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RESEARCH ARTICLE

ETHANOLIC ROOT EXTRACT OF MORINGA OLEIFERA Lam CAUSES HISTOMORPHOLOGICAL CHANGES IN THE OVARY AND UTERUS OF FEMALE WISTAR RATS

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ABSTRACT

For several centuries, evaluation of various plant species has led to the discovery of some herbal medicaments. The plant, *Moringa oleifera* is typically used traditionally as a birth control agent. This study was aimed at investigating effect of ethanolic root extract of *Moringaoleifera* lam on the morphological and histological parameters of the ovary and uterus of female Wistar rats. Thirty (30) female adult Wistar rats used for the study were divided into six (6) groups (n = 5). Rat chow and water were provided *ad libitum.* A pilot study to determine the safety of the extract was carried out using the method of Miller and Tainter. The animals received oral administration of graded doses of 100mg/kg, 1000mg/kg, 2000mg/kg, 3500mg/kg and 5000mg/kg of the extract per body weight. The control group received normal saline. Daily weights of the animals were taken throughout the duration of the experiment. On day 30, the animals were sacrificed, laparotomy was carried out and the ovary and uterus were excisedfor morphological assessment and routine histological studies. Grossly, no lesions were observed and there were no obvious changes in the morphology while histologically, the ovarian tissue showed atretic follicles with tissue engorgement. The uterus showed increase in vacuolation and size of the uterine glands. This study showed that the ethanolic root extract of *Moringaoleifera* affected the histological framework of the ovary and uterus in a dose-dependent manner and may be the basis of its usage as a traditional anticonceptive agent.

INTRODUCTION

Herbal medicine is the oldest form of health-care known to mankind and most cultures have long folk medicine histories that include the use of plants. The World Health Organization (WHO) recognizes herbal medicines as a valuable and readily available resource for primary health care and has endorsed their safe and effective use (Tilburt and Kaptchuk, 2008).

The evaluation of various plant products according to their traditional uses and medical value based on their therapeutic efficacy has led to the discovery of newer and recent drugs for treating various ailments. (Garima *et al*, 2011). This forms the basis for the development of new drugs from various plant sources. One of such plants of medicinal value is *Moringa oleifera* which is used traditionally as a birth control agent (Garima *et al*, 2011).

There are about thirteen (13) species of Moringa trees and *Moringa oleifera* is the best known belonging to the family moringacae (Aney *et al*., 2009), Genus Moringa and Species Oleifera (Ritu *et al*., 2011).

The rat estrous cycle is short, lasting four to five days. It occurs throughout the year, with no seasonal effect (Huda and Md Zuki.,2013).

The estrous cycle in the rat consists of four stages known as proestrus, estrus, metestrus and diestrus. Proestrus lasts approximately 12 h; estrus, 9 to15 h; metestrus, 21 h; and diestrus (the longest phase), over 57 h (Lohmiller and Swing, 2006).

The present study was undertaken to investigate and determine the antifertility and histomorphological effect of Ethanolic Extract of *Moringa root on* female wistar rats. Shukla *et al*, (1989), however investigated the antifertility effect of aqueous extract of *Moringa oleifera* roots histologically on the genital tract of ovariectomized rats with and without estradiol dipropionate and progesterone. Their findings on the uterine histoarchitecture revealed increase in the height of luminal epithelium, well developed glands, loose stroma and rich

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vascularity, the cervix showed metaplastic changes in the epithelium with marked keratinisation. In the vagina, cornification was very prominent, with associated increase and loose rugae. Conjoint administration of the extract with estradiol showed synergistic action, whereas inhibition was observed when administered conjointly with progesterone.

MATERIALS AND METHODS

Collection, Identification and Processing of Plant Material

Moringa oleifera roots were harvested during the rainy season from trees grown on loam soil in Dinjor village of Langtang North Local Government Area of Plateau State, Nigeria. The family and species of *M. Oleifera* was identified and authenticated by Botanists in the Federal College of Forestry, Jos and the Department of Pharmacognosy, University of Jos.
The identified *Moringa oleifera* roots, was then peeled, air–dried at room temperature at the Department of Pharmacognosy until constant weight was attained. They were kept away from direct sun light to avoid interference with the active principles. They were then pound in a metallic mortar and pestle to ease the extraction of active compounds.

Plant Extraction

500g of *Moringa oleifera* root peeled powder was weighed and soaked in 70% ethanol, shaken for three(3) hours and allowed to stand overnight, filtrered with non-absorbent cotton wool using a vaccum pump/ Buchner funnel and flask. The filtrate was concentrated to dryness on a rotary evaporator and water bath at a controlled temperature of 50^0C and stored in a desicator as ethanolic extract. The filtrate was freezed dried.

Ethical Clearance

Permission to carry out this study was sought from the Research and Ethics Committee of the University of Jos. The ethical practices that govern the use of laboratory animals for experimental purposes were strictly adhered to.

Preparation of the Animals

Thirty (30) female Wistar rats weighing between 160g and 240g to be used for this study were housed in the Experimental Animal house of the University of Jos and acclimatized for two weeks. The animals were weighed daily throughout the duration of the experiments.

Determination of LD50

The safety of the extract was evaluated by determining its LD_{50} using the Miller and Tainter method (1944).The animals receive oral administration of the extract at 100mg/kg,1000mg/kg,2000mg/kg,3500mg/kg and 5000mg/kg dose level.

The rats were divided into six(6) groups(n=5).

Group one (1) to five(5) received Oral administration of extract at doses of 100mg/kg,1000mg/kg,2000mg/kg,3500mg/kg and 5000mg/kg body weight respectively while Group six(6) which serves as experimental control receive corresponding minimum dose of normal saline.

The animals were monitored for 24 hours for any symptoms and signs of toxicity. After 24 hours, the animals were further monitored for 48 hours and 72 hours with no mortality recorded, with a follow up at 10 days,20days and 30 days.

Dissection/Harvesting of the organs

The animals were sacrificed using cervical dislocation method. The animals were then laid on their backs on a prepared operating board and dissected. The organs were harvested, weighed and immediately fixed in 10% formal saline, for routine histological studies.

RESULTS

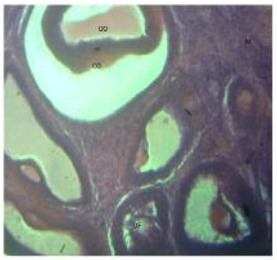

Figure 1: Light Micrograph of section of ovary from Rat treated with normal saline showing Ovarian Cortex (OC), Medullar (M), Ovarian Oocyte (OO), Cumulus oophoricus (CO) and Ovarian follicle (OF) (H&E Stain x100).

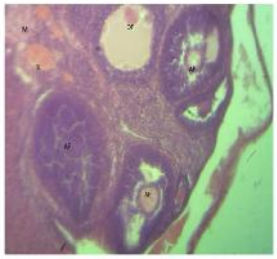

Figure 2: Light Micrograph of section of ovary from Rat treated with 100mg/kg of the Extract showing Ovarian Follicle (OF), Medullar (M), Ovarian Oocyte (OO), Atretic follicle (AF) and Tissue engorgement (TE) (H&E Stain x 100).

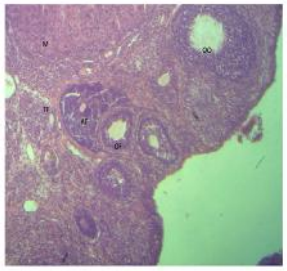

Figure 3: Light Micrograph of section of ovary from Rat treated with 1000mg/kg of the Extract showing Ovarian Follicle (OF), Medullar (M), Ovarian Oocyte (OO), Atretic follicle (AF) and Tissue engorgement (OG) (H&E Stain x100).

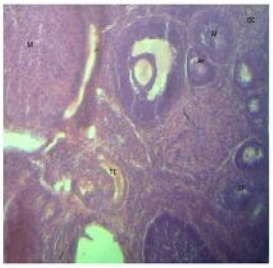

Figure 4: Light Micrograph of section of ovary from Rat treated with 2000mg/kg of the Extract showing Medullar (M), Atretic follicle (AF), Ovarian Cortex (OC) and Tissue Engorgement (TE) (H&E Stain x100).

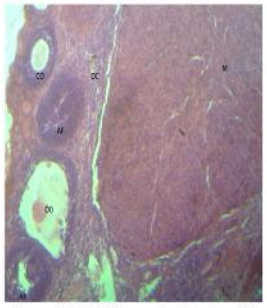

Figure 5: Light Micrograph of section of ovary from Rat treated with 500mg/kg of the Extract showing Medullar (M), Ovarian Oocyte (OO), Atretic follicle (AF) and Ovarian Cortex (OC) (H&E Stain x100).

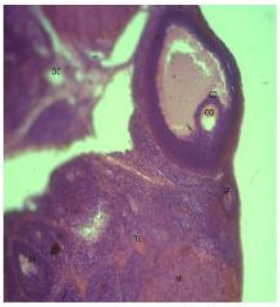

Figure 6: Light Micrograph of section of ovary from Rat treated with 5000mg/kg of the Extract showing Tissue Engorgement (TE), Medullar (M), Ovarian Oocyte (OO), Atretic follicle (AF), Cumulus Oophoricus (CO) and Ovarian Cortex (CC) (H&E Stain x100).

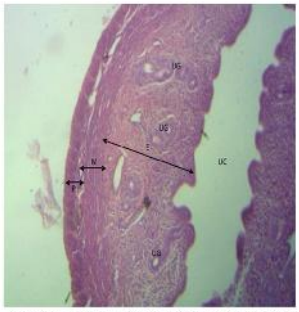

Figure 7: Light Micrograph of section of Uterus from Rat treated with normal saline showing Uterian cavity (UC), Endometrium (E), Myometrium (M), Perimetrium (P) and Uterine Gland (UG) (H&E Stain x100).

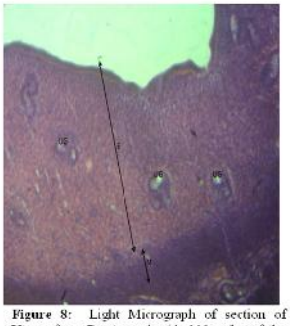

Figure 8: Light Micrograph of section of Uterus from Rat treated with 100mg/kg of the extract showing Endometrium (E), Myometrium (M) and Uterine Gland (UG) (H&E Stain x100).

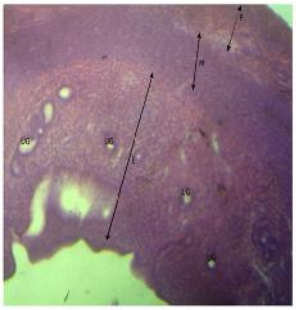

Figure 9: Light Micrograph of section of Uterus from Rat treated with 1000mg/kg of the extract showing Endometrium (E), Myometrium (M), Perimetrium (P) and Uterine Gland (UG) (H&E Stain x100).

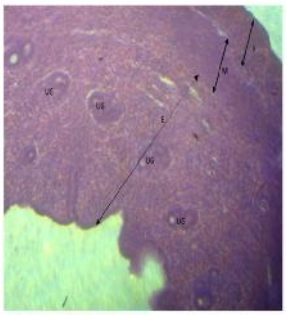

Figure e 10: Light Micrograph of section of Uterus from Rat treated with 2000mg/kg of the extract showing Endometrium (E), Myometrium (M), Perimetrium (P) and Uterine Gland (UG) (H&E Stain x100).

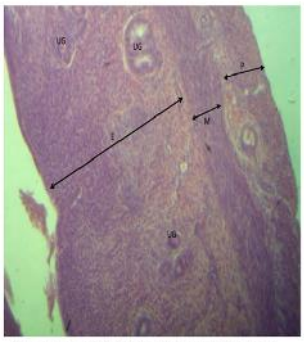

Figure 11: Light Micrograph of section of Uterus from Rat treated with 3500mg/kg of the extract showing Endometrium (E), Myometrium (M), Perimetrium (P) and Uterine Gland (UG) (H&E Stain x100).

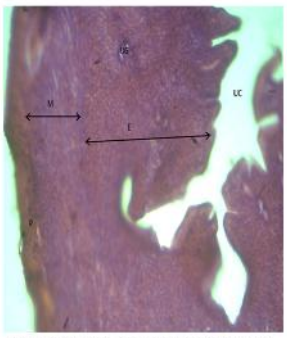

Figure 12: Light Micrograph of section of Uterus from Rat treated with 5000mg/kg of the extract showing Uterian cavity (UC), Endometrium (E), Myometrium (M), Perimetrium (P) and Uterine Gland (UG) (H&E Stain x100).

DISCUSION

Establishment of LD_{50}

The administration of the ethanolic root extract of *Moringa oleifera* orally to Wistar rats during the study at doses of 100mg/kg, 1000mg/kg, 2000mg/kg,3500mg/kg and 5000mg/kg body weight did not produce death in the treated rats. The LD_{50} was therefore concluded to be above the maximum dose used in this study. This may be an indication of the high level of safety of the extract (Dennis, 1984).

According to Bruce (2006), any substance with LD_{50} estimated to be greater than 2000-5000mg/kg body weight given orally could be considered of low toxicity and being safe. Also, the chemical labelling and classification of acute systemic toxicity based on oral LD_{50} values recommended by the Organization of Economic Co-operation and Development (OECD, Paris, France) (Walum, 1998) are as follow: very toxic, <5mg/kg; toxic, >5<50 mg/kg; harmful, >50<500 mg/kg; no label, >500<2000 mg/kg.

According to Dennis, (1984) which revealed that when extract are administered orally, absorption may not be complete due to inherent factors limiting absorption in the gastro-intestinal tract.

Histomorphological Changes

Gross Lesions

No gross lesion were observed in all the organs of the rats throughout the period of 30days, although the active principles in the extract may have been metabolized in the liver and excreted through the kidneys.

Histological Changes

Figure 1 to Figure 6 shows the histological of the ovaries of rat treated with normal saline, 100mg/kg, 1000mg/kg, 2000mg/kg, 3500mg/kg and 5000mg/kg of the extract.

In the control group, the histological sections appeared to be essentially normal showing the Medulla and ovarian cortex with follicles but at dose of 100mg/kg of the extract, the appeared to be tissue engorgement and Atretic Follicles and these features appeared throughout even at higher doses of 5000mg/kg of the extract.

Recently, it has been calculated that, in the pre-pubertal mouse ovary, 81 primordial follicle transition to primary follicle stage per day, while 155 primordial follicles are undergoing atresia daily (Tinger *et al*., 2009).

Ovarian follicular atresia is the periodic process in which immature ovarian follicles degenerate and are subsequently re-absorbed during the follicular phase of the menstrual cycle (Rolaki *et al*., 2005).

Atresia is a hormonally controlled apoptotic process (Kaipia and Hsueh, 1997) that depends dominantly on granulosa cell apoptosis.

Apoptosis is recognized as a hallmark and contributing factor of atresia of antral follicles (Inoue *et al*., 2011).

Initiating mechanisms of apoptosis include extrinsic factors such as the cytokines and intrinsic factors including oxidative stress, irradiation and the activation of tumor suppressor genes (Manabe *et al*., 2008).

Members of the tumor necrosis factor (TNF) superfamily are among the most widely-recognized cytokines triggering apoptotic events in follicles. They include TNF (Basini *et al*., 2002), Fas Ligand (Manabe *et al*., 2008) and TNF related apoptosis-inducing ligand (TRAIL) (Johnson *et al*., 2007).

Additional extrinsic factors that influence apoptosis of granulosa cells include interferon-gamma and several types of

growth factors (Quirk *et al*., 2000). Intrinsic factors of apoptosis are those that are generally provoked by aspect of stress such as nutrient deprivation, oxidative damage and genetically impairment are all cellular/molecular stress that can lead to the up regulation of intrinsic mechanism of apoptosis (Hussein, 2005).

The follicular atresia observed from this study could be due to nutritional deprivation due to tissue engorgement observed within the tissues or it could be due to TNF mediated pathway and oxidative damage secondary to exudates from the extract of Moringa Roots.

Figure 7 to Figure 12 shows sections of the uterus of rats treated with normal saline, 100mg/kg, 1000mg/kg, 2000mg/kg, 3500mg/kg and 5000mg/kg of the extract. In the control group treated with normal saline the histoarchitecture of the uterus appears essentially normal showing the Uterine Gland (UG), Endometrium (E), Myometrium (M) and Perimetrium (P) but as the dose of the extract increase, there is vacuolation of the Endometrial Gland and at higher doses, there appeared to be Tissue Engorgement (TE) and formation of polyps within the uterine cavity.

Endometrial polyps are common spontaneous reproductive tract lesion in aged nuliparous rats (Dinse *et al*., 2010; Dixon *et al*., 1999). Although the aetiology of uterine polyps is highly related to age due to the sensitivity of the uterine stroma to progesterone (and androgen) than estrogen (Knobil *et al*., 1994) but they are studies which revealed that certain chemicals are the causative agent of uterine polyps.

Results from one year neonatal mouse carcinogenicity study of quinacrine showed an increased incidence of endometrial hyperplasia and uterine stroma polyps at higher doses (Cancel *el al*., 2006).

All mammalian uteri contain Endometrial Gland (EG) that synthesized and secretes or transport a complex array of proteins and related substances termed histotroph. The idea that uterine secretion nourish the developing conceptus (Embryo and Associated placental membranes) was discussed by both Aristotle in the third century BC and by William Harvey in the 17th century. In 1882, Bonnett concluded that secretions of uterine glands were important for fetal well being.

In Rodents, Several factors, including leukemia inhibitory factor and calcitonin, are produced exclusively by uterine glands and are essential for the establishment of uterine receptivity and embryo implantation (Carson *et al*., 2000).

Increase vacuolation of the uterine gland could probably be due to hyperactivity of the gland which are usually observed in response to unopposed estrogen stimulation in the setting of polycystic ovarian syndrome or exogenous administration of hormones and these may represent early neoplastic processes (Wikipedia, 2015).

CONCLUSION

Moringa oleifera root extract has a wide safety margin with potential antifertility effect due to the formation of atretic follicles with increased vacuolation of the endometrial glands in a dose dependent manner.

Recommendation

The authors are greatful to Almighty God for the strength and wisdom to carry out this research.

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