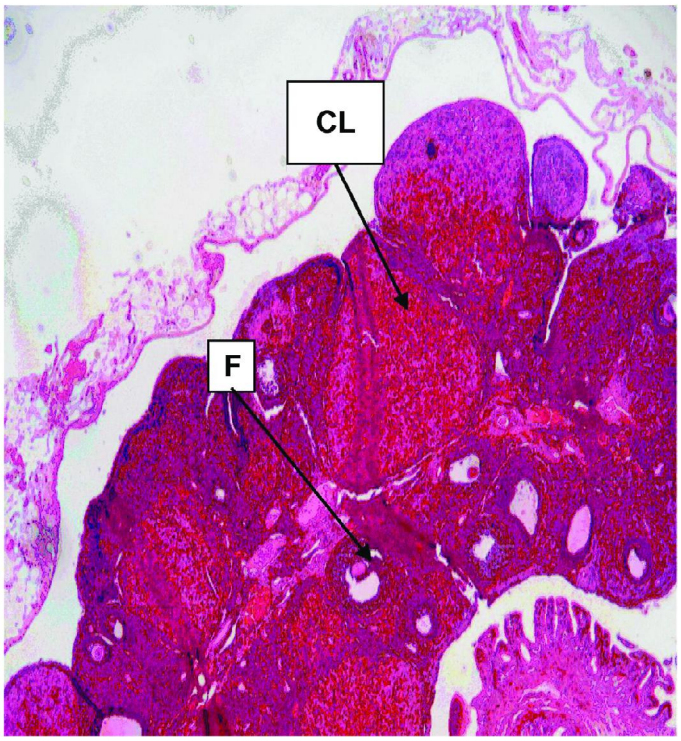


Plate 2d: Photomicrograph of cross section of right ovary of anastrozole-induced PCOS rat treated with Metformin (×400; H & E) CL-corpus luteum, F-developing follicle

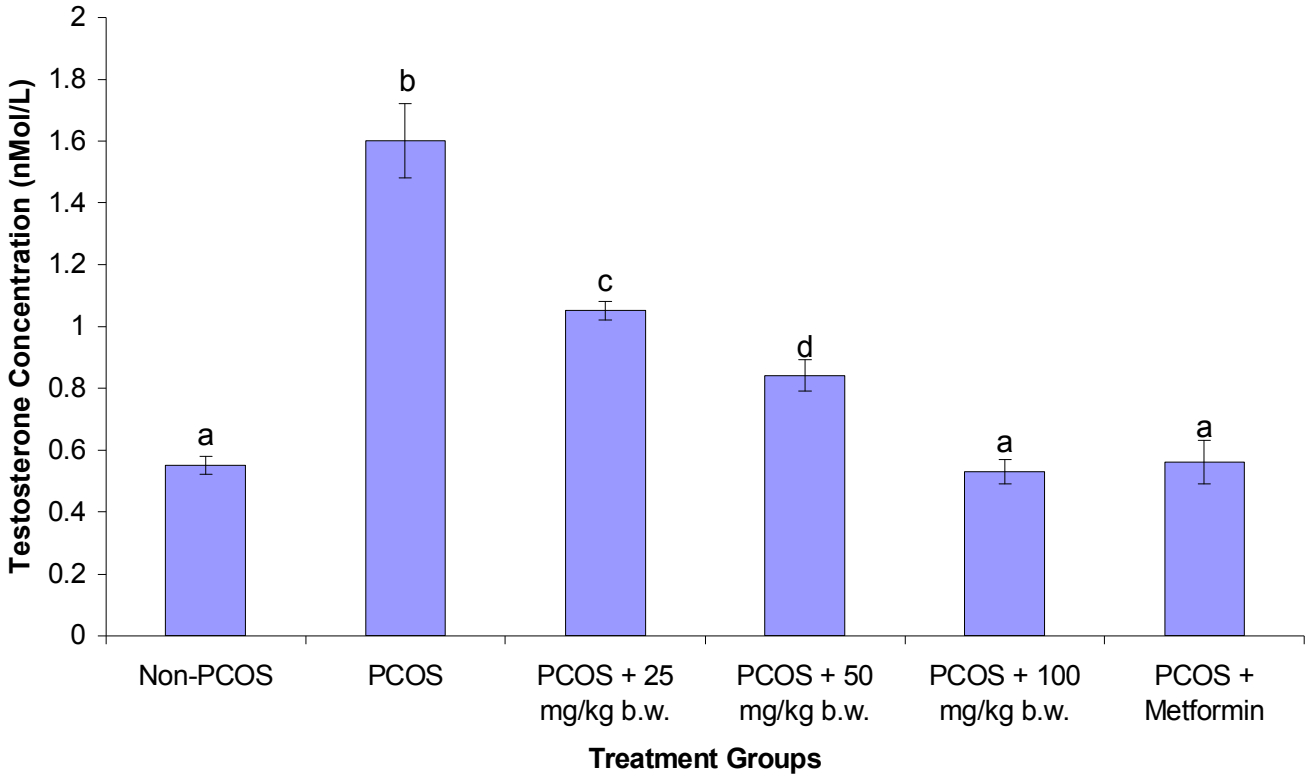


Fig 1: Testosterone concentration of PCOS female rats administered aqueous extract of *C. ferruginea* root

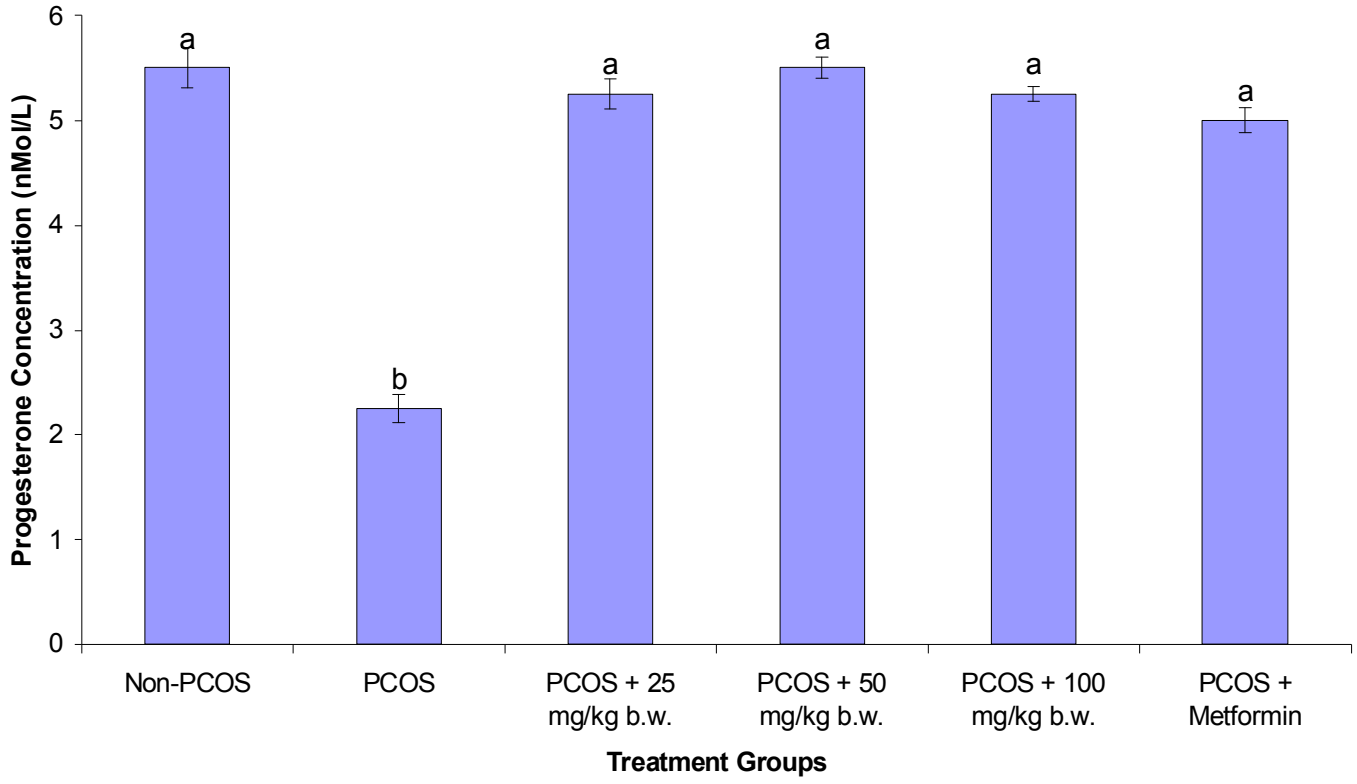


Fig. 2: Progesterone concentration of PCOS female rats administered aquoeus extract of *C. ferruginea* roots

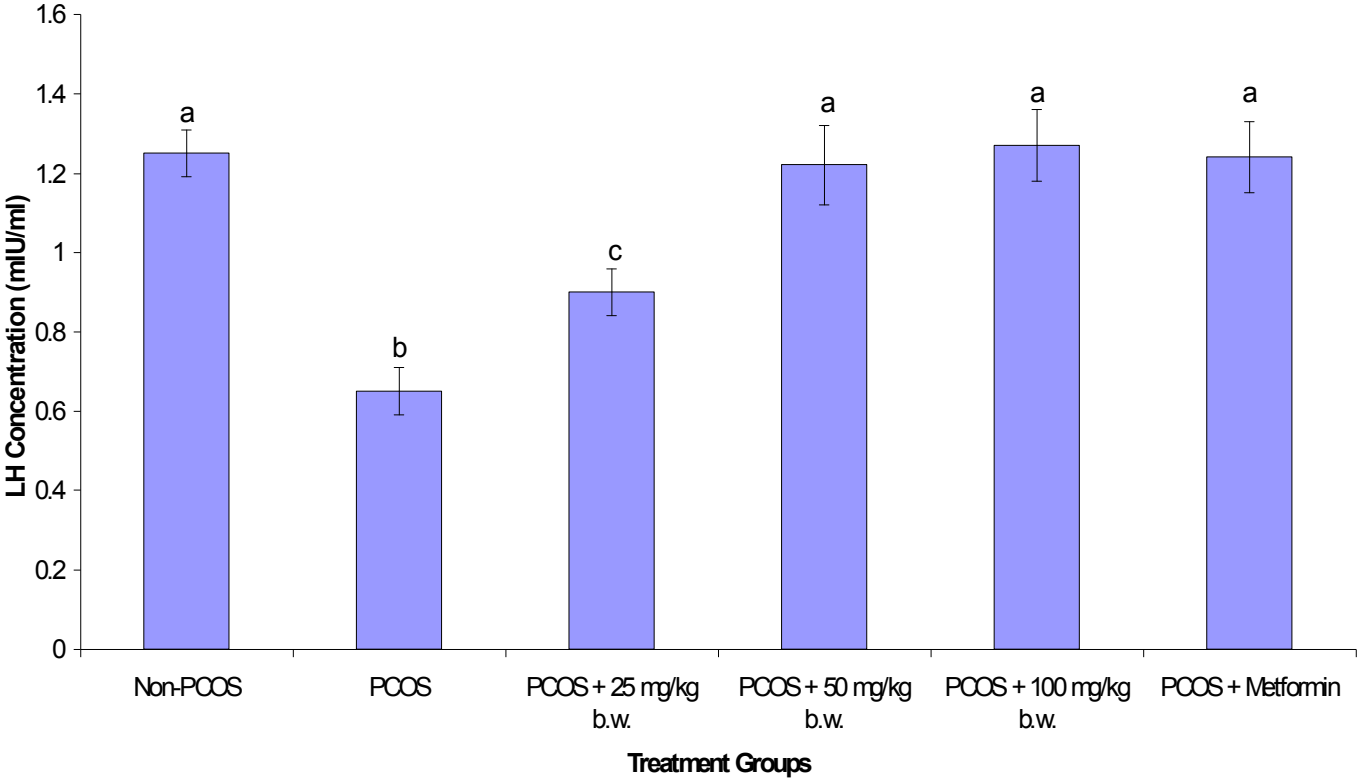


Fig. 3: Luteinizing hormone concentration of PCOS female rats administered aquoeus extract of *C. ferruginea* roots

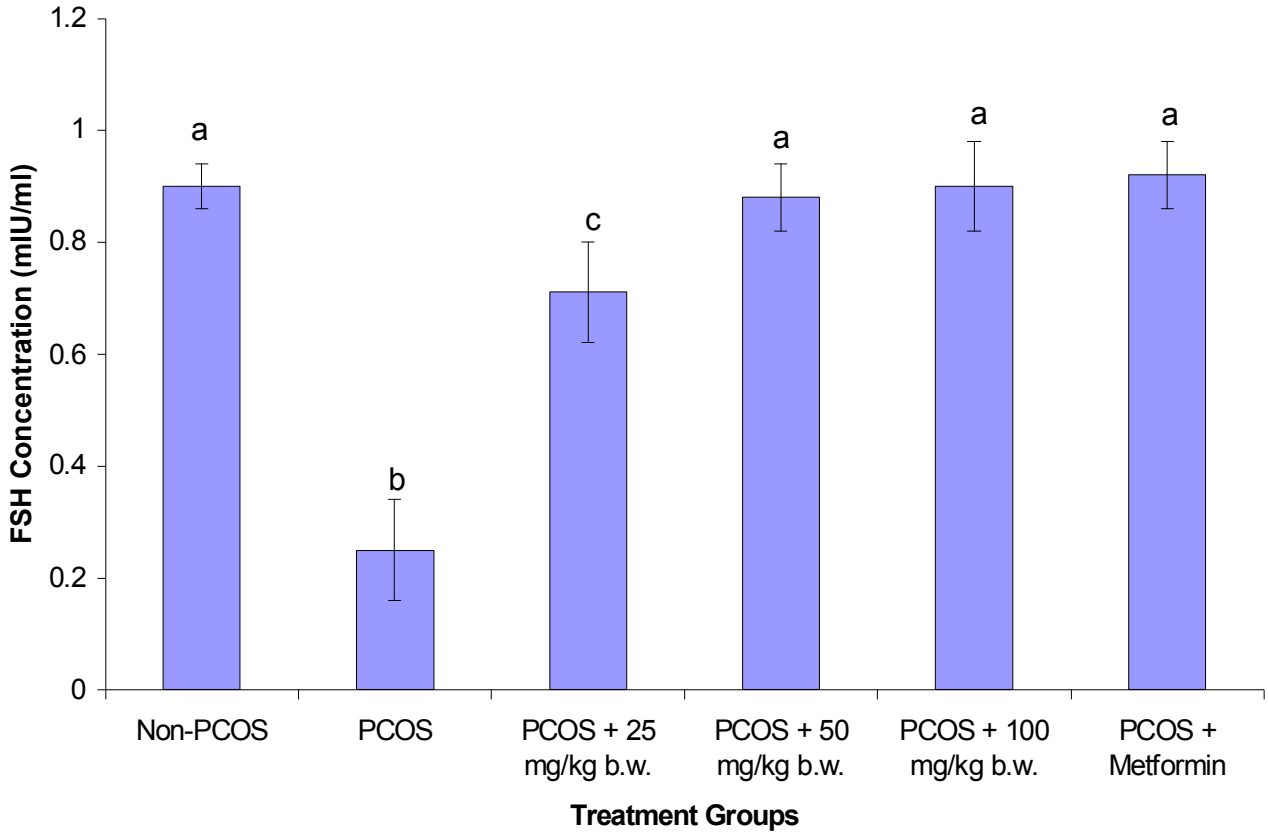


Fig. 4: FSH concentration of PCOS female rats administered aqueous extract of *C. ferruginea* roots

higher in PCOS-induced animal than those not induced into PCOS (distilled water treated). In addition, the body weight of the PCOS-induced animals administered various doses of the extract increased significantly in a manner similar to the PCOS-induced animals treated with metformin and distilled water as well as those not induced into PCOS. The weight gained by the animals was highest in the PCOS animals administered 50 mg/kg body weight of the extract (Table 3).

Anastrozole-induced PCOS animals were continuously in oestrus phase that spanned a period of 4-5 days which then prolonged the estrous cycle from the normal 4 days cycling to 8-9 days. In contrast, this was absent in the PCOS-induced animals treated with the extract. Furthermore, leukocytes, epithelial cells and cornified cells were seen in the vaginal smears of PCOS-induced animals treated with the extract and metformin (Plates 1a-c).

Administration of anastrozole alone to the animals increased the serum testosterone content by three-fold the control value whereas the extract at 25, 50 and 100 mg/kg bw significantly reduced the androgen level towards the control value. The reduction in the testosterone content of the animals at 50 and 100 mg/kg body weight of the extract as well as PCOS animals treated with metformin compared favourably with the distilled water treated non-PCOS animals (Fig. 1).

The serum progesterone level of the anastrozole-induced PCOS animals decreased significantly when compared with the distilled water treated non-PCOS rats. In contrast, administration of all the doses of the extract decreased the concentration of the hormone in a manner that compared well with both the PCOS animals treated with metformin and the distilled water treated non-PCOS animals (Fig. 2).

The levels of serum LH and FSH in anastrozole induced-PCOS animals decreased significantly when compared with the distilled water treated non-PCOS animals (Figs. 3, 4). However, the 25, 50 and 100 mg/kg body weight of the extract as well as the metformin treatment caused increase in the serum LH and FSH levels of the anastrozole-induced PCOS animals. Nonetheless, the increases in the levels of hormones did not compare favourably with the distilled water treated non-PCOS animals (Figs. 3, 4).

The histology of the right ovaries of the CMC treated control rats revealed developing and ruptured follicles as well as numerous corpora lutea in the ovarian stroma whereas the anastrazole administered animals presented with very scanty follicles and corpora lutea (Plates 2a and 2b). In contrast, the ovaries of the anastrozole-induced PCOS rats treated with aqueous extract of *C. ferruginea* and metformin had distinct and numerous developing follicles and corpora lutea (Plates 2c and 2d).

Discussion

PCOS presents with many clinical symptoms such as oligomenorrhea, hyper-androgenism, disrupted folliculogenesis, insulin resistance and chronic annovulation which leads to metabolic and reproductive dysfunction especially infertility. This study reports on the efficacy of *C. ferruginea* root in induced-PCOS rat model adopting biochemical and clinical parameters.

Anastrozole is a non-steroidal aromatase inhibitor that induces PCOS in animals by blocking the conversion of androgens to estrogens (Kafali et al., 2004). The inhibition of aromatase activity leads to increase of ovarian androgens which, in turn, lead to hyperandrogenism, a hallmark of PCOS (Diamanti et al., 2005). PCOS, according to Rotterdam criteria, is diagnosed by the presence of at least two of the following three criteria: clinical or biochemical hyperandrogenism, oligo- or amenorrhea, and the presence of PCOS. Normally, testosterone and androstenedione are converted to estradiol and estrone, respectively, with the help of cytochrome P_{450} aromatase, which plays an important role in ovary's hormonal balance. Thus, the decreased activity of aromatase results in increased ovarian production of androgens and development of PCO condition (Dunaif et al., 1996). Excess androgen level may alter gonadotropin-induced estrogen and progesterone in the follicles (Wachs et al., 2008). Therefore, anastrozole in the present study might have inhibited aromatase activity and conversion of androgens (notably testosterone) to estrogens with the clinical manifestation of the elevated levels of testosterone as well as decrease in the levels of gonadotropins (LH and FSH). This hormonal imbalance created by anastrozole resulted in irregular and/or prolonged estrous cycle as well as reduced corpora lutea and almost absence of follicles. All the changes in the present study, which are within the patho-physiological core of PCOS, suggest that the animals were induced with a complex endocrine and metabolic disorder associated with ovulatory dysfunction by treatment with anastrozole. The findings in the present study with respect to LH and FSH as well as clinical and

biochemical parameters following PCOS induction with anastrazole agree with the previous reports by Brawer et al. (1986) and Kafali et al. (2004).

Gradual and consistent increase in body weight of animals with time which culminates in obesity, a non-reproductive metabolic abnormality, is often exhibited in some women with PCOS (Pasquali et al., 2011). Although, body composition or weight of adipose depot was not analyzed, the minimal increase in weight of all the experimental animals suggests a gradual development of the adipose tissue with age and may not necessarily be due to enlargement of the adipocyte (Roland et al., 2010). In addition, such development of the adipocyte was not treatment related since the body weight increased in all the experimental animals including those treated with the extract and reference drug, metformin. It is also unlikely that the animals will be predisposed to obesity considering the minimal rate of increase in the body weight of the rats. Although the increase in weight in the PCOS-positive animals agrees with the reports by Maharjan et al. (2010), the increase in weight in the extract treated PCOS animals in the present study contradict their findings.

The constant state of vaginal cornification or persistent oestrus in the animals administered anastrozole suggest prolonged luteal phase subsequent to the lengthening of the estrous cycle (Nelson and Felicio, 1985). The ability of the extract to attenuate the lengthened estrous cycle in the anastrazole-induced PCOS animals consistently emphasize its use in managing gynaecological problems such as irregular menstruation. The normalization of the oestrous cycle in the animals which was also supported by the appearance of developing follicles that hitherto were reduced or absent in the anastrazole-induced PCOS animals may be due to the presence of flavonoids in the aqueous extract of *C. ferruginea* root which are structurally analogous to estrogens, the primary female sex hormones.

The increase in serum testosterone following the administration of anastrozole alone to the animals suggest that aromatization of androgens to estrogens was impaired leading to hyperandrogenism (Speroff et al., 2004). Such hyperandrogenism, as reflected by elevated serum testosterone level, was decreased at 25 mg/kg bw whereas the 50 and 100 mg/kg bw attenuated the hypertesosteronemia. It is possible that some phytochemical constituent of the extract may have activated aromatase which eventually converted androgens to estrogen leading to reduction or reversal of androgens to the control values. Similarly, the reduction in the level of progesterone in the anastrozole-induced PCOS animals could be responsible for the persistent oestrus phase, characterized by maintenance of corpora lutea beyond the normal 2 days life span (vom Saal et al., 1994). The elevation in the concentration of serum progesterone by *C. ferruginea* root extract may be responsible for the reversal of the luteal phase dysfunction and restoration of normalcy of the estrous cycle. Furthermore, the extract should have brought about feedback inhibition of gonadotropins (LH and FSH) since their increase resulted in corresponding decrease in the serum testosterone levels. All these together emphasize the ability of the extract in attenuating the biochemical, clinical and histological features of PCOS.

Ovulation of matured follicles is induced by a large surge of LH secretion. Therefore, the reduction in serum LH by anastrozole which could contribute to decrease or absence of matured follicles as well as disruption of the estrous cycle as evident in the present study was reversed by the extract. The restoration of normal ovarian physiology by the extract could be a consequence of stimulatory effect on the hypothalamus which enhanced the secretion or surge of LH (Park et al., 2002). The findings in the present study contradict the reports of Baravalle et al. (2006) and Zurvarra et al. (2009) and such disparity may be due to difference in the estrus cycle of the animals prior to sacrifice.

The presence of bioactive agents such as alkaloids, flavonoids, tannins, saponins and anthraquinones in the aqueous extract of *Cnestis ferruginea* root might account for the pharmacological effects. For example, saponins have been reported to enhance the synthesis of progesterone (Yang et al., 2003). Therefore, the presence of alkaloids in the extract of *C. ferruginea* root may not be unconnected with the pro-progesteronic activity displayed by the botanical. Again, since PCOS condition has been reported to reduce the level of antioxidant enzymes/molecules (Farzadi et al., 2013), the flavonoids, apart from playing an antioxidant role in the PCOS rats, may also increase the production of sex-hormone-binding globulin, by which some free testosterone can be bound, reduce not only its concentration in the serum but also some of the testosterone-related problems seen in PCOS animals. These phytochemicals may be responsible for the acclaimed use of the plant in the management of gynaecological problems.

In conclusion, aqueous extract of *Cnestis ferruginea* root not only attenuated hyperandrognemia and other reproductive hormones investigated in the present study, but also restored the irregular cycle and ovarian physiology to normal in the PCOS animals. This pharmacological activity may be attributed to the presence of alkaloids and/or flavonoids. This study justifies the acclaimed folklore use of *C. ferruginea* in the management of gynaecological problems.

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