Effect of Aqueous and Ethanolic Extract of *Mucuna Pruriens* on Ovarian Histology in Letrozole-Induced Polycystic Ovarian Syndrome in Sprague-Dawley Rats: A Preliminary Study

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ABSTRACT

Polycystic ovarian syndrome (PCOS) is recognized as one of the most common endocrinopathy accounting for about one fifth of all infertility cases in women of reproductive age. The overall aim of this study is to determine the effect of *Mucuna pruriens* on letrozole-induced polycystic ovarian syndrome using an animal model. This study explores *Mucuna pruriens* as a possible therapeutic agent in the treatment and management of PCOS.

Forty female Sprague-Dawley rats averagely weighing 180 g were used for this study. They were divided into eight groups (N=5). Letrozole (1.0 mg/kg), administered for 21 days, was used to induce PCOS. Both ethanolic (100, 200 and 400 mg/kg body weight) and aqueous fractions (100, 200 and 400 mg/kg body weight) of *Mucuna pruriens* were administered for four weeks (100, 200 and 400 mg/kg body weight). Control animals received 3 mls of distilled water. Body weights were monitored weekly throughout the study. At the end of the experiment, animals were sacrificed; ovaries were excised, trimmed off fat, weighed and fixed in 10% formal saline for histological procedure.

Body weights increased significantly across all groups when post-administration weight was compared to pre-administration weight. The ovarian weights of the 400 mg/kg groups of both aqueous and ethanolic groups were comparable with control with the same value while those of low dose (200 mg/kg) and medium dose (100 mg/kg) were slightly lower than control, although they were not statistically significant. Histological sections of the *Mucuna pruriens* treated groups showed follicles at various stages of development when compared to the letrozole-induced group which exhibited numerous subcapsular cysts, with a thin granulosa layer and absent corpora lutea indicating anovulation.

The study showed that both aqueous and ethanolic fractions of *Mucuna pruriens* seed extract ameliorated the deleterious effect of polycystic ovarian syndrome on ovarian follicles by rescuing growing follicles from cystic formation.

KEYWORDS Polycystic Ovarian syndrome, Mucuna pruriens, Letrozole, Ovary.

INTRODUCTION

Polycystic ovarian syndrome (PCOS) is one of the most common endocrine disorders affecting women of child-bearing age accounting for about 5-15% of these women globally [1,2,3,4]. Although the etiology of PCOS remains poorly understood [5], but it is usually characterized by chronic anovulation, excess androgen production, and the presence of polycystic ovaries on ultrasound. Clinically, women with PCOS present cases such as; irregular menses, hirsutism, acne alopecia, altered gonadotropin levels as well as excess insulin and androgen levels [6].

PCOS refers to the presence of multiple cysts (> 12) in an ovary [7] caused by the arrest of follicular development before

maturity. As the follicles grow, they are trapped before the point of dominant follicle selection and the negative estrogen feedback to the hypothalamus and pituitary axis is inhibited. Therefore, the LH surge is absent in patients with PCOS [8, 9, 10] making ovulation and menstrual cycles disrupted resulting into oligovulation and oligoamenorrhea.

In PCOS, excess production of androgens may alter gonadotropin-induced estrogen and progesterone synthesis in the follicles [11]. Normally, androgens are converted to estradiol and estrone, respectively, by the enzyme P450 aromatase, which plays an important role in the ovary's hormonal balance. However, steroidogenesis is thought to be affected by the decreased activity of this enzyme which might be one of the reasonable intraovarian disturbances in PCOS leading to increased ovarian androgen production and development of PCO condition [12, 13].

The wild legume, *Mucuna pruriens* are in great demand as food, livestock feed and pharmaceutically valued products [14]. All parts of Mucuna plant possess useful phytochemicals of high medicinal value [15] important to humans and constitutes raw materials for veterinary and folk Ayurvedic medicines.

Mucuna pruriens seeds are rich in high levels of protein, carbohydrates, fiber, and adequate minerals which meet the requirement of essential amino acids [14]. The seeds are a rich source of L-DOPA and contain variety of alkaloids, fatty acids, amino acids, minerals, antioxidants, antitumor, antibacterial and several nutritional elements [16, 17].

Mucuna pruriens possess valuable medicinal properties and has been investigated in several contexts. It has been reported to possess several therapeutic properties such as; anti-diabetic [18, 19] anti-neoplastic, anti-epileptic, anti-microbial [15, 20], anti-venom [21] and anti-helminthic [22] activities. *Mucuna pruriens* has also been relevant in neuroprotective studies [23], and has demonstrated analgesic and anti-inflammatory activities. [24]

Powdered seeds of *Mucuna pruriens* has also demonstrated anti-parkinsonism properties, possibly due to the presence of L-dopa. L-Dopa, a precursor of dopamine, can cross the blood-brain barrier and undergo conversion to dopamine by the enzyme dopa decarboxylase restoring neurotransmission in the brain [25].

Numerous studies have also shown that *Mucuna pruriens* seed powder increases semen volume, improves sperm quality, and combats the generation of reactive oxygen species in infertile men [26, 27]. *Mucuna pruriens* improves the quality of sperm by increasing the number of cells and providing them with greater mobility thereby resulting in increased sexual drive and power [27]. It has also been revealed in male animal studies, that *Mucuna pruriens* increases the testosterone levels as well as their sexual activity [28]. The seed powder and extract have both been seen to be effective in maintaining the antioxidant level and combating the stress mediated compromise in the process of spermatogenesis [27, 29, 30, 31].

L-DOPA and its metabolite dopamine have been reported to stimulate the hypothalamus and forebrain to secrete gonadotropin-releasing hormone (GnRH) which acts on the anterior pituitary to secrete gonadotropins; follicle stimulating hormone (FSH) and luteinizing hormone (LH) [29, 32, 33]. Previous studies have also shown that *Mucuna pruriens* increases the number of ova shed in normal and streptozotocin-induced Sprague-Dawley rats [32, 34].

Letrozole is a potent and highly specific non-steroidal aromatase inhibitor that binds to the heme of the cytochrome P450 subunit, leading to a total blockade of the aromatization in peripheral tissues without exerting effects on other steroidogenic pathways [35]. It blocks the conversion of androgens to estrogens in the ovarian follicles, peripheral tissues and the brain of experimental animals. Aromatase is responsible for catalyzing the rate-limiting step in the synthesis of estrogens from androgens which results in the decrease in local and circulating estrogens and rise in intraovarian androgens in these tissues [35].

Administration of letrozole to experimental rodents has shown increased levels of both testosterone and LH causing decrease in the activity of the aromatase enzyme resulting into increased ovarian androgen production and development of PCOS in experimental animals as seen in humans with PCOS [13, 36-37]. The use of plant-based medicine in the treatment and management of diseases is on the increase. This study is aimed at investigating the potential effects of fractions of *Mucuna pruriens* on Letrozole-induced polycystic ovarian syndrome using Sprague-Dawley rats.

MATERIALS AND METHODS

Animals

A total of forty female Sprague Dawley rats were used for this study. Animals were obtained from the Animal Holding Centre, Jide Farms Agege, Lagos State Nigeria and were transferred to the Animal House of the Department of Anatomy, College of Medicine of the University of Lagos.

The animals were housed in well ventilated cages and left to acclimatise for 2 weeks before the commencement of the experiment. Animals were kept under 12 hr light/12 hr dark cycles at standard temperature (26^{o}C-28^{o}C). They were provided with standard rat chow and water ad libitum. All experimental procedures and tissue collection were approved by the Health Research Ethics Committee of the College of Medicine University of Lagos Nigeria with ethics number: CM/HREC/12/16/102.

Drugs

Letrozole (Teva®) was obtained from Blue Seal Pharmacy Festac, Lagos Nigeria and Carboxylmethyl cellulose (CMC) was obtained from the Department of Pharmaceutics, Faculty of Pharmacy, of the University of Lagos Nigeria

Induction of Experimental Polycystic Ovarian Syndrome

Animals were induced with PCOS by oral administration of 1.0 mg/kg of Letrozole dissolved in 1% CMC (vehicle) once daily for a period of 21 days [36].

Plant Source

Mucuna pruriens with mature seeds were obtained in the early hours of the morning in the month of February from a farm at Ijan town, Ekiti State, Nigeria. They were identified and authenticated by Professor J.D. Olowokudejo of the Department of Botany, Faculty of Science of the University of Lagos, Nigeria. Voucher specimen with accession number LUH 422 was deposited in the herbarium

Seed Extraction

The extraction was carried out in the Pharmacognosy Department of the Faculty of Pharmacy, University of Lagos, Nigeria. *Mucuna pruriens* seeds were obtained from pods, air-dried and grounded into fine powder.

Ethanolic Extraction

Briefly, 1200 g of fine powder of *Mucuna pruriens* was mixed with alcohol and placed in the Soxhlet apparatus. The solution was concentrated using a rotary evaporator at 40ºC. The concentration from rotary evaporator was then dried to a semi-solid form using a regulated water bath at 40ºC for 48 hours until constant weight was obtained. The dried extract (78.52 g) was then stored in a glass airtight sample bottle and kept in the freezer at – 4ºC until usage

Aqueous Extraction

1200 g of prepared fine powder of *Mucuna pruriens* was macerated in 5000 ml of distilled water for 24 hours in a glass container. The solution was stirred frequently (3 hourly). It was firstly filtered with a muslin bag, and then, finely filtered with a Whatman No 4-filter paper. The solution was concentrated using a rotary evaporator at 40oc. The concentrate from the rotary evaporator was then dried to a semi-solid form using a regulated water bath at 40ºC for 48 hours until constant weight was obtained. The dried extract (60.78 g) was stored in a glass air-tight sample bottle and kept in the freezer at – 4ºC until usage.

Determination of Oestrous Cycle

Oestrous cyclicity was determined between 8 a.m. and 10 a.m. daily using the vaginal smear method. Vaginal secretion was collected with a plastic pipette filled with 10 µL of normal saline (NaCl 0.9%). The vagina was flushed two or three times with the pipette and the vaginal fluid was placed on a glass slide. A different slide was used for each animal. The unstained secretion was observed under a light microscope. Animals displaying two consecutive regular 4-day cycles were thereafter used for this study [38, 39].

Experimental Groupings

The Animals received a single oral dose of ethanolic or aqueous extract of *Mucuna pruriens* at 100, 200 and 400 mg/kg body weight daily [34] using an Oro-gastric tube for a period of four (4) weeks after induction. CMC was given to the control animals while negative control animals only received food and water. Animals were divided into eight (8) groups of 5 animals each after letrozole induction of PCOS.

Group 1: Served as Control group and received CMC only for 4 weeks.

Group 2: Negative control group received Letrozole for 21 days without further intervention

Group 3-5: Received oral doses of aqueous extract of *Mucuna pruriens* for 4 weeks at 100, 200, and 400 mg/kg daily after PCOS induction.

Group 6-8: Received oral doses of ethanolic extract of *Mucuna pruriens* for 4 weeks at 100, 200 and 400 mg/kg daily after PCOS induction

Sacrifice

At the end of the treatment period, animals were sacrificed by cervical dislocation. The ovaries were accessed and removed following a ventral laparotomy, trimmed off fat, rinsed in normal saline and weighed. The right and left ovaries were then fixed immediately in 10% formal saline for histological procedure.

Histological Preparation

The fixed tissues were dehydrated in increasing concentrations of ethanol and thereafter embedded in paraffin wax. Serial sections of 7 µm thick were made. These were stained with haematoxylin and eosin in the routine H&E preparation.

Statistical Analysis

The data obtained from all the groups were compiled and statistically analysed using ONE WAY ANOVA and Student T-test method on SPSS software version 22. The results of the data were expressed as mean ± SEM (standard error of mean). Statistical significance was considered at $p \leq 0.05$.

RESULTS

BODY WEIGHT

The effect of administration of aqueous extract of Mucuna pruriens on body weight

Body weight increased significantly in all groups when post-administration body weight was compared with pre-

administration body weight. When percentage difference in body weight was considered, the high dose group gained the highest weight while the low dose group gained the least weight (Table 1).

TABLE 1. The effect of administration of aqueous extract of *Mucuna pruriens* on body weight of letrozole-induced-PCOS in Sprague-Dawley rats.

Group	Aqueous extract of *Mucuna pruriens*		Percentage difference in weight (%)
	Pre-administration weight (g)	Post-administration weight (g)	
Control (CMC)	214.33 ± 3.48	235.00 ± 2.89*	8.80
LETROZOLE	181.00 ± 1.53	203.33 ± 2.96*	10.98
LMPa	212.00 ±3.46	226.00 ± 2.52*	6.19
MMPa	171.67 ± 4.63	203.33 ± 13.78*	15.57
HMPa	189.00 ± 1.73	242.00 ± 4.04*	21.90

Values are expressed as Mean ± SEM (standard error of mean) with Pvalue < 0.05. N=5

LMPa – Low dose (100 mg/kg) aqueous extract of *Mucuna Pruriens*
MMPa- Medium Dose (200 mg/kg) aqueous extract of *Mucuna Pruriens*
HMPa- High Dose (400 mg/kg) aqueous extract of *Mucuna Pruriens*

The effect of administration of Ethanolic extract of Mucuna pruriens on body weight

Body weight increased significantly in all groups when post-administration body weight was compared with pre-administration body weight. When percentage difference in body weight was considered, the letrozole group gained the highest weight while the high dose group gained the least weight (Table 2).

TABLE 2. The effect of administration of ethanolic extract of *Mucuna pruriens* on body weight of letrozole-induced-PCOS in Sprague-Dawley rats.

Group	Aqueous extract of *Mucuna pruriens*		Percentage difference in weight (%)
	Pre-administration weight(g)	Post-administration weight(g)	
Control (CMC)	214.33 ± 3.48	235.00 ± 2.89*	8.80
LETROZOLE	181.00 ± 1.53	203.33 ± 2.96*	10.98
LMPe	202.00 ± 6.03	215.00 ± 2.89*	5.89
MMPe	210.33 ± 1.20	222.33 ± 2.96*	5.40
HMPe	246.00 ± 15.26	259.33 ± 15.38*	5.14

Values are expressed as Mean ± SEM (standard error of mean) with P value < 0.05. N=5

LMPe – Low dose (100 mg/kg) ethanolic extract of *Mucuna Pruriens*
MMPe- Medium Dose (200 mg/kg) ethanolic extract of *Mucuna Pruriens*
HMPe- High Dose (400 mg/kg) ethanolic extract of *Mucuna Pruriens*

OVARIAN WEIGHT

The effect of the administration of aqueous and ethanolic extracts of Mucuna pruriens on ovarian weight

There was no statistically significant difference in the weight of the ovaries when treatment groups were compared to control. The ovarian weight of the groups which received the highest dose was comparable with control group. Also, the right and left ovaries had equal weights in all the groups. The gonadosomatic index was the same for all groups.

TABLE 3. The effect of the administration of fractions of *Mucuna pruriens* on ovarian weight of letrozole-induced PCOS in Sprague-Dawley rats.

Groups	Aqueous Weight of ovary (g)		Ethanolic Weight of ovary (g)		Gonadosomatic index
	Left	Right	Left	Right	
Control (CMC)	0.05 ± 0.00	0.05 ± 0.00	0.05 ± 0.00	0.05 ± 0.00	0.02
LETROZOLE	0.05 ± 0.01	0.05 ± 0.01	0.05 ± 0.01	0.05 ± 0.01	0.02
LMP	0.04 ± 0.00	0.04 ± 0.00	0.04 ± 0.00	0.04 ± 0.00	0.02
MMP	0.04 ± 0.01	0.04 ± 0.01	0.05 ± 0.01	0.05 ± 0.01	0.02
HMP	0.05 ± 0.01	0.05 ± 0.01	0.05 ± 0.00	0.05 ± 0.01	0.02

Values are expressed as Mean ± SEM (standard error of mean) with P value < 0.05. N=5

LMP – Low dose (100 mg/kg) of *Mucuna Pruriens*
MMP- Medium Dose (200 mg/kg) of *Mucuna Pruriens*
HMP- High Dose (400 mg/kg) of *Mucuna Pruriens*

Histological Observation

Histological analysis of ovaries from control group animals showed follicles at different stages of development [Plates 1A & 2A] whereas the PCOS positive rats exhibited numerous subcapsular cysts, with a very thin or no granulosa layer. Corpora lutea were absent indicating anovulation [Plates 1B & 2B].

Histological analysis of treated groups with *Mucuna pruriens* (aqueous and ethanolic) led to disappearance of cysts and presence of healthy follicles and corpora lutea [Plates 1C & 1E, Plates 2C & 2E] compared to PCOS positive groups [Plates 1B & 2B].

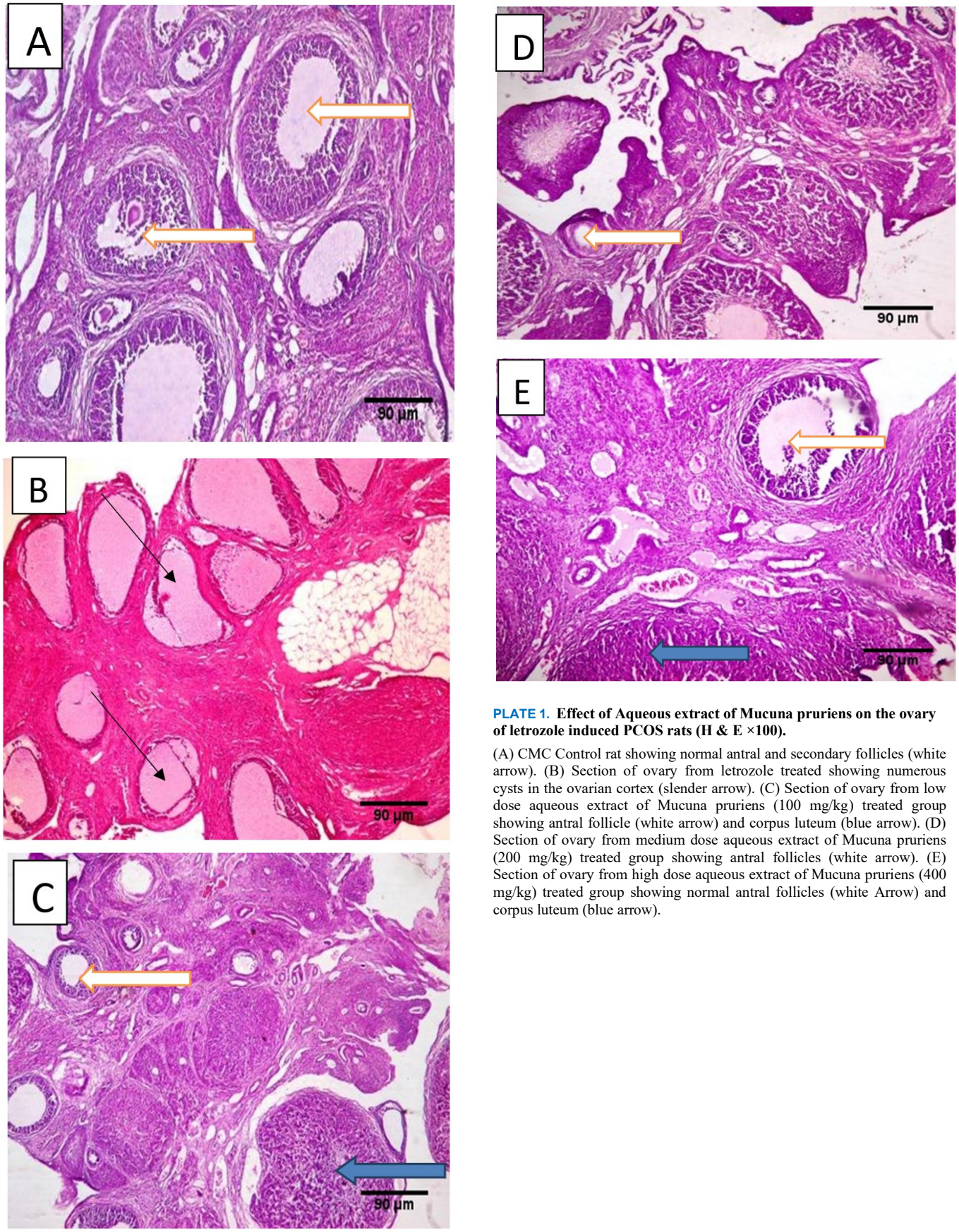

PLATE 1. Effect of Aqueous extract of Mucuna pruriens on the ovary of letrozole induced PCOS rats (H & E ×100).

(A) CMC Control rat showing normal antral and secondary follicles (white arrow). (B) Section of ovary from letrozole treated showing numerous cysts in the ovarian cortex (slender arrow). (C) Section of ovary from low dose aqueous extract of Mucuna pruriens (100 mg/kg) treated group showing antral follicle (white arrow) and corpus luteum (blue arrow). (D) Section of ovary from medium dose aqueous extract of Mucuna pruriens (200 mg/kg) treated group showing antral follicles (white arrow). (E) Section of ovary from high dose aqueous extract of Mucuna pruriens (400 mg/kg) treated group showing normal antral follicles (white Arrow) and corpus luteum (blue arrow).

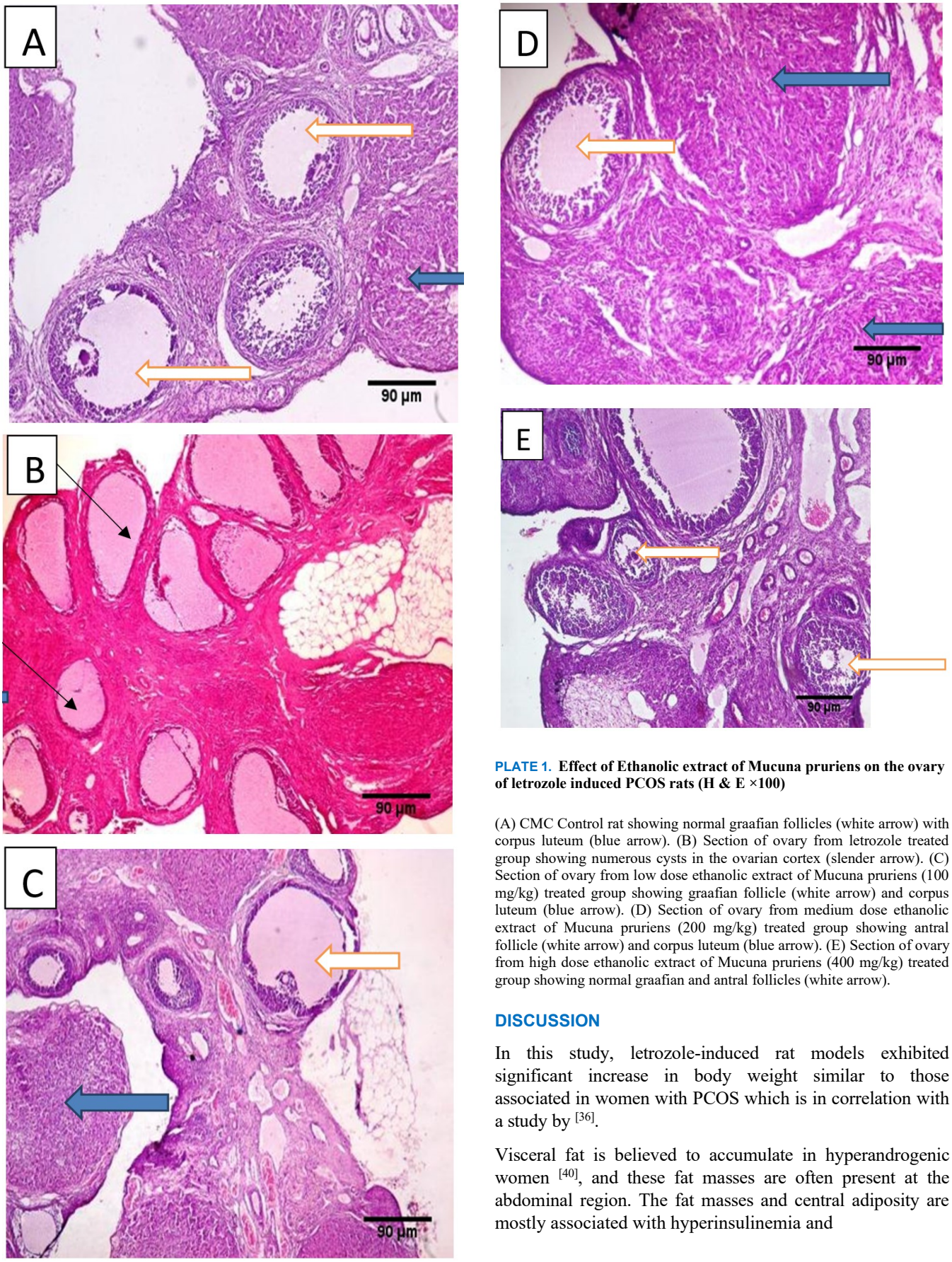

PLATE 1. Effect of Ethanolic extract of Mucuna pruriens on the ovary of letrozole induced PCOS rats (H & E ×100)

(A) CMC Control rat showing normal graafian follicles (white arrow) with corpus luteum (blue arrow). (B) Section of ovary from letrozole treated group showing numerous cysts in the ovarian cortex (slender arrow). (C) Section of ovary from low dose ethanolic extract of Mucuna pruriens (100 mg/kg) treated group showing graafian follicle (white arrow) and corpus luteum (blue arrow). (D) Section of ovary from medium dose ethanolic extract of Mucuna pruriens (200 mg/kg) treated group showing antral follicle (white arrow) and corpus luteum (blue arrow). (E) Section of ovary from high dose ethanolic extract of Mucuna pruriens (400 mg/kg) treated group showing normal graafian and antral follicles (white arrow).

DISCUSSION

In this study, letrozole-induced rat models exhibited significant increase in body weight similar to those associated in women with PCOS which is in correlation with a study by [36].

Visceral fat is believed to accumulate in hyperandrogenic women [40], and these fat masses are often present at the abdominal region. The fat masses and central adiposity are mostly associated with hyperinsulinemia and

hyperandrogenemia from prepuberty to postmenarche age as experienced in women with PCOS [41].

Mucuna pruriens, a tropical grain legume, with good nutritional qualities which makes it comparable to other conventional legumes because it contains similar proportions of protein, lipid, minerals and other nutrients [42,43] and its potential as a dietary protein source for animal feed is well recognized particularly in the developing world [44]. All treated groups in this study, showed significant increase in body weights after administration. This means that administration of *Mucuna pruriens* did not affect appetite stimulation and nutrient absorption in the small intestine and this led to increased body weights.

The gonadosomatic index (GSI) is a good indicator of the general health and gonadal maturity of an organism [45]. It is mainly used to estimate or measure the reproductive capacity in animals. Reproduction is known to be the most critical stage in the life cycle of a specie, and this determines its survival and success [46]. It is referred to as the percentage gonad weight in comparison to the somatic weight. The percentage body weight used for production of eggs is determined by this index in animals [47]. Oral administration of aqueous and ethanolic extract of *Mucuna pruriens* did not cause any variation in the gonadosomatic index of all treated groups when compared with control.

Histologically, letrozole-induced PCO rats showed the formation of empty cysts filled with follicular fluid with thin granulosa cells, hyperplasia of the theca interna cells and a thickened ovarian capsule. Furthermore, the follicular cysts had flattened epithelioid cell layers facing the antrum. These histological findings are indicative of the presence of biologically active levels of increased LH levels, reduced FSH levels, and lack of interplay between granulosa and theca cells as seen in PCOS models [13, 36, 37, 48].

Treatment with *Mucuna pruriens* for four (4) weeks following letrozole induction of PCO rats showed protective effects in the ovarian histological sections with the presence of well-developed antral follicles, graffian follicles, normal granulosa cell layer and a defined theca layer. The presence of corpus luteum also indicated the occurrence of ovulation suggesting that the extract is involved in the regulation of various factors associated with follicular development in the ovary.

Mucuna pruriens seeds are a rich source of L-dihydroxyphenylalanine (L-DOPA) and its metabolites; epinephrine and norepinephrine and a precursor to the neurotransmitter dopamine, responsible for mood, movement and sexuality [49].

Earlier studies revealed that the dopaminergic activity, as one of the circadian oscillators, may influence neuroendocrine–gonadal axis [50]. It is suggested that high levels of Dopamine resulting from L-DOPA intake may be responsible for the induction and maintenance of reproductive conditions as seen in this study.

L-DOPA is rapidly converted to Dopamine by dopa decarboxylase in the brain, which functions as one of the best natural releasers of human growth hormone by stimulating the hypothalamus and forebrain to secrete GnRH which in turn up-regulates the anterior pituitary gland to secrete FSH and LH.

In this study, an increase in dopamine level in the brain following *Mucuna pruriens* treatment may be responsible for the appearance of follicles at different stages of development as seen in the treated groups. Studies done by [32] revealed that supplementation with *Mucuna pruriens* caused an increase in the number of oocyte released at ovulation in normal Sprague-Dawley rats.

Mucuna pruriens has also been reported to contain many bioactive constituents, including alkaloids, coumarins, flavonoids and alkylamines which play an important role in increasing the antioxidant capacity in humans and animals. Antioxidants are low molecular weight molecules that are capable of mopping up free radicals and neutralize them by donating an electron thus reducing their capacity to damage tissues [51]. Such interactions can safely put an end to a series of damaging reactions that can affect vital molecules in the body. Studies done by [52] in Drosophilia Melanogaster reported that the antioxidant property of *Mucuna pruriens* seed extracts enhances sexual activity, fertility and fecundity.

Mucuna pruriens and its alcoholic extracts are a known adaptogen which reduces oxidative stress [53, 54].The increase in oocyte development as seen in the photomicrograph of this study could also be linked to the scavenging properties of this wonder plant leading to optimum protection against the deteriorating outcomes of the PCOS condition.

CONCLUSION

Mucuna pruriens seems to influence fertility by its action on the hypothalamopituitary-gonadal axis via its L-DOPA content and its precursor, Dopamine. Also, its antioxidant properties might be one of the important mechanisms for its therapeutic effect, thus, leading to the recovery of normal oocyte development and maturation by rescuing the follicles from cystic formation in the cohort. Therefore, *Mucuna pruriens* may be beneficial in the management and treatment of PCOS in women of reproductive age.

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