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Ovarian and uterine functions in female albino rats fed dietary meal supplemented with *Mucuna pruriens* (L.) DC. seed powder

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Abstract

**Background:** While the reproduction-enhancing property of *Mucuna pruriens* (MP) seed has been widely studied in males, little is known about this property in females despite the rate at which the seed is consumed by both sexes worldwide.

**Objective:** To determine the effect of MP seed powder in dietary inclusion on ovarian and uterine functions of adult female albino rats.

**Methods:** The rats were randomised into four groups. Group 1 (Control) was given standard rat chow (15g of feed/rat/day only) while groups 2, 3 and 4 were fed diets supplemented with MP seed powder at 0.75 g, 1.5g and 2.25g/day, respectively, for 12 weeks. Serum levels of oestradiol, follicle stimulating hormone, luteinising hormone, ovarian Δ5, 3β- hydroxysteroid dehydrogenase (Δ5, 3β-HSD) and 17 β hydroxysteroid dehydrogenase (17β-HSD) activities, ovarian and uterine peroxidase and tissue cytoarchitectural structures were used as diagnostic markers of reproductive function.

**Results:** Significant increases in the serum level of all hormones including ovarian Δ5, 3β-HSD, 17β-HSD activities, ovarian and uterine peroxidase activities, and improvement of the ovarian and uterine cytoarchitectural integrity of the rats fed MP at 0.75g/day compared to other groups were observed. However, MP at 2.25g/day induced reproductive dysfunction characterised by significant reductions in hormones, uterine and ovarian enzyme activities, severe degenerative cytoarchitectural lesions in tissues.

**Conclusions:** MP seed improves uterine and ovarian functions at a dose level of 0.75g/day, but a higher dose value may be toxic.

**Keywords:** Fertility, Oestradiol, Ovary, Mucuna pruriens, Reproductive hormones, Steroidogenic enzyme, Uterus.
Introduction

The progressive increase in the incidence of reproductive dysfunctions among humans in many parts of the world as a result of exposure to environmental xenobiotics has, over the years, attracted worldwide attention and is now a major concern in most countries, including Nigeria. \[1\] Most developed countries have developed effective drugs to manage reproductive dysfunction and enhance reproductive performance. However, most medications are expensive and may not be accessible to the poor in most developing nations. Hence, there is a need to consider substitutes like medicinal plants with little or no side effects, cheap and easily accessible. For several decades, Nigerian herbal practitioners have used indigenous medicinal plants in herbal formulations for treating ailments, including reproductive disorders. \[2\] Among the dominant plants commonly utilised is cowitch or velvet bean (\textit{Mucuna pruriens}), a member of the family Fabaceae, referred to as ‘werepe’ among the Yoruba tribe in Nigeria. Phytochemical analysis has revealed \textit{Mucuna pruriens} to be rich in secondary metabolites such as alkaloids, tannins, anthraquinones, saponins, flavonoids and cardiac glycosides, which contribute to its vast medicinal properties. \[3\]

Studies have also shown that the root and leaf of \textit{M. pruriens} possess anti-venom and anti-cancer properties. At the same time, the seed was anti-oxidative in nigrostriatal tissue and also improved neurobehavioral activity. \[4,5\] Moreover, the seed of \textit{M. pruriens} enhances sperm quality, quantity, and movement in male animals, including humans, due to the active compound, L-3,4dihydroxyphenylalanine (L-DOPA). \[6\] The anti-inflammatory, neuroprotective, anti-diabetic, antiepileptic, antibacterial and cardioprotective properties of \textit{M. pruriens} have been investigated. \[7,8,9\] However, the reported use of the seed of the plant in herbal formulation for the treatment of fertility-related issues among humans around the world is of major interest. \[10\]

Meanwhile, there have been conflicting opinions on the use of \textit{M. pruriens} seed in treating reproductive disorders in Nigeria. While some herbal and non-herbal practitioners claim \textit{M. pruriens} seed has the potential to enhance reproductive performance in males, resulting in increased consumption of this seed, some differ, having noticed its adverse effects on their sexual performance and reproductive functions. The conflicting opinion on the aphrodisiac property of \textit{M. pruriens} seeds laid the template of earlier research which assessed the effects of different concentrations of \textit{M. pruriens} seed powder on the reproductive functions of male albino rats fed with a diet supplemented with the seed. That study revealed that \textit{M. pruriens} seed enhanced reproductive function in male rats only at a daily dose of 0.75g, while a dose higher than 0.75 g may be toxic to the male reproductive system. \[11\] However, little is known about the effect of \textit{M. pruriens} seeds on the reproductive functions of females because both sexes consume it in several parts of the globe, including Nigeria. Therefore, this study was conducted to determine the reproductive function-enhancing potentials of the seed of \textit{M. pruriens} on female albino rats.

Methods

\textit{Seed collection and preparation}\n
The research team collected the seeds of \textit{M. pruriens} (MP) from a botanical garden in Egbe community, Kogi State, Nigeria. The seeds were de-hulled (Figure 1) and identified by Dr Ajoke S. Sanusi, a Plant Taxonomist, at the ELIKAF Herbarium of the Department of Plant Science, Olabisi Onabanjo University, with Voucher Number: EH/2019/6001. The de-hulled seeds were air-dried and ground into a powder using a Moltinex® electric blender. After that, the seed powder was screened for its phytochemical, L-dopa, mineral and proximate compositions.
Experimental animals
A total of thirty-two (32) adult female albino rats (130±8g) were used for this study. The rats were acclimatised under a standard laboratory condition (25±5°C; 65±5% Relative Humidity) in a well-ventilated experimental animal house for one week before the commencement of the study. The rats were individually housed in wooden cages (45cm × 30cm × 40cm) and were fed with standard laboratory rat chow and clean drinking water ad libitum during this acclimatisation stage. The research team conducted the experimental protocol following the regulations of the local ethics committee in the animal care unit of our university with the approval number OOU/SCIENG/EC/0003/050520. The animal experiment was also performed according to ethical guidelines of animal experimentation (regulation CEE 86/609).

Experimental design
The rats were randomly assigned into four experimental groups (Groups 1, 2, 3 and 4), comprising eight rats each. The rats in Group 1 were individually fed standard laboratory rat chow (Control: 15g feed/rat/day only), while rats in Groups 2, 3 and 4 were individually fed the rat chow containing 0.75g of MP seed powder plus 14.25g feed/rat/day, 1.5g of MP seed powder plus 13.50g feed/rat/day and 2.25g of MP seed powder plus 12.75g feed/rat/day respectively for 12 weeks as earlier described. In this study, albino rats were used following the recommendations of Organization for Economic Co-operation and Development (OECD) guidelines 453 on combined chronic toxicity\carcinogenicity studies. The quantity of food given was measured and readjusted weekly per body weight increase. Meanwhile, clean drinking water was supplied to all the rats ad libitum. The rats were well-nourished and showed no illness, and no mortality was recorded throughout the feeding regime.

Sample collections
At 24 hours after the last feeding, the research team collected blood samples from the rats in each group into plain sample tubes by retro-orbital sinus with micro haematocrit tube. The blood samples were centrifuged at 2500 rpm for 10 minutes to obtain serum samples within an hour after the blood collection. The sera obtained were later stored at -20°C for hormonal assay. The rats were later sacrificed; the ovaries and uterus were also excised. Parts of the excised ovaries and uterus were stored at -20°C and were subsequently used for the assays of ovarian Δ5, 3β-hydroxysteroid dehydrogenase and 17β-
hydroxysteroid dehydrogenase as well as ovarian and uterine peroxidase activity assay. In contrast, the remaining parts were subjected to histopathological examination.

**Phytochemical screening, mineral and proximate analysis of the seed powder**

Qualitative and quantitative analysis of the secondary metabolites (alkaloids, glycosides, flavonoids, saponins, phenols, steroid, triterpenes, coumarin, anthocyanin, terpenoids, phlobatanin and tannins contents) in the seed were carried out using the standard protocols earlier described. The L-dopa content of the seed was determined using the standard method.

The proximate analysis of the samples for moisture, ash and carbohydrate contents were done as described by the Association of Official Analytical Chemists. Crude protein, fibre and fat contents were determined by the methods of Pearson. In contrast, mineral contents were determined by atomic absorption spectrometry, flame photometry and spectrophotometry according to the methods of AOAC.

**Hormonal Assay**

The serum concentration of oestradiol was assayed by radioimmunoassay using commercial kits (Bio-Line, Brussels, Belgium) following the procedure described in the kit manual. The antisera used had the maximum cross-reactivity of 1.7% with oestrone, and the minimum detection limit of oestradiol was 5 pg/ml. The intra- and inter-assay coefficients of variation were 3.7 and 9.4%, respectively. The serum samples obtained were analysed with commercially available Enzyme-Linked Immunosorbent Assay (ELISA) kit for Follicle Stimulating Hormone (FSH) (Enzo Life Sciences, PA) and Luteinizing Hormone (LH) (Eagle Biosciences Inc., Nashua). The protocols and procedures used for the assay were as described by the manufacturer. The sensitivity of the FSH assay was 0.0017 ng/mL with a detection range of 0.02-19 ng/ml. The limit of detection of LH assay was 0.04 ng/ml with a detection range of 0.06-16 ng/ml.

Ovarian Δ5, 3β-hydroxysteroid dehydrogenase and 17β-hydroxysteroid dehydrogenase activities assay

Part of the ovary excised from the rats in each group was separately homogenised in 20% spectroscopic grade glycerol containing 5.0 mM Potassium phosphate and 1.0 mM EDTA at a tissue concentration of 15mg/ml homogenising mixture. The homogenate was centrifuged at 10,000 rpm for 30 minutes, and the supernatant obtained was used to determine the activities of ovarian Δ5 3β- hydroxysteroid dehydrogenase (Δ5, 3β-HSD) and 17, β hydroxysteroid dehydrogenase (17β-HSD). The activity of Δ5, 3β-HSD was measured spectrophotometrically following the addition of 0.5 mM NAD and 30 mg dehydroepiandrosterone to the tissue supernatant at 340nm against blank reagent (without NAD). The remaining part of the supernatant of the homogenising mixture was used to measure the activity of 17β- HSD. The tissue supernatant was mixed with 25mg of crystalline bovine serum albumin, 0.3 mM testosterone and 1.1 mM of NADP. The activity was measured at 340 nm against a blank reagent (without NADP). A unit of enzyme activity is equivalent to a change in absorbance of 0.001/min at 340 nM.

Ovarian and uterine peroxidase

The activities of ovarian and uterine peroxidase were also determined spectrophotometrically according to Lyttle and DeSombre with minor modifications. Briefly, the uteri and ovaries from the rats in each group were separately homogenised in 5% w/v of ice-cold 10mM Tris-HCl (pH 7.2), and the homogenate was later centrifuged at 15,000 rpm for 30 minutes at 4°C. The residual pellet was resuspended in 10 mM Tris-HCl (pH 7.2) with 0.5 M CaCl and recentrifuged at 15,000 rpm for 30 minutes to obtain the supernatant with solubilised peroxidase. Using guaiacol as a substrate, we
later determined the peroxidase activity at 25°C using guaiacol as a substrate. The reaction mixture consisted of 12.5 mM guaiacol, 0.33 mM \( \text{H}_2\text{O}_2 \), 10mM Tri-HCl pH (7.2) and 750-1000 pg protein in a final volume of 3 ml. The linear increase in absorbance at 460 nm resulting from the oxidation of guaiacol was recorded against a blank reagent (without \( \text{H}_2\text{O}_2 \)). The peroxidase activity was expressed as absorption change/h/mg protein. The method described earlier by Lowry was employed to determine protein concentration. \[20\]

Histopathological assessment of ovary and uterus
The histological examination of the ovary and uterus was performed according to a previous study with minor modifications. \[13\] The ovary and uterus tissues were fixed in Bouin’s fluid for 24 hours and routinely processed for paraffin embedding. The embedded tissues were subjected to serial sectioning of 4μm thickness using a Rotary Microtome and later processed in alcohol-xylene series and were stained with haematoxylin and eosin (H & E). The prepared slides were examined at ×400 magnifications. Morphometric analysis of the ovary was done according to the methods established by Hirshfield and Sanjay, and Joshi. \[21\] Micrometric measurements of the uteri were done according to Deb et al. \[22\] Both morphometric and micrometric analyses of the ovary and uterus were done using stage and ocular micrometres.

Statistical analysis
Statistical analyses of the data were performed using the Statistical Package for Social Sciences (SPSS) version 20.0. \[23\] All the data were presented as the mean ± standard deviation (SD). The mean and standard error of the mean were determined while analysis of variance was performed. The post-hoc test was performed using the Student-Newman-Keuls method. \( P \) values less than 0.05 were considered statistically significant.

Results

Phytochemical composition of Mucuna pruriens seed powder
The results of the qualitative analysis of the \( M. \text{ pruriens} \) seed powder showed the presence of twelve (12) phytochemical components (Table I). These include saponin, phenolics, steroids, flavonoids, triterpenes, coumarin, glycoside, anthocyanin, terpenoids, phlobatanin, L-dopa and alkaloids. Quantitative analysis showed that steroids (227.52±0.33 mg/100g) and triterpenes (165.77±0.42 mg/100g) were the highest phytochemical components of the MP seed powder. These were followed by flavonoids, phenolics, alkaloids and terpenoids. However, saponin had the lowest concentration.

Proximate composition in Mucuna pruriens seed powder
The \( M. \text{ pruriens} \) seeds used in this study contained moisture content, ash, carbohydrate, calorific, crude protein, crude lipids and crude fibre (Table II). The moisture content of the seed was low (10.81±0.06 %). On the other hand, the seeds contained a high calorific value (1516.65±2.32Kj/100g) and an appreciable amount of crude protein content (28.80±0.55 %) and carbohydrate content (44.70±0.64 %).

Minerals and heavy metals composition of the Mucuna pruriens seed powder
The mineral contents of \( M. \text{ pruriens} \) seed powder are shown in Table III. These include zinc, phosphorus, potassium, calcium, magnesium and iron. Trace levels of selenium and lead were also recorded in the seeds. Of all the mineral compositions of the seed, calcium recorded an appreciable higher level (125.04±2.00 ppm) than the other mineral elements. This was followed by magnesium (3.31±0.20 ppm) and iron (2.16±0.10 ppm).

Serum level of reproductive hormone in the rat
The serum levels of oestradiol, LH and FSH are presented in Figure 2. The levels of these hormones were significantly lower in the experimental groups with the increase in the dose of MP seeds fed. Oestradiol was significantly higher (p = 0.001) in the rats fed 0.75g/day of MP seed powder. This was followed by those fed a daily dose of 1.5g and the control group. However, the oestradiol levels recorded in the control group and those fed with 1.5g/day of the MP seed powder were not significantly different. The rats fed 2.25 g/day of MP seed powder had the lowest oestradiol level. There was no significant difference (p = 0.491) in the level of LH recorded in the control group and the group fed 0.75g MP seed powder. This was, however, significantly higher (p = 0.02) than those recorded in the rats fed daily doses of 1.5 g and 2.25 g of the seed powder. The rats fed a daily dose of 0.75g of the seed powder had a significantly higher level of FSH (p = 0.001). This was followed by the control rats and those fed daily doses of 1.5g and 2.25g of the seed powder.

<table>
<thead>
<tr>
<th>Secondary metabolites</th>
<th>Qualitative</th>
<th>Quantitative (mg/100g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saponin</td>
<td>+</td>
<td>0.76±0.00</td>
</tr>
<tr>
<td>Phenolics</td>
<td>+</td>
<td>44.57±0.04</td>
</tr>
<tr>
<td>Steroids</td>
<td>+</td>
<td>227.52±0.33</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>+</td>
<td>56.86±0.04</td>
</tr>
<tr>
<td>Triterpenes</td>
<td>+</td>
<td>165.77±0.42</td>
</tr>
<tr>
<td>Coumarin</td>
<td>+</td>
<td>7.61±0.00</td>
</tr>
<tr>
<td>Glycoside</td>
<td>+</td>
<td>8.71±0.02</td>
</tr>
<tr>
<td>Anthocyanin</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Terpenoids</td>
<td>+</td>
<td>22.92±0.02</td>
</tr>
<tr>
<td>Phlobatanin</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Alkaloid</td>
<td>+</td>
<td>42.31±0.00</td>
</tr>
<tr>
<td>L-Dopa (ng/ml)</td>
<td>+</td>
<td>3.11±0.03</td>
</tr>
</tbody>
</table>

-Absent; + Present

Table II: Proximate and L-Dopa compositions of Mucuna pruriens seed powder

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Mean value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture %</td>
<td>10.81±0.06</td>
</tr>
<tr>
<td>Ash %</td>
<td>4.81±0.11</td>
</tr>
<tr>
<td>Carbohydrate %</td>
<td>44.70±0.64</td>
</tr>
<tr>
<td>Calorific value Kj/100g</td>
<td>1516.65±2.32</td>
</tr>
<tr>
<td>Crude protein %</td>
<td>28.80±0.55</td>
</tr>
<tr>
<td>Crude Lipids %</td>
<td>7.63±0.02</td>
</tr>
<tr>
<td>Crude Fibre %</td>
<td>3.24±0.06</td>
</tr>
</tbody>
</table>
Table III: Minerals and heavy metals composition of *Mucuna pruriens* seed powder

<table>
<thead>
<tr>
<th>Elements</th>
<th>Mean (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Selenium</td>
<td>0.39±0.01</td>
</tr>
<tr>
<td>Zinc</td>
<td>1.06±0.04</td>
</tr>
<tr>
<td>Lead</td>
<td>0.36±0.00</td>
</tr>
<tr>
<td>Phosphorus</td>
<td>1.93±0.02</td>
</tr>
<tr>
<td>Calcium</td>
<td>125.04±2.00</td>
</tr>
<tr>
<td>Magnesium</td>
<td>3.31±0.20</td>
</tr>
<tr>
<td>Iron</td>
<td>2.16±0.10</td>
</tr>
</tbody>
</table>

Figure 2: Levels of estradiol (pg/mL), luteinising hormone (mlu/mL) and follicle stimulating hormone (mlu/mL) of female rats fed *Mucuna pruriens* seed powder daily for 12 weeks. a,b,c,d Mean having similar alphabets are not significantly different at p < 0.05.

Activities of ovarian Δ5, 3βhydroxysteroid dehydrogenase (Δ5, 3β-HSD) and 17β hydroxysteroid dehydrogenase (17β-HSD)

The activities of ovarian 17β-HSD and Δ5, 3β-HSD in the rats fed MP seed powder daily for 12 weeks are shown in Figure 3. The activities of the two ovarian enzymes in the experimental rats followed a similar pattern. The rat group fed 0.75g/day of MP seed powder had a significantly higher (p = 0.01 and 0.007 for 17β-HSD and Δ5, 3β-HSD respectively) activities of the two ovarian enzymes. These were significantly lowest in the rats fed 2.25g/day of the seed powder. On the other hand, there was no significant difference (p = 0.963 and 0.882) recorded in the activities of these enzymes between the control group and those fed 1.5g/day of the seeds powder.
Ovarian and uterine peroxidase enzymes activities
The ovarian and uterine peroxidase activities were highest in the rats fed 0.75g/day of MP seed powder (Figure 4). These were significantly higher (p = 0.001 and 0.006 for ovarian and uterine peroxidase respectively) than recorded in the control group and those fed daily doses of 1.5 g and 2.25 g of the MP seed powder. There was no significant difference (p = 0.624 and 0.078) in the activities of ovarian and uterine peroxidase in the control group and those fed 1.5 g/day of MP seeds powder. However, the activity of this peroxidase enzyme was lowest in the rats fed 2.25 g/day of the seed powder.

Ovarian and uterine histometric changes
Histological examination of the ovary and uterus showed a progressive increase in histopathological lesions along with the increase in the quantity of MP supplemented. There were severe degenerative ovarian architectural lesions characterised by a reduction in pre-antral, antral and Graffian follicles followed by a reduced number of corpora lutea along with an increasing number of regressing follicles and prominent stroma in rats fed MP seed powder at 2.25g/day (Figure 5).

The diameter of the uterus was significantly higher (p = 0.031) in the rats fed 0.75g/day of the seed powder (Table IV and Figure 6). This was insignificantly different between the control group and the rats fed 1.5g/day of MP seed powder. The rats fed 2.25g/day MP seed powder had the lowest uterine diameter. Similarly, the epithelial cell height, endometrial thickness and myometrial thickness were significantly higher in the rats fed 0.75g/day of the seed powder compared to other groups.

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Figure 3: Levels of ovarian enzymes in rats fed Mucuna pruriens seed powder daily for 12 weeks.

abcd Mean having similar alphabets are not significantly different at p < 0.05.
Table IV: Uterus histometric changes of rats fed with *Mucuna pruriens* seed daily for 16 days

<table>
<thead>
<tr>
<th>Micrometric parameters</th>
<th>Control</th>
<th>0.75 g MP</th>
<th>1.5 g MP</th>
<th>2.25 g MP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diameter (mm)</td>
<td>1.7±0.0(^b)</td>
<td>1.9±0.1(^a)</td>
<td>1.6±0.0(^b)</td>
<td>1.1±0.0(^c)</td>
</tr>
<tr>
<td>Epithelia height (µm)</td>
<td>24.4±0.3(^b)</td>
<td>25.7±0.2(^a)</td>
<td>16.4±0.0(^c)</td>
<td>12.8±0.1(^d)</td>
</tr>
<tr>
<td>Endometrial thickness (µm)</td>
<td>317.0±1.0(^b)</td>
<td>321.5±0.5(^a)</td>
<td>311.4±0.3(^c)</td>
<td>286.2±0.1(^d)</td>
</tr>
<tr>
<td>Myometrial thickness (µm)</td>
<td>290.5±0.4(^b)</td>
<td>306.2±1.0(^a)</td>
<td>272.1±0.1(^c)</td>
<td>162.8±0.0(^d)</td>
</tr>
</tbody>
</table>

\(^{abc}\)Means (±Standard deviation) in the same row having similar superscripts are not significantly different at p < 0.05; MP = *Mucuna pruriens*

*Discussions*

It has been reported that *M. pruriens* seed is a potential food source due to its richness in crude protein, essential fatty acids, starch, and essential amino acids.\(^{24}\) This claim is confirmed by the appreciable amount of the proximate and mineral compositions in the seed used in this study. Meanwhile, the findings of this study demonstrated the adverse effect of *M. pruriens* seed powder on the female gonadal steroidogenic activity at a higher dose. The vital role of gonadotropin in the reproductive system is known.\(^{25}\) An increase in the serum levels of LH and FSH in rats fed MP powder at a dose of 0.75 g could be due to the possible influence of the L-DOPA composition of the seed on the gonadotropin-releasing hormone (GnRH). L-DOPA, the active compound of *M. pruriens*, has been reported to improve reproductive function in animals by stimulating the secretion of GnRH from the hypothalamus and, consequently, inducing the release of LH and FSH from the anterior part of the pituitary gland.\(^{26}\)

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**Figure 4:** Levels of peroxidase enzymes in the uterus and ovary of rats fed *Mucuna pruriens* seed powder daily for 12 weeks.\(^{27}\)

\(^{abcd}\)Mean having similar alphabets are not significantly different at p < 0.05.
It could be suggested that the level of L-DOPA in *M. pruriens* at a dose of 0.75g might be sufficient for the stimulation and secretion of GnRH from the hypothalamus, increasing the serum levels of LH and FSH as observed in this study. In addition, the presence of some secondary metabolites (such as flavonoids) in *M. pruriens* at a dose of 0.75g may also be responsible for the higher levels of these reproductive hormones \[26\] since these compounds were noted to increase the serum level of reproductive hormones through the stimulation of hypothalamo-pituitary-gonadal axis in animals. \[27\] However, the seed's L-DOPA and secondary metabolites contents at a dose of 2.25g/day might have been in excess and, as such, toxic to the proper functioning of the hypothalamic-pituitary axis. This could explain the observed reductions in

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Figure 5: Photomicrograph of ovarian tissue of rat H & E ×400 (a) control showing normal cytoarchitecture with a normal ovarian wall (black arrow) (b) fed 0.75 g of the seed powder showing normal cytoarchitecture with Graffian follicle (green arrow), corpus luteum (blue arrow) and antral follicle (red arrow)(c) and (d) fed daily doses of 1.5 g and 2.25 g of the seed powder respectively showing severe degenerative architectural lesions with reduced and distorted antral and Graafian follicles (green arrow) as well as corpora lutea (blue arrow).
these hormones in rats fed *M. pruriens* at a dose of 2.25g/day.

Figure 6: Photomicrograph of uterine tissue of rat H & E ×400 (a) control showing normal cytoarchitecture with uterine epithelial height (black arrow) (b) fed 0.75 g of the seed powder showing normal cytoarchitecture with the uterine lumen (red star), endometrium (yellow arrow), myometrium (white arrow) (c) and (d) fed daily doses of 1.5 g and 2.25 g of the *M. pruriens* seed powder respectively showing uterine lumen width (black arrow) and epithelium (blue arrow)

Apart from the fact that ovarian 17β-HSD and Δ5, 3β-HSD are the key regulatory enzymes involved in ovarian steroidogenesis, they are also responsible for synthesising oestradiol from the ovary. [28] Therefore, a reduction in the serum level of oestradiol in rats fed *M. pruriens* at a dose of 2.25g/day may be due to the inhibition of ovarian steroidogenic enzyme activity. In addition, the steroidogenic function of the ovary is under the direct stimulatory influence of pituitary gonadotropins, and studies have demonstrated a reduction of serum levels of gonadotropins and oestradiol as a result of exposure to a toxic substance. [29] Since LH and FSH are known to regulate the activities of ovarian 17β-HSD and Δ5, 3β-HSD, a reduction in the levels of LH and FSH in rats fed *M. pruriens* at a dose of 2.25g/day may explain the alteration in their steroidogenic enzymes' activity.

*M. pruriens* at a dose of 0.75g/day increased the ovarian and uterine peroxidase activity of rats. In comparison, *MP* at a dose of 2.25g/day was significantly reduced peroxidase activity.
Peroxidase is one major component of the antioxidant defence system; thus, reducing peroxidase activity could signal oxidative stress in the tissues. [30] There is a possibility of the seed at a dose of 2.25g/day to have induced oxidative stress in the ovary and uterus of the rats. This is supported by our finding in a previous study that showed oxidative stress induction in the testis of rats fed *M. pruriens* seed powder at a dose of 2.25g/day. [11] On the other hand, studies have indicated peroxidase activity in the uterus as a viable marker for hormonal action. [31,20] Therefore, the response of the uterus to hormonal stimulation could be determined through the activity of its peroxidase enzyme. A reduction in the uterine peroxidase activity of rats fed *M. pruriens* at a dose of 2.25g/day could indicate depression of uterine response to hormonal stimulation, and possible persistence of non-functional corpora lutea could be responsible. [32]

Histologically, the ovary of rats fed *M. pruriens* at increasing dose showed the presence of arrested follicles at the pre-antral stage and virtual absence of antral follicles. A low level of FSH has been associated with a reduced number of healthy follicles in rats. [33] Therefore, the low serum level of gonadotrophins in rats fed MP at a dose of 2.25g/day corresponds with the reduction in folliculogenesis as observed in this present study.

Uterine tissue degeneration in rats fed *M. pruriens* at a dose of 2.25g/day may be due to the decreased serum oestradiol level as growth and proliferation of uterine layers is mainly under the influence of oestradiol. [34] In addition, we hypothesised in this study that a reduction in progesterone action on the oestradiol-primed uterus, which in turn, may obstruct the transition of the uterine epithelium from the proliferative to the secretory state. Studies have associated uterine endometrium degeneration with the increased production of reactive oxygen species, such as superoxide radicals, hydrogen peroxide, and hydroxyl radicals. [35] This is confirmed in this study by the observed significant decrease in the activity of uterine peroxidase in rats fed increasing doses of *M. pruriens* seed powder.

**Conclusions**

From the findings in this study, *M. pruriens* seed is phytochemically endowed with essential secondary metabolites of pharmacological importance. Moreover, its appreciable carbohydrate, protein and mineral contents are nutritionally helpful. *M. pruriens* seed powder enhanced female reproductive functions by increasing oestradiol, LH, and FSH serum levels. It also increased the activities of steroidogenic enzymes in the uterus and ovary of the rats. However, this reproductive-enhancing property was only valid at a dose of 0.75g/day, and a dose higher than 0.75g/day appeared toxic to ovarian and uterine tissues. There is a need to know the specified dose of *M. pruriens* seed powder that humans should consume based on body weight to enhance reproductive functions and prevent harmful toxicological effects.

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