

Methanolic Extract of *Moringa oleifera* Lam Roots is Not Testis-Friendly to Guinea Pigs

C.W. Paul and B.C. Didia

Department of Human Anatomy, Faculty of Basic Medical Sciences,
College of Health Sciences, University of Portharcourt, Nigeria

Abstract: The moringa tree is native to the southern foothills of the Himalayas, and possibly Africa and the middle East. The root contains an active anti-biotic principle, pterygo-spermin. The root bark contains two alkaloids viz. moringine and moringinine. The aim of the study was to investigate effect (s) of methanolic extract of *Moringa oleifera* lam root on Histo-architecture of testis of guinea pigs. The objectives were; To determine if methanolic extracts of *moringa oleifera* lam root has effect (s) on the histology of testis, of guinea pigs. To determine whether the effects are dose-dependent and/or time-dependent. To ascertain the possible mechanism (s) by which *M. oleifera* lam root extracts achieves its effects based on histologic findings on tissues. 'LD50 ip' of 223.61 mg/kg was determined using modified Lorke's (1983) method. Twenty four (24) Boars were enrolled for the study. Acclimatized and randomly distributed into groups A-C and control. They were given daily intra-peritoneal injection of methanolic extract of *Moringa oleifera* lam root for three (3) weeks. Doses of 3.6, 4.6 and 7.0 mg/kg were given to groups A, B and C, respectively. Four pigs; one from each group were sacrificed on 8th, 22nd and 43rd days, respectively. Tissues collected were immediately prepared histologically for haematoxyline and eosin stain. The photomicrographs were observed under the microscope with magnifications of X400 and X200. Sections of testis from control group showed normal histological features. Histological sections of testis from this group A showed seminiferous tubules with few spermatids and spermatozoa. Histological section of testis from group B revealed numerous seminiferous tubules with most having primary and secondary spermatogonia, spermatids and few mature spermatozoa. Group C showed seminiferous tubules with few spermatids and spermatozoa. Histological sections of testis in reversal group showed normal histology comparable to the control group. High doses and prolonged exposure to the Methanolic extract of *Moringa oleifera* lam root resulted in a spectrum of effects in the seminiferous tubules; ranging from formation of few spermatids and spermatozoa to no formation of spermatozoa the height of sertoli cells was shortened. The interstitial cells of leydig were normal in size suggesting that hypothalamus and pituitary glands are not spared. Therefore, the mechanism of action of this extract on the Boars may be due to global effects of the extract on the male reproductive system. Methanolic extract of *Moringa oleifera* lam root caused distortion of the histoarchitecture of the testis of guinea pigs. The effects of the extract increased with time and increased dose of extract administered.

Key words: Histarchitecture, methanolic, *Moringa oleifera* lam

INTRODUCTION

The moringa tree grows mainly in semi-arid, tropical and sub tropical areas; it is native to the southern foothills of the Himalayas, and possibly Africa and the Middle East (Duke, 1982). Currently it is widely grown in Africa, central and South America, Sri-lanka, India, Mexico, Malaysia and the Philippines.

The tree itself is rather slender with drooping branches that grows to approximately 10 m in height. The bark is thick, soft, corky and deeply fissured. The leaves, usually tripinnate: are elliptic and the flowers, while

fragrant have large panicles; the pods are pendulous, greenish, triangular and ribbed with trigonor's winged seeds. The fruit is long, slender and more or less straight, up to 30 cm long by 2.5 cm across, triangular in cross section and has attracted the name drumstick in India.

Principal constituents: The moringa olerifera lam root contains an active anti-biotic principle called pterygo-spermin. The root bark contains two alkaloids (total alkaloid 0.1%) viz. moringine which is identical with benzy 1 amine and moringinine belonging to the sympathomimetic group of bases. It also contains traces

of an essential oil with a pungent smell-Phytosterol, waxes and resins. An alkaloid named spirochin, has been isolated from roots of *Moringa oleifera* (Chopra and Chopra, 1957). The roots also contain phyto-chemicals like; 4-(α -L-rhamnopyranosyloxy)-benzylglucosinolate and benyl-glucinolate. (Kamel, 2009-11)

Pterygospermin (in concs of 0.5-3 kg/cc) inhibits the growth of many gram positive and gram negative bacteria, in higher concs (7-10 kg/cc) is active against fungi. It is stable in the presence of blood and gastric juice but breaks down in the presence of pancreatic juice. Its effect is counteracted by thiamine and glutamic acid but reinforced by pyridoxine (http://www.himalayahealthcare.com/herbfinder/h_moringa.htm).

The cross section of testis shows seminiferous tubules which are highly convoluted and lined by:

- Germ cells in various stages stages of spermatogenesis and spermiogenesis, which are referred to as the spermatogenetic series.
- Non-germ cells called sertoli cells, which support and nourish the spermatozoa. In the interstitial spaces between the tubules are the Leydig cells which are the supporting tissue, (Young *et al.*, 2006).

Phyto-chemicals may have no effect, positive effect (s) or negative effect (s) on the testis. The knowledge of these effects is applied in the use of these substances in humans. Anatomically, Studies are carried out to look at changes that are seen with naked eyes (gross), those that can be seen with the aid of microscope (histological), those that can be investigated by hormonal assay (hormonal changes), and effects on reproduction (reproductive ability) which may be tested with crossing the exposed animal/subjects with unexposed group. Parameters that can be considered in studying effects on testes are body weight, weight of testes, diameters of seminiferous tubule, stage of maturity of spermatozoa, and level of hormones produced by the testes. Inferences are drawn from observed changes on these parameters.

Different parts of the plant (*Moringa oleifera* lam) have different pharmacological actions and toxicity profiles which have not yet been completely defined. *Moringa oleifera* has been used over the years for different ailments, out of which only few were investigated. *Moringa oleifera* is one of the leading names recently in plants and drug research. A large number of reports on the nutritional qualities of Moringa 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).

Moringa oleifera tree has been reportedly shown to have various biological activities such as Hypocholesterolemic, antitumor and antihypertensive agents (Richa *et al.*, 2005), as well as antibiotic and anticancer properties. (Mazumder *et al.*, 1999; Ramachandran *et al.*, 1980) The methanol fraction of *Moringa oleifera* leaf extract (at 100 and 150 mg/kg) was found to possess significant protective actions in acetylsalicylic acid, serotonin and indomethacin-induced gastric lesions in wistar rats. A significant enhancement of the healing process in acetic acid-induced chronic gastric lesions was also observed with the extract-treated animals (Pal *et al.*, 1995).

Moringa oleifera Lam is a rapidly growing tree that is native to India (Tsaknis *et al.*, 1999), endemic in Asia (Duke, 1982) and most widely cultivated species of a mono generic family, the moringaceae (Fahey, 2000). The role of the plant in improving male reproduction has been a focus of interest. This accounts to the fact that male infertility is one of the problems faced by 10% of couples in the society and within a population; incidence rate of male infertility is approximately 1/136 individuals or 0.74% (based on NWHIC).

Moringa 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). *Moringa oleifera* Lam has been described as having medical and health importance such as Abortifascient (Nath *et al.*, 1992; Tarafder 1983), Aphrodisiac (Fuglie, 1999), Birth Control (Shukla *et al.*, 1988a, b, c, 1989; Faizi *et al.*, 1988).

Lilibeth and Glorina (2010) studied the effects *Moringa oleifera* Lam (Moringaceae) on the reproduction of male mice. (mus musculus), using hexane extracts of the leaves. Data on body weights, weights of reproductive organs, diameter of seminiferous tubule, stage of maturity, and levels of FSH & LH was analyzed. The significant findings were; increased thickness of epidermal wall (at medium and high doses); higher score for lumen formation (at high dose) and epidermal inactivity (at all doses). No significant effects on the levels of both hormones.

The bark of the tree may cause violent uterine contractions that can be fatal (Bhattacharya *et al.*, 1978). Methanolic extract of *Moringa oleifera* root was found to contain 0.2% alkaloids. Effects of multiple weekly doses (35, 46, 70 mg/kg, respectively) and daily therapeutic (3.5, 4.6 and 7.0 mg/kg, respectively) 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 *Moringa oleifera* Lam. are 500 and 184 mg/kg, respectively, when used intraperitoneally in rodents (mice). Changes in clotting factor, changes in serum composition (e.g., total protein, bilirubin, cholesterol), along with enzyme inhibition, induction, or change in blood or tissue levels of other transferases have been noted (Woodard *et al.*, 2007).

However, the interior flesh of the plant can also be dangerous if consumed too frequently or in large amounts. Even though the toxic root bark is removed, the flesh has been found to contain the alkaloid spirochin, which can cause nerve paralysis (Morton, 1991).

In Siddha medicine, the drumstick seeds are used as sexual virility drug for treating erectile dysfunction in men and also in women for prolonging sexual activity (Wikipedia, 2008).

The aim of the study was to investigate effect (s) of methanolic extract of *Moringa oleifera* lam root on Histology of testis of guinea pigs. The objectives were;

To determine if methanolic extracts of *Moringa oleifera* lam root has effect (s) on the histology of testis, of guinea pigs. To determine whether the effects are dose-dependent and/or time-dependent. To ascertain the possible mechanism (s) by which *M. oleifera* lam root extracts achieves its effects based on histologic findings on tissues.

METHODOLOGY

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 *Moringa oleifera* lam was collected from Port Harcourt in January, the roots were identified by Professor Obute Of 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 suscinat extraction using methanol. The extract was weighed and stored in sub-zero temperature in the refrigerator.

'LD50 ip' of 223.61 mg/kg was determined using Lorke's (1983) method and as used by (Azikwe *et al.*, 2007; Azikwe *et al.*, 2009; Basse *et al.*, 2009) was adapted for the LD50 study. Twenty four (24) Male

guinea pigs (Boars), weighing between 200 and 500 g, 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 *Moringa oleifera* lam root was administered for three (3) weeks. Doses of 3.5, 4.6 and 7.0 mg/kg, respectively was given to groups A, B and C, respectively. Four (4) Boars; one from each group (groups A, B, C and control) was sacrificed on 8th, 22nd and 43rd days, respectively. 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 haematoxyline 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 collected from the guinea pigs.

RESULTS

Histological findings of control and experimental groups:

Control group (boars treated with 0 mg/kg of extract): Sections of testis from control group (Fig. 1 and 2) showed normal histological features which served as reference points for comparing and interpreting the experimental groups.

Group A (boars treated with 3.5 mg/kg of extract): Histological sections of testis treated with 3.5 mg/kg of extract (Fig. 3 and 4) showed seminiferous tubules with few spermatids and spermatozoa.

Group B (boars treated with 4.6 mg/kg of extract): Histological section of testis treated with 4.6 mg/kg of extract (Fig. 5 and 6) revealed numerous seminiferous tubules with most having primary and secondary spermatogonia, spermatids and few mature spermatozoa

Group C (boars treated with 7.0 mg/kg of extract): Histological sections Testis treated with 7.0 mg/kg of extract (Fig. 7) showed seminiferous tubules with few spermatids and spermatozoa.

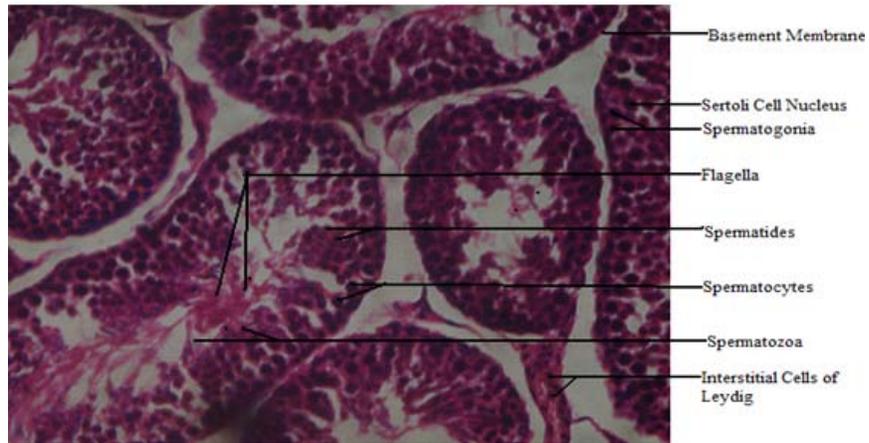


Fig. 1: A photomicrograph of guinea pig testis from the control group showing normal architecture. Magnification X100 H & E

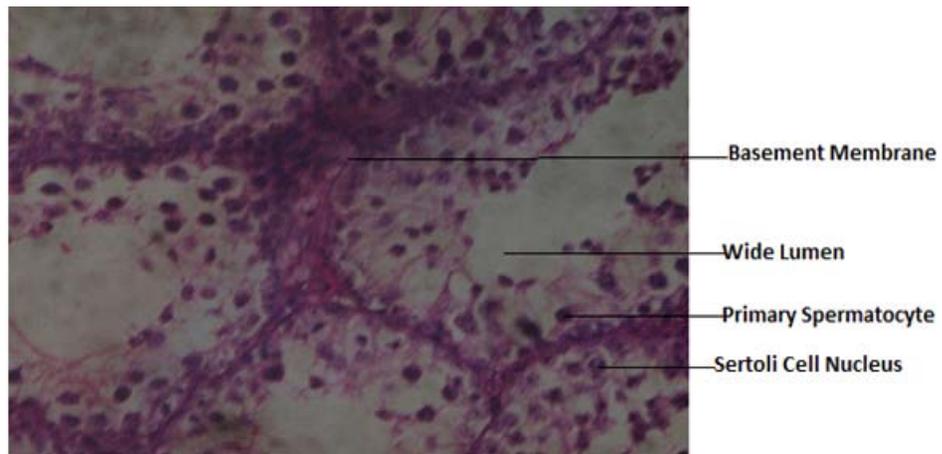


Fig. 2: A photomicrograph of guinea pig testis treated with 3.5 mg/kg showing seminiferous tubules without spermatozoa. Magnification; X400. H & E

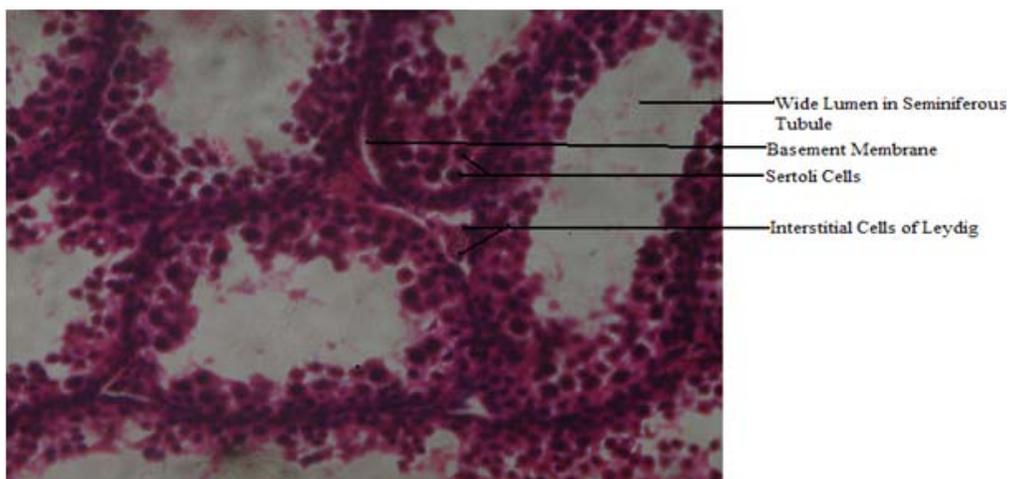


Fig. 3: A photomicrograph of guinea pig testis treated with 3.5 mg/kg showing numerous seminiferous tubules without spermatogonia. Magnification; X400. H & E

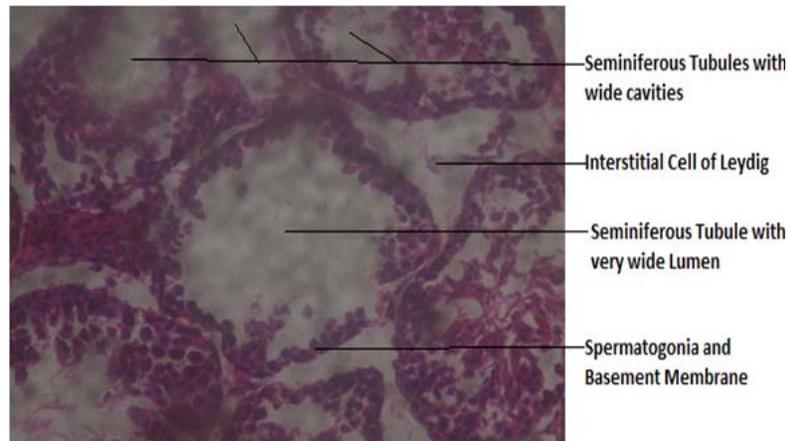


Fig. 4: A photomicrograph of guinea pig testis treated with 4.6 mg/kg of extract showing some seminiferous tubules with few spermatides and spermatozoa. Magnification X400 H & E

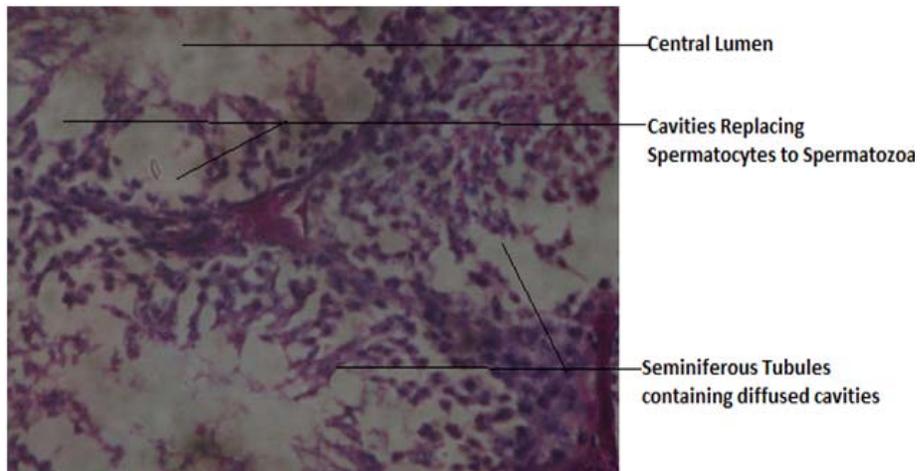


Fig. 5: A photomicrograph of guinea pig testis treated with 7.0 mg/kg of plant extract showing seminiferous tubules with patchy areas replaced with cavities. Magnification X400 H & E

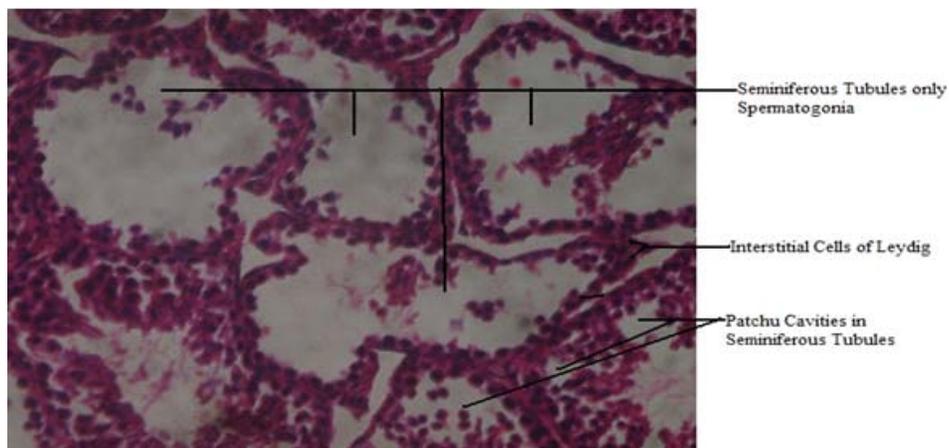


Fig. 6: A photomicrograph of guinea pig testis treated with 7.0 mg/kg of plant extract showing some seminiferous tubules with only few spermatogonia and spermatocytes. Magnification X400 H & E

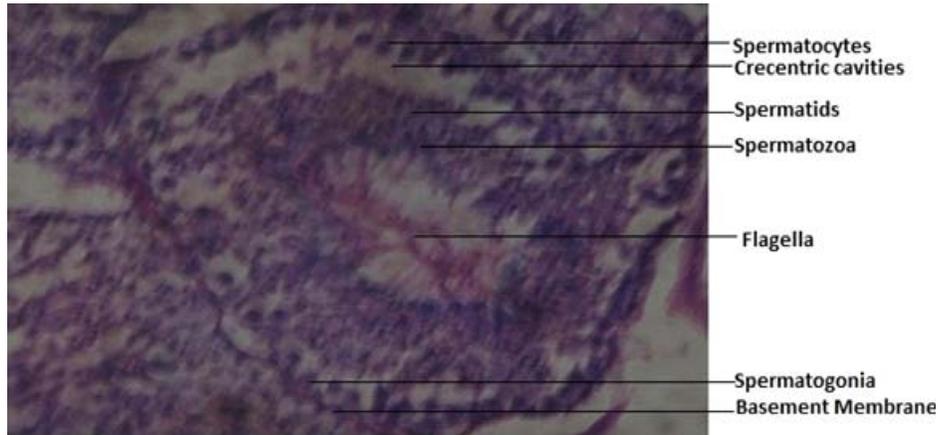


Fig. 7: A photomicrograph of the guinea pig testis from the reversal group. Magnification X400 H & E

Reversal group: Histological sections of testis after eight weeks of cessation of treatment (Fig. 8) showed almost normal histo-architecture which is comparable to the control group.

DISCUSSION

Both negative and neutral effects were noticed on exposure to Methanolic extract of *Moringa oleifera* lam root. Short duration and/or low doses were found to have neutral effects on histo-architecture of the testis. Among the control group it was noticed that both seminiferous tubules and interstitial cells are normal throughout the duration of the study. Considering the negative effects; high doses and/or prolonged exposure to the plant extract (Methanolic extract of *Moringa oleifera* lam root) resulted in a spectrum of effects in the seminiferous tubules; ranging from destruction of spermatozoa at the luminal border alone to destruction of all the cells in the seminiferous spering only the spermatogonia at the basement membrane. Figure 2 and 3 represent photomicrographs of testes exposed to 3.5 mg/kg of methanolic extract. At the early stage (Fig. 2) only the luminal layer of spermatozoa was destroyed, but with longer duration of exposure (Fig. 3) the layer of spermatids was destroyed in addition to the layer of spermatozoa. Figure 4 represents photomicrograph of a testis from a group exposed to 4.6 mg/kg of extract; concentric layers of cells of the seminiferous tubules, from spermatozoa through spermatids to spermatocytes have been destroyed. At the earlier stage only few cells at the luminal border were destroyed similar to Fig. 2. The implication is that, low doses administered at the beginning of study only destroys cells at the luminal surface, but with increased duration, the cellular

destruction continued centripetally towards the tunica albuginea, which means at low doses, there is an inverse relationship between the ages of cells destroyed and the duration of exposure (i.e., mature cells are eliminated first, and younger cells later). It can also be interpreted that, there is lack of maturation of spermatozoa which directly proportional to the dose of plant extract administered. Figure 5 and 6 are photomicrographs of testes from Guinea pigs treated with 7.0 mg/kg of the plant extract; at the early stages (Fig. 5) all the layers of cells in the seminiferous tubules from spermatogonia to spermatozoa were seen, interspersed with patchy cavities extending from basement membrane to the luminal border. However, with increased duration, (Fig. 6) the cavities expanded laterally to occupy the entire seminiferous tubules and only spermatogonia seen because spermatogonia regenerate themselves. Which means that at higher doses cell destruction is in lateral direction and not related to the age of the cells. The basement membrane had its fair share of the toxic effect of the plant extract, (Figure 4 and 5) as evidenced by increasing irregularity of the basement membrane with increasing dose and duration of exposure to the plant extract. The sertoli cells are not spared by the effects of the plant extract. The interstitial cells of leydig remained normal (no hypertrophy) in all the photomicrographs despite the doses of plant extract administered. Figure 7 represents photomicrograph of guinea pig's testis collected from the reversal group; it is similar to Fig. 1, but for the presence of crescentic cavity between the layers of spermatogonia and primary spermatocyte, suggesting that the effect of the extract on the testis is not permanent. This study therefore considers the mechanism of action of this extract on the male pigs (Boars) to be due

to global effects of the extract on the male reproductive system affecting spermatogenesis, production and maintenance of sertoli cells for nourishing the spermatozoa and sparing the interstitial cells of leydig which is supposed to generate testosterone needed in spermatogenesis in the testis. The decrease in testicular function is in keeping with Bose (2007) who opined that root extracts of *Moringa oleifera* inhibits maintenance and growth and of reproductive organs. The findings supports the possibility of low sperm count, other components of the seminal fluid analysis include; sperm motility, (there are motile and none motile spermatozoa) sperm cell-morphology (which could be normal or abnormal).The aforementioned in addition to hormonal assay are suggested to be looked into to determine the global picture of the effect (s) of Methanolic extracts of *Moringa oleifera* Lam root in the fertility of male guinea pig which in turn can be extrapolated to man.

CONCLUSION

From the present study we infer that Methanolic extract of *Moringa oleifera* lam root causes distortion of the histo-architecture of the testis of guinea pigs, which is time and dose-dependent. We also concluded that the mechanism of action of this extract on the Boars is due to global effects of the extract on the male reproductive system.

REFERENCES

- Azikwe, C.C.A., L.U. Amuzu, P.C. Unekwe, M.J. Nwankwo, S.O. Chilaka and O.J. Afonne, (2007). Anticoagulation and antithrombotic effect of *trichlisia dictyophylla*, moonseed. Niger J. Nat. Prod. Med., 11: 26-29.
- Azikwe, C.C.A., L.U. Amuzu, P.C. Unekwe, P.J.C. Nwosu, M.C. Ezeani, M.I. Siminialayi, S.O. Obidiya and J.E. Arute, (2009). Antidiabetic fallacy of *vernonia amygdalina* (bitter leaves) in human diabetes. Asian Pa. J. Trop. Med., 2(5): 54-57.
- Bhattacharya, J., G. Guha and B. Bhattacharya, (1978). Powder microscopy of bark-poison used for abortion: *Moringa pterygosperma* gaertn. J. Ind. Forensic Sci., 17: 47-50.
- Bassey, A.S., J.E. Okokon, E.I. Etim, F.U. Umoh and E. Bassey, 2009. Evaluation of the *in vivo* antimalarial activity of ethanolic leaf and stem bark extracts of *Anthocleista djalonensis*. 41(6): 258-261.
- Bose, C.K., 2007. Possible role of *Moringa oleifera* lam. Root in epithelial ovarian cancer. Medscape, 9(1): 26.
- Chopra, R. N. and I.C. Chopra, 1957. Ind. J. Med. Res. Med., 5: 123.
- Duke, J.A., 1982. Handbook of energy crops: *Moringa oleifera*. From the produce center for new crops Website.
- Fahey, W.J., 2005. *Moringa oleifera* : A review of the Medical evidence for its nutritional,therpeutic and prophylactic properties, Part 1. Tree life J., 1: 5-5.
- Faizi, S. B.S. Siddiqui, R. Saleem, K. Aftab and F. Shaheen, 1988. Bioactive compounds from the leaves and pods of *Moringa oleifera*. N. Trends in Natural Products Chemistry, pp: 175-183.
- Fuglie, L.J., (1999). The Miracle Tree: *Moringa oleifera*: Natural Nutrition for the Tropic. Revised in 2001 and published as Miracle Tree: The Multiple Attributes of Moringa, Church world service, Dakar, pp: 68. Retrieved from: http://echotech.org/bookstore/advanced_search_result.php?
- Garima, M., P. Singh, R. Verma, S. Kumar, S. Srivastav, K.K. Jha and R.L. Khosa, 2011. Traditional uses, phytochemistry and pharmacological properties of *Moringa oleifera* plant; An overview. Der. Pharmacia. Lett., 3(2): 141-164.
- Lilibeth, A.C. and L.P. Glorina, 2010. Effects *Moringa oleifera* lam (moringaceac) on the reproduction of male mice (*mus musculus*). J. Med. Plant Res., 4(12): 115-1121.
- Lorke, D.A., 1983. A new approach to practical acute toxicity testing. Arch. Toxicol., 54: 275-287.
- Mazumder, U.K., M. Gupta, S. Chakrabarti and D. Pal, 1999. Evaluation of hematological and hepatorenal functions of methanolic extract of *Moringa oleifera* lam. Root Treated Mice. Ind. J. Exp. Biol., 37: 612-614.
- Kamel, M., 2009-11. Nutrition Information. In: John Hopkin, I.J., Med. plant. Res. I Ratty Donovan.
- Morton, J.F., 1991. The horseradish tree, *moringa pterygosperma* (Moringaceae)-A boon to arid lands? Econ. Botany, 45: 318-333.
- Nath, D., N. Sethi, R.K. Singh and A.K. Jain, 1992. Commonly used Indian abortifacient plants with special reference to their teratologic effects in rats. J. Ethnopharmacol., 36: 147-154.
- Pal, S.K., P.K. Mukherjee and B.P. Saha, 1995. Studies on the antiulcer activity of *Moringa oleifera* leaf extract on gastric ulcer models in rats. Phytother. Res., 9: 463-465.
- Ramachandran, C., K.V. Peter and P.K. Gopalakrishman, 1980. Drumstick (*Moringa oleifera*).
- Richa, G., M.K. Guruswamy, S. Mamta and J.S.F. Swaran, 2005. Therapeutic effects of *Moringa oleifera* on arsenic induced toxicity in rats. Environ. Toxicol. Pharm., 20: 456-464.
- Shukla, S., R. Mathur and A.O. Prakash, 1988a. Biochemical and physiological alteration in female reproductive organs of cyclic rats treated with aqueous extract of *Moringa oleifera* lam. Acta Europ. Fertil., 19: 225-232.

- Shukla, S. and R. Mathur and A.O. Prakash, 1988b. Anti-implantation efficacy of *Moringa oleifera* lam and moringa concanensis nimmo in rats. Int. J. Crude Drug Res., 26 (1): 29-32.
- Shukla, S., R. Mathur and A.O. Prakash, 1988c. Anti-fertility Profile of the Aqueous Extract of *Moringa oleifera* Roots. J. Ethnopharmacol., 22:51-62.
- Shukla, S., R. Mathur and A.O. Prakash, 1989. Biochemical alterations in the female genital tract of ovariectomized rats treated with aqueous extract of *Moringa oleifera* lam. Pak. J. Scient. Indust. Res., 32(4): 273-277.
- Tarafder, C.R., 1983. Ethnogynaecology in relation to plants: 2 plants used for abortion. J. Econ. Taxon. Bot., 4(2):507-516.
- Tsaknis, J., G. Lalas, V. Gergis, V. Dourtoglou and V. Spilotis, 1999. Characterisation of *Moringa oleifera* variety mbololo seed oil in Kenya. J. Agric. Food Chem., 47: 4495-4499.
- Wikipedia, 2008. *Moringa oleifera*. Retrieved from: http://en.wikipedia.org/wiki/Moringa_oleifera) Web site, Assessed on 6/23/2008.
- Woodard, D. and R. Stuart, 2007. The Vermont Safe Information Resources. Inc. Material Safety Data Sheet Collect., Retrieved from: <http://hazard.com/msds/tox/f/q77/q479.html>.
- Young, B., J.S. Lowe, A. Stevens and J.W. Heath, 2006. Wheater's Functional Histology; A Text and Colour Atlas. 5th Edn., Churchill Livingstone, 348: 304-305.