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The Effect of Methanolic Extract of *Moringa oleifera* Lam Roots on the Histology of Ovary and Female Reproductive Tract of Guinea Pigs.

*CW PAUL, BC DIDIA

[1]Department of Human Anatomy, Faculty of Basic Medical Sciences, College Of Health Sciences, University of Port Harcourt-Nigeria
[2]Department of Anatomy, Faculty of Basic Medical Sciences, College of Health Sciences, University of Portharcourt-Nigeria

*Author for Correspondence

ABSTRACT

M. oleifera lam has various pharmacological actions yet the toxicity profiles has not received a robust attention. Hence the need for a study of this kind, which aims at determining the effect(s) of methanolic extract of *M. oleifera* lam root on the histo-architecture of ovary and reproductive tracts of albino winter rats considering specific goals as time and dose dependence and possible mechanism of action. 'LD50 Ip" of 223.61mg/kg was determined using modified Lorke's 1983 method. Twenty four (24) Sows were used for the study, they were acclimatized and randomly distributed into groups A-C and control group. Daily intra-peritoneal injection of methanolic extract of *M. oleifera* root was administered for three (3) weeks. Doses of 3.6mg/kg, 4.6mg/kg and 7.0mg/kg were given to groups A, B and C respectively. Intra-peritoneal injection of extract was administered once daily. Four Sows were sacrificed on the 8th, 15th and 22nd days. Tissues collected were prepared for staining with haematoxylene and eosin. . Normal histological features were seen in Sections of ovary and reproductive tract from groups A and control as well as ovary from group B. Distortion of histo-architecture was noted in group B ovary, and histological sections of ovary and reproductive tract from group C. Sections from reversal group showed normal histology of ovary comparable to the control group while the genital tracts retained the distortion in histo-architecture. Ovarian distortion resulted in reduced viability of the follicles, which in turn cannot produce hormones to effectively sustain fertilized ovum. The effect on ovarian follicles is probably the most important mechanism of action. The effects of dis-functional ovary on the reproductive tracts are; fusion of plicae in fallopian tubes, contraction of the myometrum, dilated endocervical glands with increased mucin secretion and keratinization and desquamation of vaginal epithelial cells. These histological findings have been correlated with the anti-implementation action of the extract in the light of its normal properties. Methanolic extracts of *M. oleifera* root causes distortion of the histoarchitecture of the ovary and female reproductive tract. The effect is more pronounced on tissues exposed to higher doses and for longer duration. The extract acted through several mechanisms to bring about the anti-fertility effects and these effects were found to be reversible.

Key words: *Moringa oleifera*, Methanolic,Histoarchitecture, guinea pigs.

Different parts of the plant (*M. oleifera*) have different pharmacological actions and toxicity profiles which have not yet been completely defined. *M. oleifera* has been used over the years for different ailments, out of which only few were investigated. *M. oleifera* is one of the leading names recently in plants and drug research. A large number of reports on the nutritional qualities of plant now exist in both the scientific and the popular literature. However, the outcome of well controlled and well documented clinical study are still clearly of great value. (Mazumder *et al* 1999).

M. oleifera an important medicinal plant, is one of the most widely cultivated species of the family Moringaceae. It has vast medicinal properties and every part is said to have beneficial properties (Garima *et al* 2011). *M. oleifera* has been described as having medical and health importance such as Abortifascient (Nath D *et al* 1992, Tarafder and Srivastava 1997), Aphrodisiaec (Fuglie 1999), Birth Control (Shukla *et al* 1988, 1989, Faizi *et al* 1988, Galnani *et al* 1988). Indian medica described its use in abortion. The root and bark are abortificient and dried powder of leaf extract produces abortificient activity in rats (Talalay and Talalay 2001, Shukla *et al* 1988, Tarafder 1983).

Nath *et al* (1992) and Shukla *et al* (1989) studied the Biochemical alterations in the female genital tract of ovariectomized rats treated with aqueous extract of *M. oleifera*. While Shuklas, R mathur, AO Prakash (1980) looked at Biochemical and physiologic alteration in the female reproductive organs of cyclic rats treated with aqueous extract of moringa oleifera lam.

Devendra *et al* (2008) undertook a study to elucidate the possible mechanism of

uterotonic effect of aqueous extract of *M. oleifera* flower(mofe) on Buffalo myometral strips and inferred that mofe possesses promising uterotonic potential and the effect seems to be mediated through excitatory muscarinic, histaminic Hi and x, adrenergic receptors, however, involvement of cyclic adenosine monophosphate (C-AMP) and Nitric oxide pathway in antagonizing its uterotoic activity cannot be ruled out. Aqueous extracts of *M. oleifera* roots, bark and flowers have been reported to possess estrogenic, anti-estrogenic, progestational and anti-progestational activities and anti fertility effect (Jadhav *et al* 2000*,* Shukla *et al* 1989) and induce abortion in rats (Nath *et al* 1992).

Ravi and Bhagwat (2007) carried out efficacy study of Him Rop VET LIQUID in the management of retained placenta and post parturcent septic in bovines. Livestock line, the aim of the trial was to study the efficacy of HimRop VET LIQUID, a polyherbal formulation (mainly contains moringa oleifera(Shigra), Adhatoda Vasica (vasaka),Gloriosa superba(Kalihari), ruta graveolens (Sudapa), Penganum harmala (Harmala), and cyperus rotundus (musta) of the Himalaya Drug company, Bangalore, in the management of retained placenta as ecbolic in dairy cows. The results of the study showed that Him Rop VET LIQUID is effective and safe as an ecbolic in post partum uterine health of bovines.

Health help, an Herbal monograph documented that Oral administration of Aqueous extracts of Moringa pterygosperma at dose of 200mg/kg to different groups of rats for 6, 12, 18 and 24 days parallel control group was provided. Each group received gum acacia suspension as vehicle. During the experimental period, vaginal smear of each rat was collected and examined. It was observed that the dose of 200mg/kg disturbed the normal estrous cycle in all the animals and the response was dependent on the duration (Shukla *et al* 1987).

Aqueous extracts of the roots and root bark of *M. oleifera* were effective in preventing implantation. The anti implantation activity of *M. oleifera* root was consistent regardless of its time and place of collection (Shukla *et al* 1988). With regard to the female reproductive system, *M. oleifera* root is shown to have unique estrogenic, antiestrogenic, progestational, and antiprogestational activities (Lampronti *et al* 1988). Root-bark yields two (2) alkaloids: moringine and moringinine. Moringinine acts as a cardiac stimulant, produces a rise in blood pressure, acts on sympathetic nerve endings as well as smooth muscles throughout the body, and depresses the sympathetic motor fibers of vessels in large doses only.

Some years ago, hormonal property of this miracle tree was shown to cause biochemical and physiologic alterations in the female reproductive organs of cyclic rats (Shukla *et al* 1988). Initially, its administration stimulated the uterine structures, caused metaplastic changes in the cervical epithelium, and provoked considerable cornification in the vaginal epithelium. After more days of treatment, significant inhibition in the general histo-architecture was observed. Biochemical observations and histologic findings have been correlated with the anti-implantation action of the aqueous extract in light of its hormonal properties. These and other experiments can easily explain its use as an abortifacient (Nath *et al* 1992). However, the active mechanism that produces such effects is still unknown, it appears to be acting on some receptor, most likely the follicle-stimulating hormone receptor (FSHR), because it initially increases estrogenic action when the uterus is enlarged. Then, it may inactivate this receptor locally or with the help of a central mechanism through nerve growth factor (NGF)-mediated pathways. Of interest, the central inhibitory effect of *M. oleifera* root extract and a possible role of neurotransmitters have also been proposed. Dopamine and norepinephrine levels were studied in Holtzman strain adult albino rats. The results revealed that pretreatment with *M. oleifera* inhibited penicillin-induced seizure and markedly reduced locomotor activity. Chronic treatment with *M. oleifera* significantly increased the 5-HT and decreased the dopamine level in the cerebral cortex, midbrain, caudate nucleus, and cerebellum. The norepinephrine level was significantly decreased in the cerebral cortex (Ray *et al* 2003 and 2004). As dopamine and norepinephrine influence Nerve

GrowthFactor(NGF) and Follicular Stimulating Hormone(FSH) release through central mechanisms (Bose *et al* 2005), this result may corroborate their possible role in epithelial ovarian cancer. Vascular endothelial growth factor (VEGF) might also be involved via Nerve Growth Factor (Selman *et al* 2007). Hormonal action may be mediated through the estrogen or progesterone receptor as well. *M. oleifera* inhibits maintenance and growth of reproductive organs. In fact, in rural and tribal areas of the West Bengal province in India, the root of this plant is taken by women, especially prostitutes, as permanent contraception, and it has been shown to totally inactivate or suppress the reproductive system. (Chinmoy K. Bose 2007).

The bark of the tree may cause violent uterine contractions that can be fatal (Bhattacharya *et al* 1978). Methanolic extract of *M. oleifera* root was found to contain 0.2% alkaloids. Effects of multiple weekly doses (35, 46, 70 mg/kg) and daily therapeutic (3.5, 4.6, and 7.0 mg/kg) intraperitoneal doses of the crude extract on liver and kidney function and hematologic parameters in mice have been studied. The results indicate that weekly moderate and high doses (> 46 mg/kg body weight) and daily/therapeutic high doses (7 mg/kg) of crude extract affect liver and kidney function and hematologic parameters, whereas a weekly dose (3.5 mg/kg) and low and moderate daily/therapeutic doses (3.5 and 4.6 mg/kg) did not produce adverse effects on liver and kidney function (Mazumder *et al.,*1999). LD50 and lowest published toxic dose (TDLo) of root bark extract *M. oleifera* Lam. are 500 mg/kg and 184 mg/kg, respectively, when used intraperitoneally in rodents (mice).

The aim of the study was to investigate effect(s) of methanolic extract of M. oleifera lam root on Histo-archtechture of Ovary and Female reproductive tract of guinea pigs. Objectives were; To determine if methanolic extracts of moringa oleifera lam root has effect(s) on the histology of Ovary and female reproductive tract of guinea pigs. To determine whether the effects are dose-dependent and/or time-dependent. And to ascertain the possible mechanism(s) by which *M. oleifera* lam root extracts achieves contraception/ abortifascience based on histologic findings on tissues.

MATERIALS AND METHOD

Ethical Clearance for research was obtained from the College of health sciences ethical committee and the protocols were strictly adhered to. Also the internationally accepted principles for laboratory animal use and care were adopted.

Fresh roots of *M. oleifera* lam was collected from Port Harcourt in January, the roots were identified in the Department of Plant Science and Biotechnology, University of Port Harcourt, after which, the roots were washed and cut into very small pieces, blended into fine texture, dried in the room, and sent to phytochemistry laboratory of the university of port Harcourt for cold suscinate extraction using methanol. The extract was weighed and stored in sub- zero temperature in the refrigerator. The extract was dissolved in injection water and constituted to the required doses for administration the animals in the pilot study and the different experimental groups except the control group.

"LD50 ip' of 223.61mg/kg was determined using Lorke's 1983 method and as used by Azikiwe et al 2007, Azikiwe et al 2009 and Bassey et al (2009) was adapted for the LD50 study Twenty four (24) Female guinea pigs (Sows),weighing between 200g and 500g,said to be between three(3) and fifteen(15) weeks of age were purchased and housed in plastic cages with steel nettings and solid bottom, in the animal house of the faculty of basic medical sciences, University of Port Harcourt. The acclimatization period was for two (2) weeks. The animals were weighed and randomly distributed into groups A-C and control group. They were also weighed on weekly basis. To identify the animal groups, fur dye was applied. Daily intra-peritoneal injection of methanolic extract of *M. oleifera* lam root was administered for three (3) weeks. Doses of 3.6mg/kg, 4.6mg/kg and 7.0mg/kg, was given to groups A,B and C respectively. Four Sow were sacrificed on the 8th day; one from each female group (A.B,C and control).Same number of sows from each group were sacrificed on the 15th and 22nd days. These animals were weighed anesthetized with

chloroform in desiccators and dissected, tissues collected were immediately fixed in formalin, dehydrated in graded alcohol (50% alcohol-absolute alcohol),cleared in xylene, embedded in paraffin wax, sectioned with the microtome, mounted on slides, stained with haematoxylene and eosin dye and photomicrography done. The photomicrographs were observed under the microscope magnifications of X400 and X200 for possible effects of methanolic extracts Moringa oleifera lam roots on tissues harvested from the guinea pigs.

RESULTS

Histological Findings of control and experimental groups

Control group (0mg/kg);

Sections of ovary (fig.1) and reproductive tract (figs.7 and 10) from control group showed normal histological features which served as reference points for comparison.

Group A (Sows treated with 3.5mg/kg of extract)

Histological sections of ovary and reproductive tract treated with 3.5mg/kg of extract (Fig.2) showed normal histo-architecture.

Group B (Sows treated with 4.6mg/kg of extract)

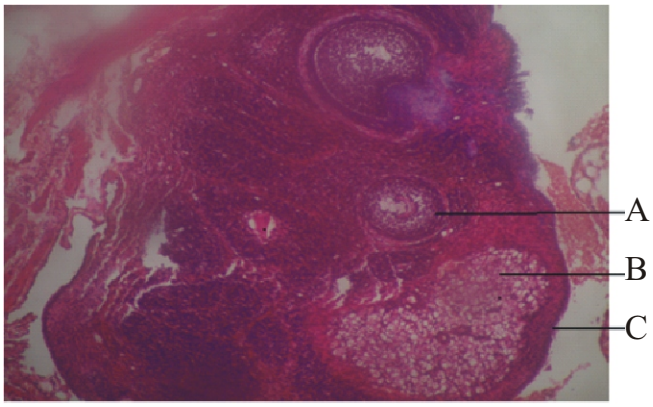
Histological section of ovary treated with 4.6 mg/kg of extract (fig.3) revealed normal histology, however histological section of the vagina (fig.13) showed distorted epithelial lining.

Group C (Sows treated with 7.0mg/kg of extract)

Histological section of ovary treated with 7.0mg/kg of extract (fig.5) showed distorted ovarian follicles and fusion of the plicae in the fallopian tubes (figs. 8 and 9). Endometrial hyperplasia with crowded dilated glands in the uterus (fig.11) and dilated endocervical glands with prominent mucin vacuoles in their lining epithelial cells (fig.12). In the vagina, (figs. 14 and15) it showed abnormal epithelial festures (keratosis,vesicular nuclei, eosinophilic cytoplasm infiltrated with adult inflammatory cells),desquamated epithelial cells and mucinous keratin layer.

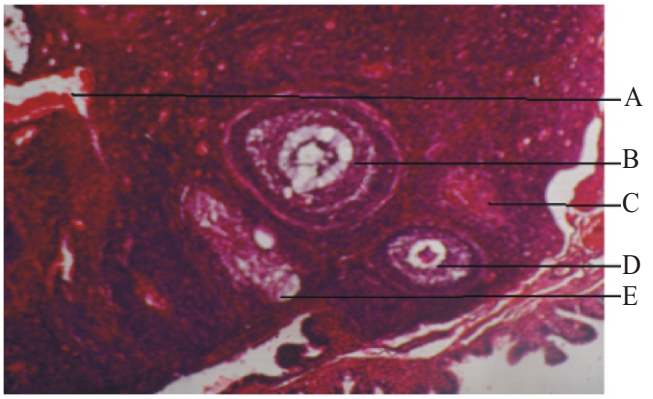
Reversal group

Histological sections of ovaries (fig.6) and reproductive tract after eight weeks of cessation of treatment showed normal histology of ovary comparable to the control group while the genital tracts retained the distortion in histo-architecture.



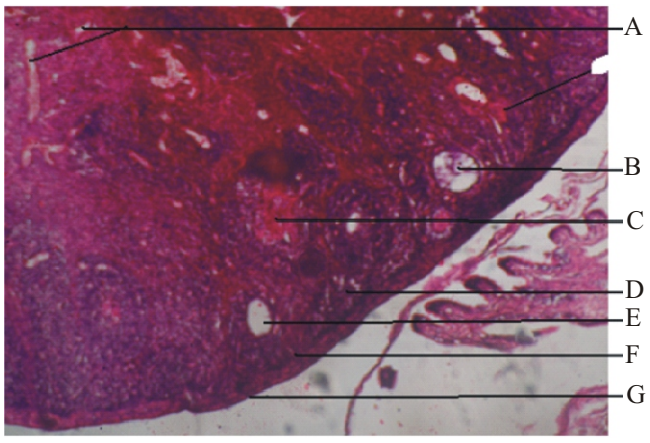
A. = Graffian Follicle B. = Corpus Luteum C. = Tunica Albuginea

Figure 1: A Photomicrograph of guinea pig ovary from control group, showing normal follicles. Magnification X400 H & E



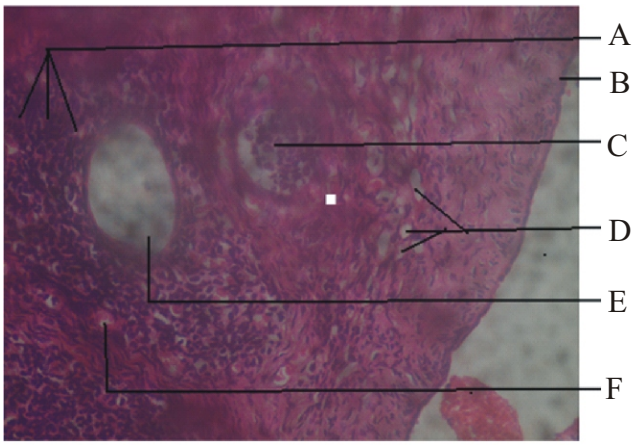
A = Blood Vessel, B = Secondary Follicle,
C = Corpus Luteum, D = Secretary Follicle,
E = Graffian Follicle

Figure 2: A Photomicrograph of guinea pigs Ovary treated with 3.5mg/kg of extract showing few pre-anthral follicles and primary follicles Magnification X200



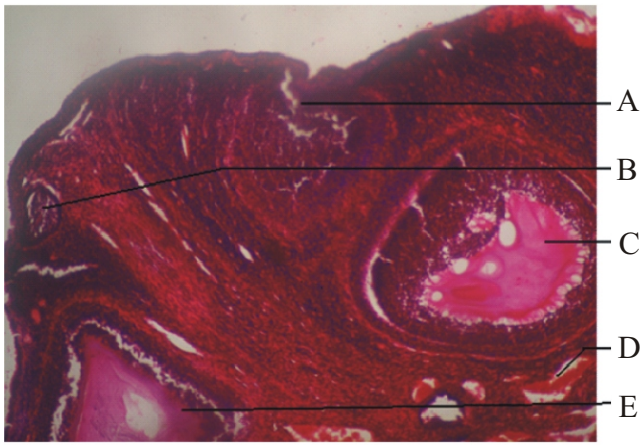
A. = Blood Vessels, B. = Secondary Follicle, C. = Corpus Luteum, D. = Primordial Follicle, E. = Primary Follicle, F. = Germinal Epithelium, G. =Tunica Albuginea.

Figure 3: A Photograph of guinea pig ovary treated with 4.6mg/kg of Extact, showing follicles and luteinized stroma. Mag. X400 H & E.



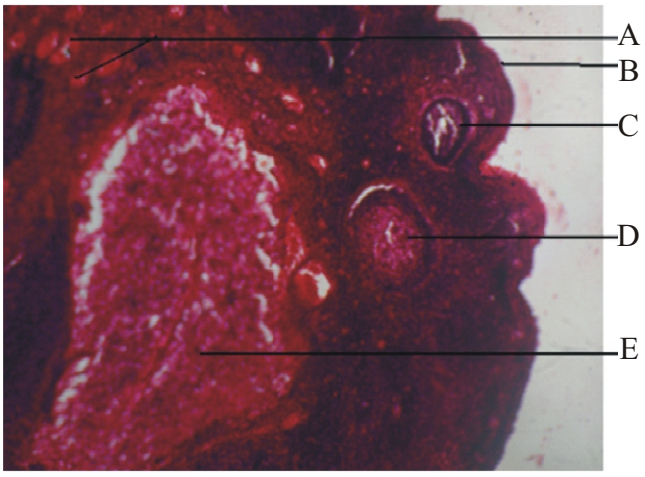
A. = Inflammatory Cells, B. = Tunica Albuginea C. = Primary Follicle, D. = Primordial Follicles E. = Primary Follicle, F. = Blood Vessel

Figure 4: A Photograph of guinea pig ovary treated with 4.6mg/kg of Extract, showing only Primordial and Primary Follicles. Magnification X400 H & E. .



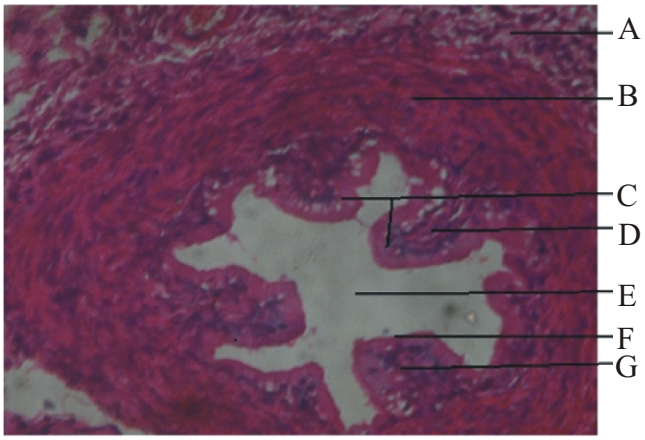
A. = Corpus Luteum, B. = Corpus Luteum C. = Distorted Graffian Follicle, D. = Blood Vessel E. = Distorted Secondary Follicle.

Figure 5: A Photomicrograph of guinea pig ovary Treated with 7.0mg/kg of extract, Mag. X200 H & E.



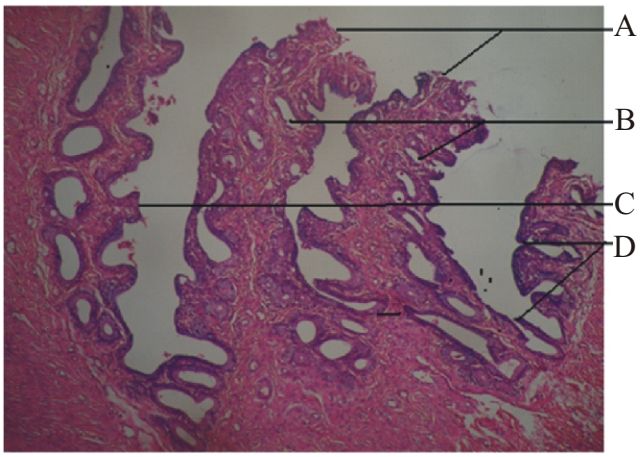
A. = Blood Vessels, B. = Tunica Albuginea C. = Primary, D. = Primary Follicle E. = Corpus Luteum.

Figure 6: A Photomicrograph of guinea pig ovary from the reversal group Magnification X200 H & E.



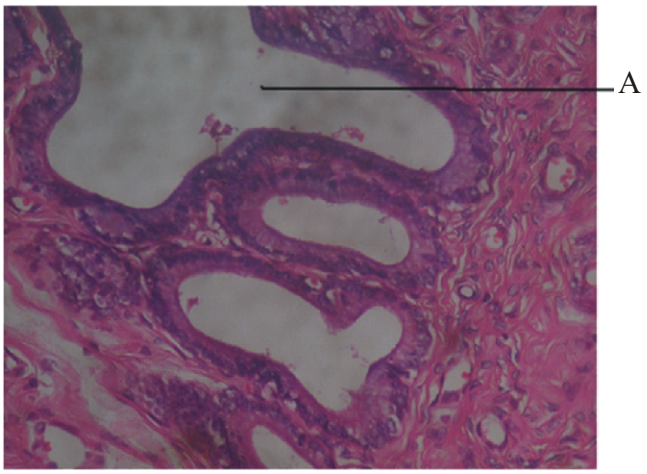
A. = Outer Longitudinal Muscle, B. = Inner Circular Muscle, C. = Plicae, D. = Supporting Tissue E. = Cavity of Oviduct, F. Epithelium of Oviduct G. = Supporting Tissue

Figure 7: A Photomicrograph of guinea pig's fallopian tubes. From the control group. Magnification X200 H & E.



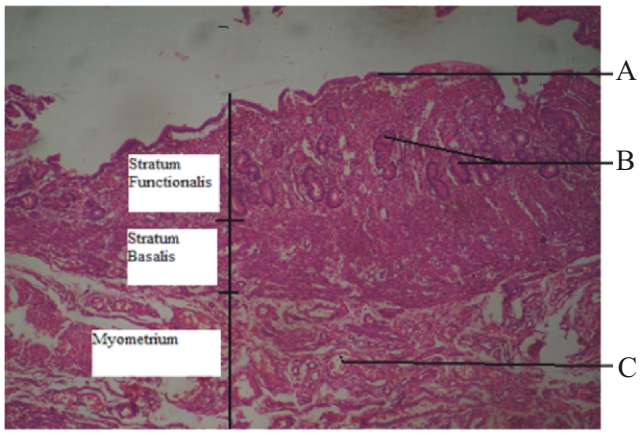
A. = Destroyed Epithelial Lining of the Plica B.= Fusion of Plicae, C. = Destroyed Epithelial Lining of the Plicae, E. Fusion of Plicae

Figure 8: A Photomicrograph of guinea pig fallopian tube treated with 7.0mg/kg of extract, showing fusion of plicae which appears glandular. Magnification X200 H & E

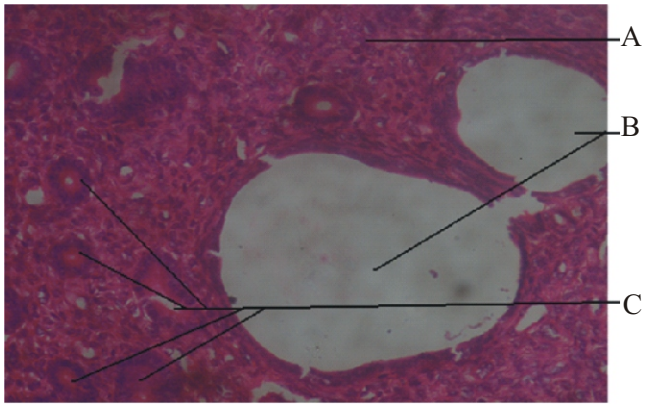


A. = Glandular structure resulting from fused plicae

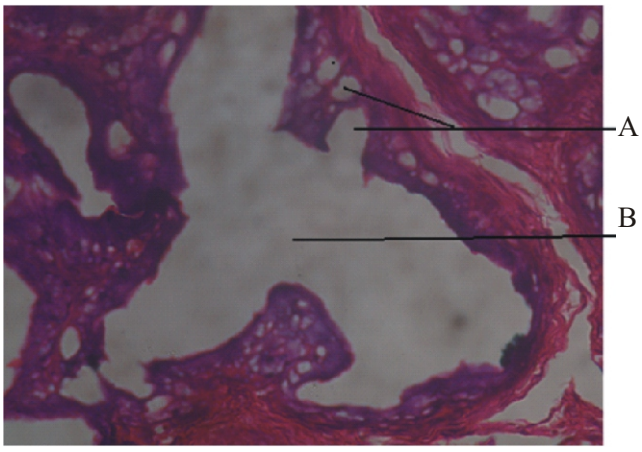
Figure 9: A Photomicrograph of guinea pig's fallopian tube treated with7.0mg/kg of extract, showing fusion of plicae which appears glandular. Magnification X400 H & E



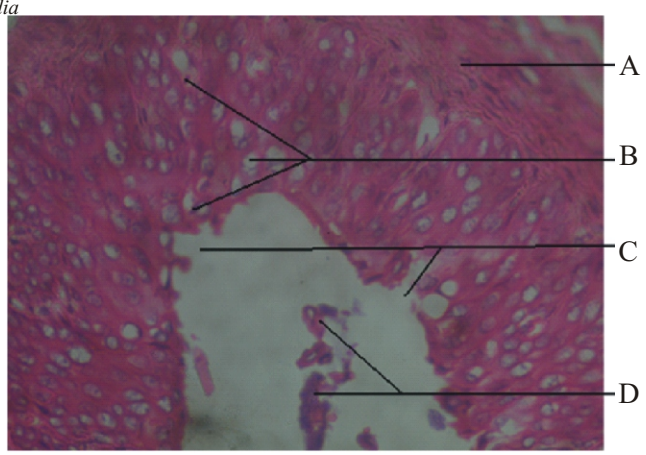
A. = Epithelium, B. = Uterine Glands
C. = Blood Vessel
Figure 10: A Photomicrograph of guinea pig uterus showing tubular glands containing eosonophilic materials and the stroma is compact.Magnification X400 H & E



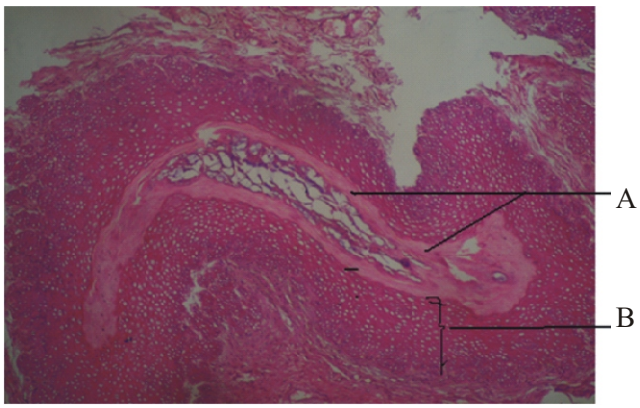
A. = Compact Stroma, B. = Dilated Glands
C. = Crowded Glands
Figure 11: A Photomicrograph of guinea pigs uterus treated with 7.0mg/kg showing endometrium with features of simple endometrial hyperplasia. Magnification X400 H & E



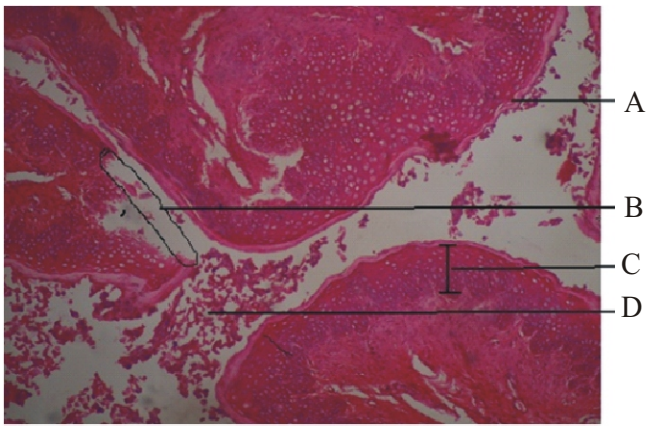
A. = Prominent Mucin-secreting Vacoules,
B. = Dilated Endocervical Gland,
Figure 12: A Photomicrograph of guinea pigs cervix exposed to 4.6mg/kg of Extract showing dilated endocervical glands with prominent mucin vacuoles in their lining epithelial cells. Magnification X400 H & E



A. = Normal Muscular Layer, B. = Vesicular Nuclei,
C. = Destroyed Epithelial Lining,
D. = Dykeratotic Cells
Figure 13: A Photomicrograph of guinea pig cervix/vagina showing disorder surface epithelial cells. Mag. X400. H & E



A. = Hyperkeratotic Epithelial Surfaces,
B. = Stratified Squamous Epithelium with Cesicular nuclei
Figure 13: A Photomicrograph of guinea pig cervix/vagina showing disorder surface epithelial cells. Mag. X400. H & E



A. = Hyperkeraosis
B. = Desquamated Surface
C. = Straified squamous Epthelium
D. = Desquamated Tissues
Figure 15: A Photomicrograph of guinea pig vagina with features of abnormal histo-architecture. Magnification X400. H&E.

DISCUSSIONS

Fig.1 was obtained from the control group and it revealed ovarian follicles at different stages of development. Fig.2 was prepared from a group of guinea pigs exposed to 3.5mg/kg of the plant extract. It showed pre-anthral follicles and primary follicles. Figs. 3 and 4 were prepared from guinea pigs group exposed to 4.6mg/kg of plant extract; features of fig.3 include few follicles and luteinized stroma, while features of Fig.4 include primordial follicles and primary follicles. Fig.5 was prepared from guinea pigs ovary exposed to 7.0mg/kg of plant extract, and it revealed distorted follicles. Fig.6 was obtained from the reversal group and it showed distorted primary follicles and corpus luteum.

This study has revealed that the histology of ovarian sections made from Sows exposed to the plant extracts did not differ from the control group much, except for those exposed to 7.0mg/kg of extract where the follicles are distorted. Most of the groups showed the different stages of development of the ovarian follicles, from the primary oocytes to the graffian follicles and corpus luteum. With most of the graafian follicles located at the cortical region of the ovaries. The interpretation is that the extract does not cause burning-out of the ovary (ovariectomy) .In as much as the number and locations of these ovarian follicles are not adversely affected, our study suggest that the injection of *M. oleifera* root extract has negative effect on the integrity and viability of the follicles. Non-functional follicles cannot produce hormones effectively, and when fertilized will usually abort. The effect on ovarian follicles is probably the most important mechanism of action, as most of these other tissues depend on the ovarian hormones for their growth and sustenance. We suggest further research on comparism of moringa root extracts and anti-estrogens (eg Tamoxifen)

Fallopian tubes: - The tubal mucosa is invaginated to form the major plicae, each with secondary and tertiary folds; the tubal epithelium is also highly plicated. In our study, these folds are found to be fused (Figs.8 and 9). This fusion will likely hinder transport of ova, spermatozoa, zygote, the pre-implantation morula and blastocyst. This however, may not be absolutely certain, because it is generally agreed that muscular movements are the most important factor in the transport of ova and spermatozoa. The muscles; inner circular and outer longitudinal are seen to be essentially normal in all the slides.

The cause of fusion of plicae could be the effect(s) of *M. oleifera* extracts on nerves, hormones (estrogens and progesterone) or neurotransmitters. So, further investigations can be done around these areas. The fact that there is possibility of hindrances to movements of ova and spermatozoa in the fallopian tube creates a tendency of abnormally implanted blastocyt resulting in ectopic pregnancy.

Uterus and Cervix: - In most of the photomicrographs of guinea pigs (sows) uterus exposed to methanolic extract of *M. oleifera* root, the endometrial stroma was found to retain normal histo-architecture, a finding that agrees with Shukla *et al*(1989). However, at the dose of 7.0mg/kg, (fig.11) features of endometrial hyperplasia (crowded glands, dilated glands with breakages on the walls of the glands) were observed. This is likely to create a form of porosity and instability in the endometrium, affecting the spiral and straight arteries of the endometrium, which in-turn affects nutrition of the endometrium and conceptus. This agrees with (Shukla *et al* 1988) who worked on aqueous extracts of the roots and root bark of *M. oleifera* and found that they were effective in preventing implantation. They also stated that the anti implantation activity of *M. oleifera* root was consistent regardless of its time and place of collection.

Cervix:- with higher doses (4.6mg/kg as in fig.12) and prolonged exposure to the plant extract the endo-cervix became dilated and the number of mucin-secreting glands were increased. This is a possible site for the anti-fertility activity of the plant extract. A school of thought may consider the dilatation to be advantageous for the semen to have easy access to the uterine cavity; however, high content of mucin secreted by the glands may end up blocking the endocervix, hence preventing passage of semen through the cervix. The changes in the number of mucin secreting cells can be supported by the fact that

Shukla S *et al* (1988) concluded that initial administration stimulated metaplastic changes in cervical epithelium.

Vagina:- The Epithelium is stratified squamus with vesicular nuclei and eosinophilic cytoplasm. There is keratinization and desquamation of epithelial cells (figs.14 and 15). The keratinization, desquation, vesicular nuclei and oesinophilic cytoplasm are the effects of plant extract in this region; they may be due to estrogen withdrawal secondary to premature menopause. Oestrogen is needed to maintain the non-keratinized state of the epithelial lining and the elasticity of the vaginal walls. The present state of the vagina will not favour mating in the animals, because the vaginal walls are liable to trauma during intercourse which will in turn discourage intercourse and possibly prevent pregnancy. This agrees with Shukla S et al (1988) who stated that initial exposure to the plant extract provoked considerable cornification in the vaginal epithelium. After more days of treatment, significant inhibition in the general histoarchitecture was observed. This agrees with the submissions of some researchers (Jadhav *et al* 2000*,* Shukla *et al* 1989) who alluded that *M. oleifera* Lam root has shown unique anti-estrogenic, estrogenic, anti-progesteronal, and progesteronal activities which in turn causes biochemical and physiologic alterations in the female reproductive organs of cyclic rats. That the initial effect was stimulation of uterine structures, metaplastic changes in cervical epithelium and provoked considerable conification in the vaginal epithelium. After more days, it caused significant inhibition of the general histo-architecture of organs. These histological findings have been correlated with the anti-implementation action of the extract in the light of its normal properties.

The entire picture of effects of the extract on the female genital tract (Fallopian tubes, uterus, cervix, and vagina) can be accounted for by its effects on the ovary. As the ovary is destroyed by the extract, it results in inability to grow or sustain reproductive organs. This is in agreement with the works of Chinmoy K. Bose (2007) who stated that moringa olerifera inhibits maintenance and growth of reproductive organs. In fact in rural and tribal areas of the west Bengal Province in India, the root of this plant is taken by women, especially prostitutes, as permanent contraceptive and it has been shown to totally inactivate or suppress the reproductive system and generally its use in family planning.

CONCLUSION.

Methanolic extracts of *M. oleifera* lam root causes distortion of the histo-architecture of the ovary and female reproductive tract. The effect is more pronounced on tissues exposed to higher doses and for longer duration. The extract acted through several mechanisms to bring about the anti-fertility effects and these effects were found to be reversible. The fact that methanolic extract of *M. olerifera* lam extract has been demonstrated to affect the histo-architecture of both ovary and female reproductive tracts, it would have been necessary to recommend that further research be done on how to apply this on human to stem the tide of over-population/rising population of the world. But it has also been shown that it has toxic effects on other organs, so it cannot make a good drug for contraception/ family planning, etc since it is harmful to other organs.

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