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AQUEOUS EXTRACT OF *SOLANUM NIGRUM* LEAF REVERSED REPRODUCTIVE DYSFUNCTIONS ASSOCIATED WITH ANASTROZOLE-INDUCED POLYCYSTIC OVARIAN SYNDROME IN RATS

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Abstract

Background: Polycystic Ovarian Syndrome (PCOS), also referred to as Stein-Leventhal syndrome, is a common endocrine disorder that impacts 12-21% of women in their reproductive years. The aqueous extract of *Solanum nigrum* leaves (AEoSNL) was investigated for its therapeutic effects on reproductive dysfunctions associated with anastrozole-induced PCOS in Wistar rats.

Method: Sixteen female Wistar rats (160.46 ± 4.11 g) were divided into four groups (A-D): group A were not induced into PCOS, while those in groups B, C and D 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, C and D received 0.5 ml co-administration of metformin (7.14mg/kg/day) and clomiphene citrate (2mg/kg/day) metformin, and 200 mg/kg body weight (bw) of AEoSNL once daily for fourteen days post-induction. Vaginal cytology, ovarian histology and levels of some reproductive hormones in the serum were determined.

Result: Anastrozole administration resulted in disrupted oestrous cyclicity, ovarian cyst formation and altered hormonal levels thereby replicating PCOS-like symptoms. The administration of 200mg/kg (bw) AEoSNL to PCOS rats significantly decreased ($P \leq 0.05$) serum testosterone, follicle stimulating hormone, progesterone and luteinizing hormone (LH)

concentration but there was no significance difference (P≥0.05) in the prolactin level when compared with the control. The AEoSNL reversed the hyperandrogenemia, LH hypersecretion and irregular estrous cycle in PCOS-induced rats.
Conclusion: This study suggests that, 200 mg/kg(bwt) of AEoSNL exhibit therapeutic functions in anastrozole-induced PCOS in rats and can be explored in an anti-PCOS drug design subject to further experimentation.
Key words: Anastrozole, Clomiphene citrate, Metformin, Oestrous, Polycystic Ovarian Syndrome, *Solanum nigrum*

Introduction

Polycystic Ovary Syndrome (PCOS) is a hormonal disorder prevalent in females primarily in those of reproductive age[1]. PCOS is characterized by an imbalance in sex hormones, particularly an excess of androgens (male hormones like testosterone) in the body and this hormonal imbalance can disrupt the normal functioning of the ovaries[1]. One of the hallmark features of PCOS is anovulation, which connotes that the ovaries do not consistently release eggs during the menstrual cycle. This can lead to irregular or absent menstrual periods[2]. PCOS can manifest with various symptoms, including irregular menstrual periods, heavy or prolonged bleeding, acne, excess facial and body hair (hirsutism), hair thinning on the scalp, weight gain as well as metabolic disorders such as insulin resistance, type 2 diabetes, and cardiovascular problems[3]. The available treatment options involve lifestyle changes (dietary modifications), drugs (such as metformin), exercise to help with weight management, medications to regulate the menstrual cycle (clomiphene citrate), and medications to manage the hormonal and metabolic aspects of the condition[1]. However, some of the medications used in the treatment of PCOS have been reported to have various side effects[4]. This has necessitated the need scientifically explore alternative therapies for the treatment of PCOS. Folklore medicine has been in existence for years and its usage by women has been on the increased in the recent times[5].

Solanum nigrum (Black nightshade) is a multipurpose plant species belonging to Solanaceae family[6]. The leaves of *Solanum nigrum* are notable for their anti-inflammatory, antioxidant, antibacterial, anti-diabetic, and maybe anti-cancer properties[6,7,8]. Some studies have reported *Solanum nigrum* having the potential to improve insulin resistance and enhance lipid metabolism[9]. However, in the management of polycystic ovary syndrome and fertility disorders, there is limited scientific reports on the efficacy of *Solanum nigrum* leaves on reproductive dysfunctions. Thus, this study was aimed at evaluating the therapeutic effect of *Solanum nigrum* leaves on certain reproductive dysfunctions associated with anastrozole-induced PCOS in female Wister rats.

Materials And Methods

Plant Material

Fresh leaves of *Solanum nigrum* plant were collected from Mountain Top University permanent site, Ogun State, Nigeria. The plant leaf was authenticated at the

Department of Plant Biology, University of Lagos, Nigeria. A voucher specimen number LUH 10037 was prepared and deposited at the herbarium of the department.

Animals

Sixteen healthy female Wistar rats (190.56±5.35g) were obtained from the animal holding Centre of the Mountain Top University, Ogun State, Nigeria. The animals were kept in a well-ventilated house condition (temperature: 22±3°C; photoperiod: 12h/12h light/dark cycle; humidity: 45-50 %) and fed with rat pellets (New Hope, Grand Cereals, Lagos, Nigeria) and water *ad libitum*.

Drugs, Assay Kits, and Chemicals

Progesterone, Insulin, Prolactin, Follicle Stimulating Hormone, Testosterone and Luteinizing Hormone assay kits were manufactured by Perkin Elmer Laboratories, Freiburg, Germany. Anastrozole was a product of Ani Pharmaceuticals, Minnesota, USA. All other reagents used were from Sigma Chemicals, St. Louis, USA.

Preparation of Extract

A known weight (2 kg) of *Solanum nigrum* leaves was washed, air-dried and pulverized in a warring electric blender. The powdered leaves (715 g) was extracted in distilled water using the ratio (1:4)[3] for 48 hours and filtered with Whatman No. 1 filter paper. The filtrate was lyophilized to give a yield of 17.4 g which corresponded to 2.43%. The resulting powder was reconstituted in distilled water to obtain the dose, 200 mg/kg body weight used in this study.

Animal grouping and extract administration for pharmacological study

A total of 16 female rats were acclimatized for 2 weeks and divided into 4 groups of four (4) animals each (A-D). PCOS was induced in twelve female Wistar rats in groups designated B - D with 1 mg of anastrozole described by Yakubu and Ibiyo (2013)[10] for a period of 21 days. The extract administration was done as follows:

Group A (non PCOS induced control) received 0.5ml of distilled water

Group B (untreated PCOS) received 0.5ml of distilled water

Group C (PCOS induced) received 7.14mg/kg body weight of metformin and 2mg/kg

body weight clomiphene citrate (Reference drug)

Group D (PCOS induced) received 200mg/kg body weight of *Solanium nigrum* leaves extract.

The extract, distilled water and reference drugs were once daily administered for fourteen days. Twenty-four hours after the last administration (end of the experimental period), the rats were anesthetized using diethyl ether and sacrificed by jugular puncture. The blood samples and ovaries were collected using the procedures described by Femi-Olabisi *et al.* (2023)[11]. Thereafter, the serum was used to carry out hormonal assays.

Vaginal Cytology

Vaginal Cytology Using a light microscope, the vaginal cytology of the stages of estrous cycle in female rats was monitored to observe the predominant cell type in the vaginal smears which were obtained daily for 21 days during the induction period[12].

Determination of reproductive hormones

The concentrations of Progesterone, Insulin, Prolactin, Follicle Stimulating Hormone, Testosterone and Luteinizing Hormone, were determined by adopting standard procedures. Also, Histopathology of the ovaries were determined

Histopathological examination

The histological examination was carried out as described by Yakubu and Ibiyo (2013)[10]. The animals were quickly dissected and the ovaries were 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)[13].

Statistical Analysis

Data were expressed as the mean ± standard error mean of four determinations and data were analysed for statistical significant at P≤ 0.05 using One Way Analysis of Variance and Duncan Multiple Range Test performed with Statistical Package for Social Sciences, version 21.0 (IBM Inc., Chicago, USA).

Results

The administration of anastrozole significantly increased (p≤ 0.05) the serum testosterone and LH concentrations while the serum progesterone, FSH and prolactin concentrations were significantly decreased (p≤ 0.05) when compared with that of normal control. The administration of 200mg/kg B.W of AEoSNL significantly decreased (p≤0.05) the elevated levels of testosterone and LH in anastrozole-induced PCOS animals in a manner similar to the reference drug-treated group. compared favourably the glucose level to the anastrozole -treated rats (Table 1). The reduction in the prolactin content of the animals at 200 mg/kg body weight of the AEoSNL as well as PCOS animals treated with MET and CC compared favourably with the distilled water treated non-PCOS animals (Table 1). However, the extract significantly decreased the FSH levels of the PCOS rats while the reference drug increased the serum levels of FSH compared to the normal control animals (Table 1).

The ovarian histology of the normal control rats revealed developing follicles with numerous corpora lutea in the ovarian stroma whereas the distilled water-treated anastrazole-induced PCOS animals had very few developing follicles and corpora lutea (Plates 1a and 1b). The ovaries of the anastrozole-induced PCOS rats treated with metformin and clomiphene citrate and AEoSNL had numerous developing follicles, many corpora lutea and few atretic follicles (Plates 1c and 1d)

From the obtained as plotted in the line graphs, the control rats showed regularity of

the estrous cycle and were in the diestrus phase three times over the period of the experiment. The control rats had 4-6 days of oestrus cycle (Figure 1). In contrast to the normal control animals, group B, the distilled water-treated PCOS rats, had irregular cycles characterized with persistent estrus and metestrus phases, and cyclical disruptions ranging from 5-9 days (Figure 2). Compared to the control group, the PCOS-induced rats were consistently at the metestrus phase, with irregular intervals for extended periods within the experiment. In the proestrus phase, all the rats exhibited similar cyclical patterns. The administration of extract at 200mg/kg body weight reversed the trend of persistent presence of cornified epithelial cells in the oestrous cycle of the anastrozole induced rats in a manner that was similar to that of the control group and the reference drug-treated PCOS animals (Figure 3 and 4).

Table 1: Effect of aqueous extract of *Solanum nigrum* leaves on reproductive hormone

Parameters / Groups	Testosterone (nMol/L)	Progesterone (nMol/L)	Follicle Stimulating Hormone (mIU/ml)	Luteinizing Hormone (mIU/ml)	Prolactin (mg/mL)
Control	6.13 ± 0.125^{a}	44.00 ± 0.41^{a}	62.50 ± 1.44^{a}	25.00 ± 0.04^{a}	9.50 ± 0.29^{a}
PCOS +Distilled water	27.75 ± 1.30^{d}	33.50 ± 0.29^{c}	51.25 ± 1.25^{d}	30.03 ± 0.20^{b}	9.00 ± 0.01^{b}
PCOS+MET+CC	14.38 ± 0.13^{b}	31.50 ± 0.87^{b}	151.75 ± 1.18^{b}	27.01 ± 0.13^{c}	9.25 ± 0.14^{c}
PCOS + 200 mg/kg b.wt. of AEoSNL	23.99 ± 0.01^{c}	34.50 ± 0.29	33.75 ± 1.25^{c}	$27.05\pm0.03c$	9.50 ± 0.29^{a}

Data are means of four determinations ± SEM. Values with different superscripts in each column are significantly different ($P \leq 0.05$). **Metformin- MET, clomiphene citrate- CC**

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 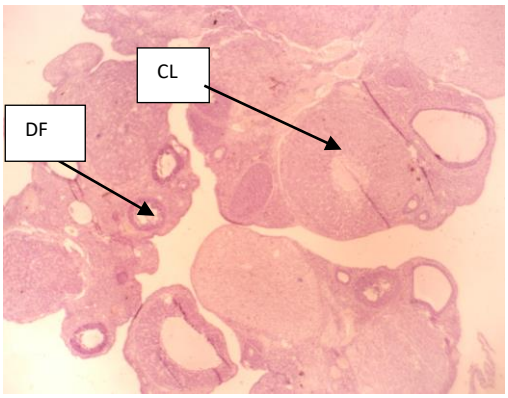

Plate 1a: Photomicrograph of cross section of ovary of normal rat administered carboxymethyl cellulose (×400; H & E) CL-corpus luteum, DF-developing follicles</td>
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 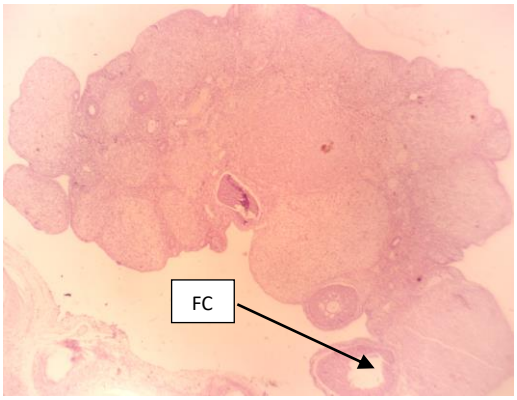

Plate 1b: Photomicrograph of cross section of ovary of the untreated anastrozole-induced PCOS rat administered distilled water (×400; H & E) FC- follicular cyst</td>
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 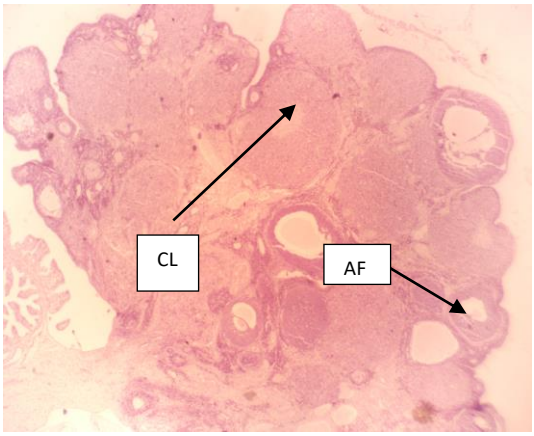

Plate 1c: Photomicrograph of cross section of ovary of anastrozole-induced PCOS rat co-administered metformin and clomiphene citrated (×400; H & E) CL-corpus luteum, AF-Atretic follicles</td>
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 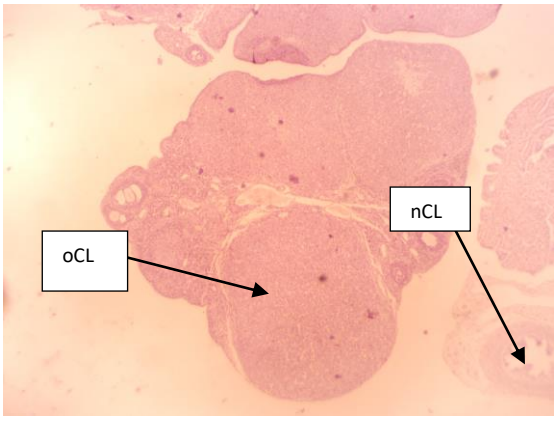

Plate 1d: Photomicrograph of cross section of ovary anastrozole-induced PCOS rat administered 200 mg/kg bwt. Of AEoSNL (×400; H & E) oCL- old corpus luteum, nCL-new Corpus Luteum</td>
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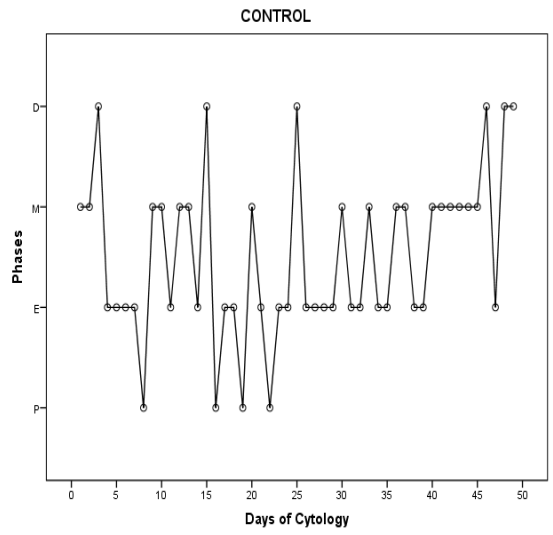

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Figure 1: Oestrous cyclicity of rats of normal control rats

Proestrous, E: Estrous, M: Metaestrous, D: Diestrous

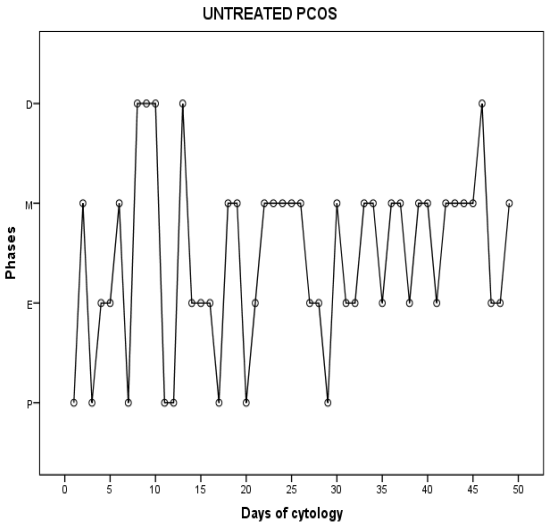

Figure 2: Oestrous cyclicity of anastrozole -induced female rats untreated group

Proestrous, E: Estrous, M: Metaestrous, D: Diestrous

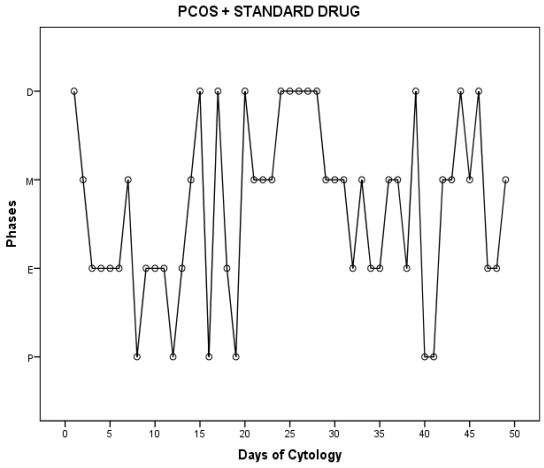

Figure 3: Oestrous cyclicity of anastrozole - induced female rats treated with reference drugs (metformin and clomiphene citrate).

: Proestrous, E: Estrous, M: Metaestrous, D: Diestrous

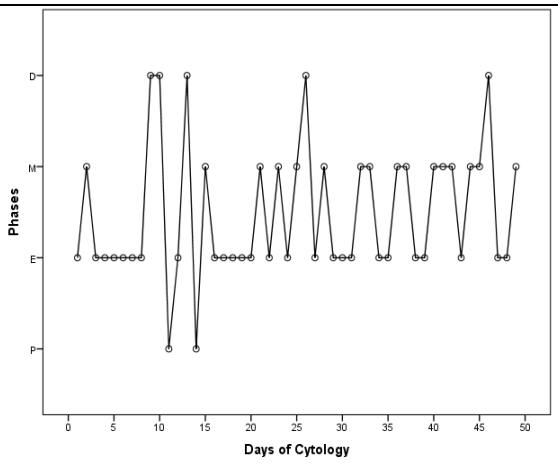

Figure 4: Oestrous cyclicity of anastrozole - induced female rats treated with 200mg/kg body weight of AESN LEAVES

P: Proestrous, E: Estrous, M: Metaestrous, D: Diestrous

Discussion

There non-availability of cure for PCOS necessitated the use of drugs to manage this condition symptomatically but without a variety of adverse effects. Drugs made from natural plant products may help treat PCOS because several plant extracts have been widely recognized to attenuate the symptoms of PCOS[14]. Measurement of sex hormone levels reveal the presence of PCOS and this is a consistent parameter needed to diagnosis a woman with PCOS exhibiting elevated serum testosterone, LH concentrations, and low progesterone and FSH levels[15]. High testosterone levels indicated a buildup of androgens, presumably as a result of the inhibition of androgen substrate conversion to estrogens[16]. Increased testosterone secretion, the elevated serum LH concentrations could be related to a reduction in estrogen synthesis in the brain and pituitary and this validates the usage of anastrozole for the induction of PCOS[17]. Elevated blood levels of androgens are also the one of the major etiologies behind PCOS. Therefore, therapeutics with anti-androgen activity are used in the treatment of PCOS[18]. The decrease in the testosterone and LH concentration in PCOS rats administered 200 mg/kg body weight of AEoSNL suggests that the extract possesses anti-androgenic activity (Table 1). Changes in prolactin levels and hormonal imbalances will have a significant impact on ovulatory cycles. Decreasing prolactin levels or improving the hormonal balance have a positive impact on ovulatory cycles and the treatment of PCOS. The prolactin levels of PCOS rats treated with the extract compared favourably with the control and also reversed the acyclicity in their oestrus cycle characterized by persistent estrus and metestrus phases and this implies that the AEoSNL possesses anti-PCOS activity in ameliorating hormonal imbalances as well as reversing acyclicity in the ovulatory cycles. Metformin's insulin-sensitizing qualities have been found in studies to enhance menstrual periods and ovulation rates in women with PCOS especially when co-administered with Clomiphene citrate. Metformin have been reported affect hyperandrogenism, metabolic changes, and, most critically, fertility[19]. The extract may have similar mechanism of action with metformin due to its effects on hyperandrogenism and improvement of the oestrus cycle (Figure 3 and 4).

Acyclicity and the presence of polycyctic ovary are crucial parameters used in measuring reproductive abnormalities in PCOS[20]. The control animals exhibited regular 3–5 days oestrus cycle, and their histologically examined ovaries contained new corpora lutea which indicates recent ovulations (Plate 1a). The untreated PCOS rats ovaries had follicular cyst which are atretic and this establishes the induction of PCOS using anastrozole. The administration of AEoSNL to PCOS rats reflected in the ovarian histology with the presence of few old atretic follicles and new corpus lutea which indicates that ovulation has recently occurred (Plate 1d).

Conclusion

The administration of 200 mg/kg BW of aqueous extract of *Solanum nigrum* leaf to anastrole-induced PCOS rats reversed reproductive dysfunctions such as elevated testosterone, LH hypersecretion, oestrous acyclicity and the presence of cystic ovaries which are features common with PCOS. Therefore, the use of aqueous extract of *Solanum nigrum* leaf can be explored in novel drug design for the treatment of PCOS subject to further experimentation.

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