Effects of Ethanolic Extract of *M. Oleifera* Seeds and Leaves on the Reproductive System of Female Albino Rats

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**Abstract**

Infertility has remained a major health problem among couples and sexually active individuals who seek to procreate, and the search for therapeutic solutions have remained endless. Therefore, this study investigated the effects of ethanolic extract of *M. oleifera* seeds and leaves on the reproductive system of female albino rats. Eighty-four (84) albino rats comparing of 56 females and 28 males, which acclimatized for two weeks and mated in ratio of 2 females to 1 male, the pregnant female rats were then divided into 7 Groups of 8; Group 1- Control (10 ml/kg body weight/day of vehicle [Tween 80] orally). Groups 2-4 (Seed extract at dose level of 100, 200 and 400 mg/kg body weight/day respectively), Groups 5-7 (Seed extract at dose level of 100, 200 and 400 mg/kg body weight/day respectively). Administration was carried out throughout the gestation period. The blood samples were collected for hormonal assay and under standard aesthetic conditions, the reproductive organs (uterus, ovaries and fallopian tube) excised for histological examination. From the results it was observed that the *M. oleifera* leave and seed extract caused resorption of the foetus with decrease in weight in a dose dependent manner; however, there was no disruption of the normal gestation. The levels of FSH and LH for animals treated with 400 mg/kg were significantly lower than those of 100 mg/kg, 200 mg/kg and the control group. From the histological slides, there was degeneration or atretic follicles in animals treated with extracts, which was intense at 400 mg/kg dose. In conclusion, the ethanol extract of both the leaf and seed of *M. oleifera* has shown abortifacient effect and therefore not advise for consumption during pregnancy.

**Keywords:** *Moringa oleifera;* abortifacient effect; albino rats; reproductive system

**Introduction**

For centuries and up until date, plants have remained an important and dependable source of medicine. World Health Organization (WHO) estimated that about 80% of the global population depends absolutely tradomedicine [8]. The therapeutic value of these plants is because of the variety of active phytochemicals and their essential composition. The role medicinal plants play in fighting and managing diseases have been attributed to presence of antioxidant in their constituents, often linked to numerous types of polyphenolic compounds [6]. Thus, the global interest in understanding the nature and dynamic of these natural antioxidants obtained from therapeutic plant materials for health care use has continued to grow.

Among the numerous medicinal plants which have shown great potentials is Moringa oleifera Lam (*M. Oleifera*); commonly known as Moringa [2]. Moringa is a versatile tropical tree popularly known for its culinary uses; however, it has wide range of application in the industry, medicine and agriculture, including animal feeding. For this purposes it has become increasingly popular in Asian, European and African continents, where its economical valuable is unprecedented [16, 19]. It has been dubbed the “Miracle tree” or “tree of life” by the media as every part of *M. oleifera* have been reported for one or more therapeutic uses as well as pharmaceutical and industrial byproducts [6,18].

Aside the culinary and other local uses various researchers have reviewed the numerous biochemical properties of various parts of *M. oleifera* in the past [6,10,15,16,18]. These parts contain both macro- and micro-nutrients, which are rich sources of natural antioxidants, which wide range of hormone modulation [9, 12, 14].

Across the globe, the importance of the reproductive system cannot be overlooked, as it is one of the most significant characteristics of humans and essential for the continuity of life, because of the continued exposure to life and attacks from environmental agents. The disease of the disease of the reproductive system is infertility and it has resulted in large cases of marital problems (WHO, 2013). This high burden of infertility has lead couples and individuals, who yearn but are unable to realize and sustain desired pregnancy to sort for assistance in tradomedicine especially in low resources countries.

The key hormones of the reproductive system are the Follicle-Stimulating Hormone (FSH) and Luteinizing Hormone (LH) are
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Materials and Methods

Purchase, Identification and Extraction of Plant (M. Oleifera)

Moringa oleifera seed and leaf were purchased at Moringa Purchase, Identification and Extraction of Plant (Moringa Oleifera). The seeds and leaves of M. oleifera were properly processed, ground to powdered and subjected ethanol soxhlet extraction. The seed and leaf extracts were evaporated to near dryness on rotary evaporator (40°C), weighed and preserved at -4°C in a refrigerator until needed.

Experimental Design

Fifty-six (56) non-pregnant female albino rats (weighing between 180-200g) and twenty-eight (28) male albino rats (weighing between 200-220g) were obtained from the animal farm, University of Port Harcourt. The rats were housed four per cage and maintained under natural conditions. They were fed with laboratory feeds and clean tap water. They were allowed to acclimatize to laboratory environment for 14 days before commencement of research. All experimental protocols were in line with the approved guidelines of the University’s Research Ethics Committee. After the 2weeks of acclimatization, the female animals were mated with males (2:1). Animals were checked for the presence of vaginal plug to confirm pregnancy. The pregnant rats were separated out for the main research. The pregnant female rats were divided into 7 groups of 7 animals each. The animals were treated with different dose of Moringa oleifera seed and leaf extract. Group 1 represented the control group, which was administered 10 ml/kg body weight/day of vehicle (Tween 80) orally. Groups 2-4 were administered suspension of ethanolic extract of Moringa oleifera seed in tween 80 orally at dose level of 100, 200 and 400 mg/kg body weight/day respectively. Groups 5-7 were administered suspension of ethanol extract of Moringa oleifera leaf in tween 80 at dose level of 100, 200 and 400 mg/kg body weight/day respectively. Extracts and vehicle were administered throughout the gestation period. The mothers were sacrificed after delivery under deep diethyl ether anaesthesia and the histology of the uterus and ovaries were conducted.

Determination of Lethal Dose

Doses used were based on the LD50 of the plant and previous studies done. The oral LD50 of the leaves of the plant in rats has been recorded as 6616.67 mg/kg while it is 5000mg/kg for the seeds [17,21]. The doses chosen are approximately 2, 4 and 8% of the LD50 respectively.

Histological Analysis of Reproductive Organs

The reproductive organs (uterus, ovaries and fallopian tube) of both the control and experimental groups were removed immediately after sacrifice and fixed in Bouin’s solution for 24hrs and then dehydrated with ascending grade of alcohol (80% ethanol), cleared in xylene and embedded in paraffin wax. Thin sections of 7 microns thick were sectioned using a rotatory microtome. The sections were then de-paraffinized and stained using the routine haematoxylin and eosin (H & E). The sections were then examined under bright field light microscopy, and Photomicrographs of the results were obtained using digital research photographic microscope.

Hormonal Assay

The blood samples collected from the animals were subjected to hormonal analysis.

Data Analysis

All data generated were computed and analysed using Microsoft Office Excel 2013 and IBM SPSS version 23.0. Data was presented in tables of descriptive statistics as mean SEM (Standard Error of Mean). Analysis of Variance (ANOVA) was done to determine if significant difference exist between the groups, while Dunnett’s multiple comparison test was done to determine the pair that differs (each group will be compared against the control; typical of Dunnett’s). Comparison was carried out at three significant levels (95%, 99% and 99.9%). Hence P < 0.05, P < 0.01 and P < 0.001 respectively will be considered significant.

Discussions

Ethanol extract of Moringa oleifera was involved in the study, with their effects examined on the following: number of pups delivered, FSH, LH as well as the histology of the uterus, fallopian tube and ovaries of the female albino rats.

The ethanolic extract of the seed and leaf of Moringa oleifera was found to decrease the number of pups delivered in a dose dependent manner (Table 1). This may be related to the reported abortifient effect of the plant. On this regard several works have reported similar effect. Some of them include the report of and These works have also reported a dose dependent effect of the stem bark of the plant of the number of resorption of the pregnant animals, i.e. the higher the dose the greater the number of animals that experience resorption [1,22].

Table 1: Number of Pups delivered by female albino rats treated with extract of Moringa oleifera

<table>
<thead>
<tr>
<th>Group</th>
<th>Leaf Extract (µg/ml)</th>
<th>Seed Extract (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>7.14 0.34</td>
<td>7.14 0.34</td>
</tr>
<tr>
<td>100mg/kg</td>
<td>3.29 0.29***</td>
<td>4.14 0.51***</td>
</tr>
<tr>
<td>200mg/kg</td>
<td>2.14 0.26**,***</td>
<td>2.14 0.26**,***</td>
</tr>
<tr>
<td>400mg/kg</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

SEM = Standard Error of the Mean, * = P < 0.05, ** = P < 0.01, *** = P < 0.001

Table 2 revealed that there was also a dose dependent decrease in the levels of FSH and LH in the tested animals; higher doses produce low hormonal level while the lower doses produce higher hormonal levels. The levels of FSH and LH for animals treated with 400 mg/kg were significantly lower than those of 100 mg/kg, 200 mg/kg and the control group. This is indicating that the ethanolic extract of both leaf and seed of Moringa oleifera have the capacity of reducing the level of both hormones (FSH and LH) in biological systems.

Table 2: Level of FSH in albino rats treated with extract of Moringa oleifera

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean  SEM</th>
<th>Leaf Extract (µg/ml)</th>
<th>Seed Extract (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>9.89 0.03</td>
<td>9.89 0.03</td>
<td></td>
</tr>
<tr>
<td>100mg/kg</td>
<td>6.11 0.17***</td>
<td>7.47 0.14***</td>
<td></td>
</tr>
<tr>
<td>200mg/kg</td>
<td>5.44 0.15**,***</td>
<td>6.83 0.13**,***</td>
<td></td>
</tr>
<tr>
<td>400mg/kg</td>
<td>3.13 0.04**,***</td>
<td>4.26 0.05**,***</td>
<td></td>
</tr>
</tbody>
</table>

SEM = Standard Error of the Mean, * = P < 0.05, ** = P < 0.01, *** = P < 0.001

Table 3: Level of LH in albino rats treated with extract of Moringa oleifera

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean  SEM</th>
<th>Leaf Extract (µg/ml)</th>
<th>Seed Extract (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>11.51 0.14</td>
<td>11.51 0.14</td>
<td></td>
</tr>
<tr>
<td>100mg/kg</td>
<td>9.76 0.07***</td>
<td>10.16 0.03***</td>
<td></td>
</tr>
<tr>
<td>200mg/kg</td>
<td>8.53 0.27**,***</td>
<td>8.62 0.09**,***</td>
<td></td>
</tr>
<tr>
<td>400mg/kg</td>
<td>5.34 0.10**,***</td>
<td>6.82 0.02**,***</td>
<td></td>
</tr>
</tbody>
</table>

SEM = Standard Error of the Mean, * = P < 0.05, ** = P < 0.01, *** = P < 0.001

The result of the effect of the leaf and seed extract of Moringa oleifera on the ovaries showed different pathological changes when compared to the control group (Plates 1 - 7). There was no observed pathological change in the animals of the control group. Both the theca externa and the theca interna were intact. There was no degeneration of the ovarian follicles as well as distortion of the ovarian follicles. Animals treated with 100 mg/kg of the leaf and seed extract of the plant showed degeneration of the ovarian follicles or atretic follicles. The animals treated with 200 mg/kg of both the leaf and seed of the extract also showed the presence of follicular degeneration of atretic follicles. The presence of degeneration or atretic follicles in animals treated with ethanolic extract of Moringa oleifera indicates that the extract enhances the degeneration of pre-ovulatory follicles. This is in agreement with findings [13]. The animals in the group treated with 400 mg/kg of the extract also showed both distortion and degeneration of the ovarian follicle as well as atretic follicles thus indicating degeneration in the pre-ovulatory follicle. The degeneration of pre-ovulatory follicles occurs because of non-availability of steroidal hormones. This also agrees with result of the FSH and LH which are responsible for the production of both oestrogen and progesterone.

Plate 1: Photomicrograph normal histology of Ovary Showing Follicles (OF) in the cortical region. (Control)

Plate 2: Photomicrograph of Ovary Showing Follicles (OF) in the cortical region and Blood vessels (BV). No ova are seen in the follicles may be due to degeneration (100 mg/kg of leaf extract)
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Plate 3: Photomicrograph of ovary showing elongated ovarian follicles (OF) in the cortical area with dense granulosa cells. No ovum is seen in the follicle may be due to degeneration (200 mg/kg of leaf extract).

Plate 4: Photomicrograph of ovary showing Ovarian Follicles (OF) with proliferation of granulosa cells. The ovarian follicles are distorted. (400 mg/kg of leaf extract).

Plate 5: Photomicrograph of ovary showing Ovarian Follicles (OF) in the cortical region. No ovum is seen in the follicle. There is also proliferation of granulosa cells and slight distortion of the ovarian follicle (100 mg/kg of seed extract).

Plate 6: Photomicrograph of ovary showing elongated Ovarian Follicle (OF) with proliferation of the granulosa cells. The ovarian follicle is distorted. (200 mg/kg of extract).

Plate 7: Photomicrograph of ovary showing elongated Ovarian Follicle (OF) with proliferation of granulosa cells. The ovarian follicle is distorted. No ovum is seen in the follicles may be due to degeneration. (400 mg/kg of seed extract).

The result of histological examination of the uterus (Plates 8 - 14) showed no observable changes in the control group, it showed intact and normal simple cuboidal epithelial layer of the luminal border (C). the endometrium (E) contain Blood Vessels (BV) and endometrial glands (G) in the proliferative phase of development. Animals treated with 100mg/kg of leaf extract showed shrunken endometrial glands (G), while those treated with 100 mg/kg of seed extract showed engorged endometrial glands (G). Also animals treated with 200 mg/kg of leaf extract showed fewer endometrial glands (G) that may be due to shrinkage of other glands, while those treated with 200 mg/kg of seed extract no observable gland which may have occurred due to shrinkage. The animals treated with 400 mg/kg of leaf showed lumen filled with mucus. Similarly, animals treated with 400 mg/kg of seed extract showed lumen mucus and engorged glands; these could be considered as polyps.
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Plate 8: Photomicrograph of normal histology of fallopian tube showing simple columnar ciliated epithelium (A) at the luminal border with layer of smooth muscles containing Blood Vessels (B) on the outside (Control)

Plate 9: Photomicrograph of fallopian tube showing simple columnar ciliated epithelium (A) with an area (B) of slightly distorted smooth muscles. (100 mg/kg of leaf extract)

Plate 10: Photomicrograph of fallopian tube showing inner layer of simple columnar ciliated epithelium (A) and slightly distorted outer layer of smooth muscles (B) (200 mg/kg of leaf extract)

Plate 11: Photomicrograph of fallopian tube showing slight distortion of the tube of inner layer of simple columnar ciliated epithelium (A) and outer layer of smooth muscles (B) (400 mg/kg of leaf extract)

Plate 12: Photomicrograph of fallopian tube showing the outer/smooth layer of smooth muscles containing numerous Blood Vessels (BV). The tube section not clearly shown but with a slight distortion (100 mg/kg of seed extract)

Plate 13: Photomicrograph of fallopian tube showing the outer/smooth layer of smooth muscles containing numerous Blood Vessels (BV). The tube section not clearly shown but with a slight distortion (200 mg/kg of seed extract)
According to endometrial polyps are common spontaneous reproductive tract lesions that occur in aged rats, but because the animals used in this research are young, the presence of these lesions may be due to the effect of the extract. Another study from [7,4], revealed that certain agents such as quinacrine, can cause an increase in the incidence of endometrial hyperplasia and uterine stroma polyps. This also suggested that the phytochemicals in the ethanolic extract of both leaf and seed of Moringa oleifera are responsible for this effect.

In this study there was also an observed shrinkage of the uterine gland with increase in dose with the absence of extensive folding of luminal epithelium. In the higher dose of the extract the musculature was seen to be highly affected and stroma was compact with poor vascularity. The above changes in the uterine histology, after treatment with the extract may cause the endometrial milieu to become unfavourable for the implantation of the fertilized ovum and hence their antifertility effect. This agrees with other studies made by on Rumexsteudelii. These effects were seen to be dose dependent [11].

The histology of fallopian tube (Plates 15 - 21) in the control group show normal simple columnar ciliated epithelium at the luminal border with layer of smooth muscles containing blood vessels on the outside. On the contrary, the histology of fallopian tube in animals treated with 100 mg/kg of both leaf and seed extracts showed slight distortion of the smooth muscles. Also in animals treated with 200 mg/kg of both leaf and seed extracts, there was an observable slight distortion of the outer layer of smooth muscles. In animals treated with 400 mg/kg of leaf and seed extracts, there were observable distortion of the tube of inner layer of simple columnar ciliated epithelium and outer layer of smooth muscles. All these changes can cause obstruction of the smooth movement of a fertilized egg in the uterus.
Summary of Findings

Dose dependent decrease was observed in the levels of FSH and LH in the treated animals; the higher the dose the lower the hormonal levels and vice versa.

Different pathological changes (degeneration of the ovarian follicles or atretic follicles) were observed on the ovaries of the rats treated with the extracts (leaf and seed) as compared to the control group.

There were no observable histological changes in the uterus of the control group, while changes ranges from shrunken endometrial to engorged as well as fewer endometrial glands as the dose increases (from 100 mg/kg to 200 mg/kg as well as 400 mg/kg).

Conclusions

The ethanol extract of both the leaf and seed of moringa oleifera has shown abortificient effect in that they caused a decrease in the number of litters from animals treated with the extract. The ethanol extract also causes atretic follicle and tissue engorgement follicle in a dose dependent manner in the ovaries as a sign of high levels of degenerating pre-ovulatory follicle and an absence of the steroid hormones. The abortificient effect was also observed in the uterus where it causes endometrial polyps and shrinkage of the uterine gland as well as making the endometrial milieu to become unfavourable for the implantation of the fertilized ovum. While in the fallopian tube, the both extract caused distortion of the tube of inner layer of simple columnar ciliated epithelium and outer layer of smooth muscles.

Recommendations

Pregnant mothers no matter the anticipated beneficial effect should not use the plant. More research should be conducted to know the effects on other organs and hormones not covered in this work.
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References