Fertility Enhancing Potential of Mucuna Pruriens Seeds in Female Sprague-Dawley Rats.

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ABSTRACT (ARIAL, BOLD, 11 FONT, LEFT ALIGNED, CAPS)

Aims: To determine the effect of oral administration of methanolic seed extract of *Mucuna pruriens* (*M. pruriens)* on oestrous cycle, ovulation, reproductive hormones and oxidative stress in the ovary of cyclic Sprague-Dawley (S-D) rats.

Design: Prospective animal study related to *M. pruriens* in reproductive area. .

Place and Duration: Animal Facility of the Department of Anatomy, Faculty of Basic Medical Sciences, College of Medicine of the University of Lagos, Nigeria between the months of June 2012 and August, 2012.

Methodology: Forty female S-D rats with regular 4 days cycle averagely weighing 145 g were used. Methanolic extract of *M. pruriens* was given orally at 50, 100 and 200 mg/kg body weight. Oestrous cycle was monitored daily. At the end of the experiment animals were sacrificed by cervical dislocation. Oocytes were counted, blood and ovaries were assayed for hormonal and biochemical studies respectively.

Results: Oestrous cycle remained unchanged in the treatment groups. Catalase and superoxide dismutase levels were increased slightly compared to control. A dose dependent increase in FSH and LH ($p < 0.05$ at 200 mg/kg) levels were observed with an increase in the number of oocytes released at ovulation compared to control.

Conclusion: *M. pruriens* seed extract has the potential to enhance fertility by increasing serum levels of FSH and LH which in turn increases the number of oocytes released at ovulation possibly through its antioxidant properties.

Keywords: [Mucuna pruriens, ovulation, oxidative stress markers, FSH, LH}

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1. INTRODUCTION (ARIAL, BOLD, 11 FONT, LEFT ALIGNED, CAPS)

Human health is of prime importance to a country's development and progress. Herbal preparation and medications have been in use for the treatment of diseases and various ailments since ancient times in many parts of the world. In developed countries, despite newer formulations of effective conventional drugs, the treatment of diseases and other ailments with herbal remedies is still very popular (1). In developing countries, the use of herbal remedies as alternatives to

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modern medicine is on the increase. In Nigeria, many indigenous plants have been used in herbal medicinal preparations to cure sicknesses and diseases and to heal injuries (2), (3). *M. Pruriens* is one such plant; it is a tropical legume known as velvet bean *('Agbara'*- Igbo, *'Yerepe'* - Yoruba). It is found in Africa, India and the Caribbean's; where it is widely known for its uses in various ailments as reported in literature (4), (5), (6). It is a constituent of more than 200 indigenous drug formulations (6), (7). Some authors have reported that all the various parts of the *Mucuna* plant possess valuable medicinal properties (6), (8), (9). Following the discovery, that *Mucuna* seeds contain l-dopa which is used in the treatment of parkinson's disease; its demand even in the international market has increased considerably (6). This demand has motivated Indian farmers to start commercial cultivation of the *Mucuna* plant. It has widespread cultivation over most of the subcontinent and is found in bushes, hedges and dry deciduous low forests throughout the plains of India (7), (10), (11).

***M. Pruriens* has been reported to enhance fertility in male rats (12-18) however; there is a dearth of literature on the effect of *M. pruriens* on the function of the female reproductive system. This study was carried out to evaluate the effect of *M. pruriens* on the reproductive function of the mature female Sprague-Dawley rats.**

2. MATERIAL AND METHODS / EXPERIMENTAL DETAILS / METHODOLOGY (ARIAL, BOLD, 11 FONT, LEFT ALIGNED, CAPS)

2.1 Plant source

The *M. pruriens* plant with mature seeds was harvested from Mowe area of Lagos, Nigeria. Both plant and seeds were identified and authenticated by Professor J.D. Olowokudejo of the Department of Botany of the University of Lagos. Voucher specimen with accession number LUH 4922 was deposited in the herbarium of the Department of Botany.

2.1.1 Seed extraction

The extraction was carried out in the Pharmacognosy Department of the Faculty of Pharmacy, University of Lagos. Briefly, seeds were obtained from the pods, air-dried and grounded into fine powder using the mortar and the pestle. 450 g of fine powder was mixed with alcohol and placed in the Soxhlet apparatus. The mixture was heated at 60 ^{0}C and the extract was obtained by distillation. The powder obtained (107.6 g, 23.9% yield) was stored at room temperature of 25 ^{o}C before use. All dilutions of the extract were made in distilled water.

2.2 Animals

Forty mature female S-D rats of two months old weighing 140 - 150 g obtained from the Animal House of the College of Medicine, University of Lagos, Nigeria were used in this study. They were housed five animals per cage at the Animal Facility of the Department of Anatomy, College of Medicine of the University of Lagos, Nigeria. The animals had free access to water and rat chow purchased from Pfizer Nigeria Limited and was maintained at 12-h light/12-h dark cycle and at temperatures between 25 to 28 ^{o}C. The animals were

allowed to acclimatize for two weeks before the commencement of the experiment. Throughout the duration of the experiment, the animals were observed for adverse effects such as fur loss, diarrhea, bleeding, ataxia, morbidity and mortality resulting from administration of the extract. All procedures involving animals in this study conformed to the guiding principles for research involving animals as recommended by the Declaration of Helsinki and the Guiding Principles in the Care and Use of Animals (19) and were approved by the Departmental Committee on the use and care of animals and tissue collection.

2.3 Determination of the oestrous cycle

Oestrous cycle was monitored for 24 days. Oestrous cyclicity was determined daily between 8 a.m. and 9:30 a.m. 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. Only animals with a 4-day oestrous cycle were selected for this study.

2.3.1 Oestrous Cyclicity Study

Twenty rats divided into 4 groups of 5 rats in each were used – A, B, C and D; they were given daily dosages of *M. pruriens* orally using an oro-gastric tube for 24 days at: 50 mg, 100 mg and 200 mg/kg body weights respectively while group D animals received distilled water and served as control. The dosages were determined by a previous study (15). Animals were sacrificed by cervical dislocation. Laparotomy was performed; ovaries were removed, trimmed of fat and stored at -80 ℃ for biochemical analysis.

2.3.2 Ovulation study

Twenty animals were used for this study. The animals received a single oral dose of *M. pruriens* at 9 a.m. on the day of proestrus using an oro-gastric tube. The animals in this group were divided into 4 groups (E, F, G and H). *M. pruriens* was given at a dose of 50, 100 and 200 mg/kg bodyweights to groups E, F and G respestively while group H served as control and received distilled water. The rats were sacrificed by cervical dislocation the next day (estrus) at 10 a.m. A ventral laparotomy was performed and the oviduct was dissected out, placed on glass slides with a drop of saline and covered with cover-slips. This was squeezed with both sides being gently rocked and each ovum found in the distended ampulla was counted under a light microscope (20). Control animals received equivalent volume of distilled water.

2.4 Biochemical analysis

The right ovaries were homogenized using a Potter–Elvehjem homogenizer. A 20% (1/5 w/v) homogenate of the tissue was prepared in 50 mM Tris-HCl buffer (pH 7.4) containing 1.15% potassium chloride and centrifuged at 10,000 rpm at 4 ^{o}C for 10 min.

Superoxide dismutase (SOD) was assayed utilizing the technique of (21). A single unit of enzyme was expressed as 50% inhibition of Nitroblue tetrazo-lium (NBT) reduction/min/mg/protein.

Catalase (CAT) was assayed colorimetrically at 620 nm and expressed as µmoles of H2O2 Consumed/min/mg/protein as described by (22).

2.5 Hormonal assay studies

Blood was obtained from the angular vein of the eye of the S-D rats at 6 p.m. in the evening of proestrus and collected into heparinised bottles. Each blood sample was spun at 2,500 rpm for 10 minutes in an angle-head desktop centrifuge at temperatures of 25 ^{o}C. Serum samples were assayed in batches with control sera at both physiological and patho¬logical levels by Standard Quantitative Enzyme- Linked Immunosorbent Assay (ELISA) technique with Microwell kits from Syntro Bioresearch Inc., California, USA.

2.6 Statistical analysis

Results were analyzed and expressed as Mean ± SD and were subjected to one-way ANOVA with Newman-Kenls post hoc test version 5.0 for windows. Statistical significance was considered at P = .05.

3. RESULTS AND DISCUSSION

All the treated rats showed normal behaviour throughout the study. No signs of adverse effects were observed; no fur loss, diarrhoea, bleeding, ataxia, morbidity nor mortality.

3.1 Oestrous Cycle

Analysis of the oestrous cycle revealed that oral administration of 50 mg, 100 mg and 200 mg/kg body weight of methanolic seed extract of *M. pruriens* did not produce any irregularity/derangement in the cycle pattern. Also, length of cycle remained unchanged in all the treated rats; animals showed a regular four days cycle as shown (Table 1). Estrogens and progesterone are important in the normal functioning of the female reproductive system. They are responsible for the development and maturation of reproductive organs and also provide the proper environment required for the transport of gametes and nidation. The balance in hormonal interplay between estrogens and progesterone is responsible for a normal regular cycle (23), (24). This study revealed that the oral administration of M. pruriens seed extract did not alter the oestrous cycle in all the treated animals throughout the treatment period of 24 days. The treated animals maintained a normal cycle pattern and cycle length that was comparable with the control animals. Although authors did not determine the levels of progesterone and estrogens in this study however, we can deduce from our findings that *M. pruriens* did not produce any negative effect either directly on the pituitary or indirectly on the hypothalamus to disrupt the intricate balance in hormonal interplay between progesterone and estrogens levels that is necessary to maintain a normal cycle.

Table 1: Effect of the oral administration of M. pruriens for 24 days on the length of the oestrous cycle in S-D rats.

Treatment groups	Length of oestrous cycle in days
Control	4.0 ± 0.00
50 mg/kg	4.0 ± 0.10
100 mg/kg	4.0 ± 0.20
200 mg/kg	4.0 ± 0.40

n = 5. Values are expressed as mean ± standard deviation

3.2 Antioxidant status of CAT and SOD

The extract exhibited a dose dependent increase in CAT and SOD activities in the treatment groups compared to the control group however, this increase was not statistically significant (Table 2).

Literature is rife with studies reporting that *M. pruriens* has an excellent scavenging ability that mops up excessive production of reactive oxygen species (ROS) and free radicals (17), (25), (26), (27), (28). ROS plays both a physiological as well as a pathological role in the female reproductive tract. Numerous animal and human studies have demonstrated the presence of ROS in the female reproductive tract such as in the ovaries (29), (30), (31), the fallopian tubes (32) and in embryos (33). ROS is involved in the modulation of an entire spectrum of physiological reproductive functions such as oocyte maturation, ovarian steroidogenesis, corpus luteal function and luteolysis (29), (31), (34). On the other hand, the pathological effects are exerted by various mechanisms including lipid damage, inhibition of protein synthesis, and depletion of ATP (35). ROS have been implicated in more than 100 diseases (36), (37), (38). The superoxide radical is formed when electrons leak from the electron transport chain (39). SOD decomposes superoxide anion into hydrogen peroxide and oxygen at very high rates. Superoxide radical is involved in diverse physiological and pathophysiological processes (40). CAT catalyses the decomposition of hydrogen peroxide to water and oxygen. High concentration of hydrogen peroxide is deleterious to cells such as DNA, proteins, and lipids, leading to mutagenesis and cell death (41).

The slight increase in the activities of SOD and CAT observed in the ovary in this study is an indication of the antioxidant properties inherent in *M. pruriens*. The upregulation in these markers of oxidative stress is in response to ROS and free radicals. As earlier stated, both animal and human studies have demonstrated the presence of ROS in the ovary. Phytochemical analysis has shown that *M. pruriens* seeds contain flavonoids (27), (42) and tannins (6). Flavonoids and tannins are phenolic compounds, and plant phenolics are a major group of compounds that act as primary antioxidants or free radical scavengers (43). In addition, *M. pruriens* seeds are a rich source of l-Dopa and its metabolites. In vitro antioxidant assays have supported the antioxidant property of l-Dopa (44). Dopamine, a product of l-Dopa metabolism, has also been found to possess strong anti-oxidant capacity and free radical scavenging activity (45), (46). Antioxidants prevent oxidative stress caused by free radicals which damage cells and vital biomolecules. They terminate chain reactions triggered by free radicals by removing free radical intermediates and inhibit other oxidation

reactions (47). The antioxidant capacity of the extracts may be attributed to the presence of l-Dopa and its metabolite, dopamine and also, the identified phytochemicals.

Table 2: Effect of the oral administration of *M. pruriens* on the enzymatic antioxidant activities of CAT and SOD in the ovary of S-D rats.

Treatment groups	SOD (min/mg protein)	CAT (Mmol/min/mg protein
Control	1.50 ± 0.30	60.17 ± 16.50
50 mg/kg	1.65 ± 0.37	60.83 ± 16.57
100 mg/kg	1.67 ± 0.56	61.14 ± 15.30
200 mg/kg	1.88 ± 0.90	62.67 ± 17.40

n = 5. Values are expressed as mean ± standard deviation.

3.3 Serum concentrations of Follicle Stimulating (FSH) Hormone and Luteinizing Hormone (LH)

A dose dependent increase in serum concentrations of FSH and LH was observed. This increase was significant for LH at 200 mg/kg (Table 3). Our study showed a dose dependent increase in the levels of FSH and LH compared to the control. Increase in LH was significant at 200 mg/kg body weight of the extract. Treatment with *M. pruriens* significantly improved blood levels of dopamine, adrenaline and noradrenaline in infertile males (13). L-Dopa and its metabolite dopamine have been reported to stimulate the hypothalamus and forebrain to secrete gonadotropin-releasing hormone (GnRH) (13), (17), (48). This ultimately will activate the anterior lobe of the pituitary gland to secrete FSH and LH. The report of this study is in agreement with studies carried out by elegant researchers in other parts of the world on both animal and human males in which FSH and LH levels increased significantly following the administration of *M. pruriens*. (13), (14), (16), (26).

Table 3: Effect of the oral administration of *M. pruriens* on serum concentrations of FSH and LH at 6.00 p.m. on proestrus.

Treatment groups	FSH	LH
Control	1.83 ± 0.77	1.13 ± 0.15
50 mg/kg	1.87 ± 0.04	1.56 ± 0.81
100 mg/kg	1.95 ± 0.63	1.90 ± 0.51

200 mg/kg	2.01 ± 0.02	2.03 ± 0.76*

n = 5. Values are expressed as mean ± standard deviation. * P < 0.05

3.4 Ovulation and number of ova shed

A slight increase in the number of oocytes released in the oviduct was observed in the treated animals compared to the control (Table 4). LH is critical to ovulation because it is responsible for all the processes and events that accompany ovulation. The rapid surge of LH that occurs between 5 to 7 p.m. in the evening of proestrus induces follicular rupture and ovulation in rats (49). The present study showed a dose dependent increase in LH levels and a slight increase in oocyte number at 50 and 100 mg/kg body weights. However, at the highest dosage of 200 mg/kg we recorded a significant increase in the levels of circulating LH and also a concomitant increase in the number of oocytes released at ovulation compared to the control. Increase in testosterone levels resulting from increase in circulating LH levels have been recorded following treatment with *M. pruriens* in both human and animal studies. This in turn has increased fertility indices such as sperm count, sperm motility, sperm morphology and libido (12), (16), (17), (26). Therefore, the report of this study suggests that the increasing levels of circulating LH from the anterior pituitary produced by the administration of *M. pruriens* was responsible for the increase in the number of oocytes released at ovulation.

Table 4: Effect of the oral administration of a single dose of *M. pruriens* on the number of ova shed in the oviduct in the morning of estrus in S-D Rats.

Treatment groups	Number of ova shed in the oviduct
Control	7.5 ± 2.40
50 mg/kg	7.6 ± 1.30
100 mg/kg	7.6 ± 1.50
200 mg/kg	8.1 ± 2.50

n = 5. Values are expressed as mean ± standard deviation.

4. CONCLUSION

M. pruriens enhances fertility in female Sprague-Dawley rats by producing a dose dependent increase in FSH and LH which in turn increased the number of oocytes released at ovulation possibly through its rich source of L-Dopa and its metabolite, dopamine. At higher dosages than that was administered in this study, it is possible that a significant increase in the number of ova shed may be observed. Thus, the use of *M. pruriens* in the treatment of female infertility caused by anovulation seems promising.

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COMPETING INTERESTS

"Authors declare that no competing interests exist".

AUTHORS' CONTRIBUTIONS

First author wrote the first part of the manuscript, managed the analysis of the study and performed the statistical analysis. Second author designed the study, performed literature search and wrote the final manuscript in accordance with the guideline of this journal. Third author wrote the protocol and edited the manuscript for submission.
"All authors read and approved the final manuscript."

ETHICAL APPROVAL (WHERE EVER APPLICABLE)

"ALL AUTHORS HEREBY DECLARE THAT "PRINCIPLES OF LABORATORY ANIMAL CARE" (NIH PUBLICATION NO. 85-23, REVISED 1985) WERE FOLLOWED, AS WELL AS SPECIFIC NATIONAL LAWS WHERE APPLICABLE. ALL EXPERIMENTS HAVE BEEN EXAMINED AND APPROVED BY THE APPROPRIATE ETHICS COMMITTEE"

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DEFINITIONS, ACRONYMS, ABBREVIATIONS

Here is the Definitions section. This is an optional section.

Term: Definition for the term

APPENDIX