

The Effect of Methanolic Extract of *Moringa oleifera* Lam Roots on the Histology of Kidney and Liver of 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: *Moringa oleifera* lam has Horseradish tree, Drumstick tree and Ben oil tree, as its English names. Principal Constituents of the roots include an active anti-biotic principle, pterygo-spermin, two alkaloids viz; moringine and Moringinine contained in the root bark. The aim of the study is to investigate effect(s) of methanolic extract of *Moringa oleifera* lam root on Histo-architecture of Liver and Kidney, considering objectives such as determining if the effect of the extract is dose and time-dependent, and determination of LD50. 'LD50 ip' of 223.61 mg/kg was determined using modified Lorke's (1983) method. Twenty four (24) guinea pigs were used for the study. They were 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 was done 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, 15th and 22nd days. Tissues collected were immediately prepared histologically for haematoxyline and eosin stain. The photomicrographs were observed under the microscope with magnifications of X400 and X200. Histological sections of group A revealed that kidney sections did not differ from the control group, while liver sections had balloon degeneration. For guinea pigs in group B, histological sections of liver showed balloon degeneration, while kidney sections showed mild tubular damage with interstitial inflammations. For guinea pigs in group C, histological sections of liver had balloon degeneration with microvesicular steatosis and sections of kidney had infiltration of interstitium with inflammatory cells as well as tubular Lumina filled with amorphous eosinophilic materials. Histological sections of liver and kidney of guinea pigs after eight weeks of cessation of treatment did not show normal histo-architecture. Histological sections of all treated groups had ballooning degeneration of the liver, means that hepatotoxicity is not dose-dependent, but time-dependent. Concerning the kidneys, sections from group B showed mild tubular damage with tubular cast and interstitial inflammation while group C had infiltration of interstitium by inflammatory cells and amorphous eosinophilic materials In the Lumina of the tubules. Methanolic extracts of *Moringa oleifera* lam roots was found to distort the histo-architecture of both liver and kidneys of guinea pigs. All the reversal groups retained histo-architectural distortions.

Key words: Histo-architecture, methanolic, *Moringa oleifera* lam

INTRODUCTION

Moringa oleifera lam has Horseradish tree, Drumstick tree and Ben oil tree, as its English names. While the local names in Nigeria include; Fulani; Gawara, Konamarada, Kini maka. Hausa; Zogall, Zogalla-gandi, Bagaruwar. Ibo; Ikwe oyibo. Yoruba; Ewe ile, Ewe igbale. The moringa tree grows mainly in semi-arid, tropical and sub tropical areas, it is native to the southern foothills of the Himalayes, and possibly Africa and the middle East (Duke James, 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: the leaves elliptic and the flowers, while fragrant in large panicles; the pods; 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 oleifera* lam root contains an active anti-biotic principle, pterygo-spermin. The root bark contains two alkaloids (total alkaloid 0.1%) viz. moringine which is identical with benzyl 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 (Chopra and Chopra, 1957). The roots also contain phytochemicals like; 4-(α -L-rhamnopyranosyloxy)-benzylglucosinolate and benyl-glucinolate (Menhnaz, 2009-2011).

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 aim of the study was to investigate effect(s) of methanolic extract of *Moringa oleifera* lam root on Histology of Liver and Kidney. Objectives were; To determine if methanolic extracts of *Moringa oleifera* lam root has effect(s) on the histology of Liver and Kidney of guinea pigs. And to determine whether the effects are dose-dependent and/or time-dependent.

Different parts of *Moringa oleifera* lam have different pharmacological actions and toxicity profiles which have not yet been completely defined. It 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 lam has vast medicinal properties and every part is said to have beneficial properties (Garima *et al.*, 2011). It 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.*, 1988, 1989; Faizi *et al.*, 1988). Nikkon *et al.* (2009) worked on Benzylcarbamothioethionate from root bark of *Moringa oleifera* Lam, and its toxicological effects. They concluded that histopathology of the liver, kidney, heart and lung did not reveal acute toxicity. 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).

Nikken *et al.* (2009) considered the new compound, benzylcarbamothionate Isolated from the chloroform soluble fraction of the ethanolic extract of the root bark of the *Moringa oleifera* Lam. The acute toxicity studies of the extract on long Evan's rats were carried out using four groups of animals. The haematological parameters, biochemical study and histopathology of the liver, kidney, heart and lung did not reveal acute toxicity. They concluded that both chloroform soluble fraction and compound I of *Moringa oleifera* Lam. Had no toxic effects in the experimental model.

Kumar *et al.* (2010) Compared the hepato-protective activity of leaves and roots of *Moringa oleifera* lam against carbon tetrachloride induced hepatotoxicity in albino rats. The hepatoprotective activity of the both extracts were tested using histopathological parameters. The liver section of methanolic extracts of *Moringa oleifera* Lam (leaves and roots) treated groups clearly showed normal hepatic cells and central veins, which are comparable with silymarin treated group of animals. The potent hepatoprotective activity of leaves of *Moringa oleifera* was confirmed from this study.

METHODOLOGY

The internationally accepted principles for laboratory animal use and care were adopted, and Ethical Clearance for research was obtained from the University of Port Harcourt ethical committee and the protocols were strictly adhered to.

'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, 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. 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 done 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 Boars; one from each group (groups A, B, C and control) were sacrificed on 8th, 15th and 22nd days. They were weighed, anesthetized and dissected, tissues collected were immediately fixed in formalin, prepared histologically for haematoxyline and eosin stain. The photomicrographs were observed under the microscope with magnifications of X400 and X200.

Fresh roots of *Moringa 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. Cold suscinat extraction was done using methanol. The extract was weighed and stored in sub-zero temperature in the refrigerator. The extracts were administered once daily, slowly, and intra-peritoneally to the healthy guinea pigs, using insulin syringes and needles.

RESULTS

Histological findings of control and experimental groups:

- **Control group (Administered with 0 mg/kg of plant extracts):** Histological sections of liver and kidney from control group showed normal histological features which served as reference points for comparing with experimental groups.
- **Guinea pigs treated with 3.5 mg/kg of extract:** Histological sections of the kidney of guinea pigs treated with 3.5 mg/kg of extract did not differ from the control group (normal histo-architecture). While histological sections of liver treated with 3.5 mg/kg of plant extract had balloon degeneration.
- **Guinea pigs treated with 4.6 mg/kg of extracts:** For guinea pigs treated with 4.6 mg/kg of plant extracts; histological sections of liver showed balloon degeneration, while kidney sections showed mild tubular damage with interstitial inflammations.
- **Guinea pigs treated with 7.0 mg/kg of extract:** For guinea pigs treated with 7.0 mg/kg of extract, histological sections of liver had balloon degeneration with microvesicular steatosis and sections of kidney had infiltration of interstitium with inflammatory cells as well as tubular Lumina filled with amorphous eosinophilic materials.
- **Reversal group:** Histological sections of liver and kidney of guinea pigs after eight weeks of cessation of treatment did not show normal histo-architecture.

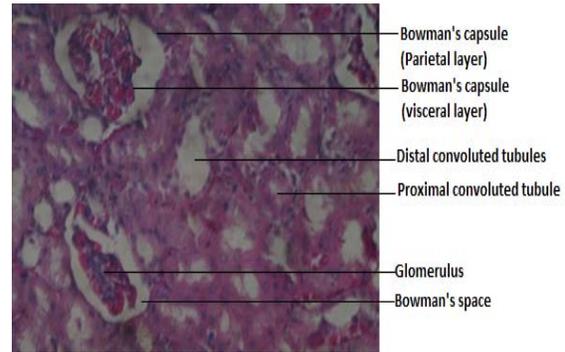


Fig. 1: A Photomicrograph of guinea pig kidney from the control group showing normal glomeruli and tubules. Magnification X400 H & E

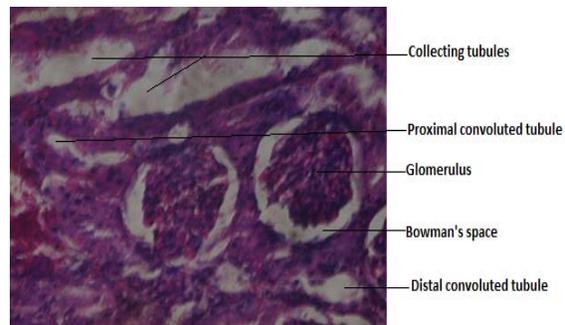


Fig. 2: A Photomicrograph of guinea pig kidney treated with 3.5 mg/kg of extract showing mild tubular damage (tubulointerstitial nephritis). Magnification. X400. H & E

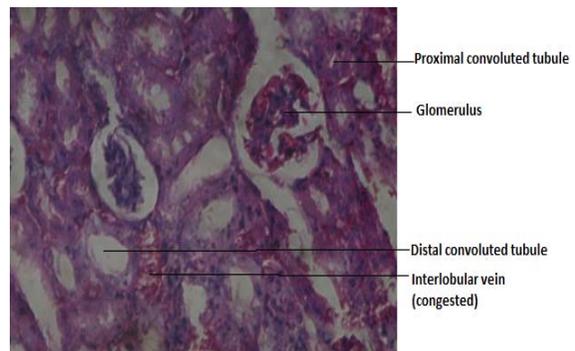


Fig. 3: A photomicrograph of guinea pig kidney treated with 3.5 mg/kg of extract showing mild glomerular damage (glomerulonephritis). Magnification X400 H & E

DISCUSSION

Histological sections of guinea pig's kidneys treated with methanolic extracts of *Moringa oleifera* lam roots (Fig. 1); Fig. 2 and 3 were collected from guinea pigs treated with 3.5 mg/kg of plant extract, Fig. 2 was collected early in the study and it revealed little or no damages, Fig. 3 exhibited mild glomerular damage (glomerulonephritis)

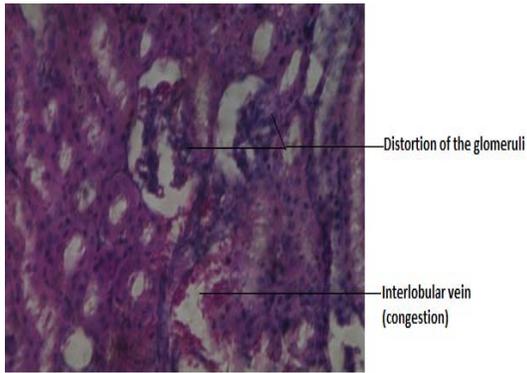


Fig. 4: A photomicrograph of guinea pig kidney treated with 4.6 mg/kg of extract showing glomerular damages (glomerulo-nephritis). Magnification X400 H & E

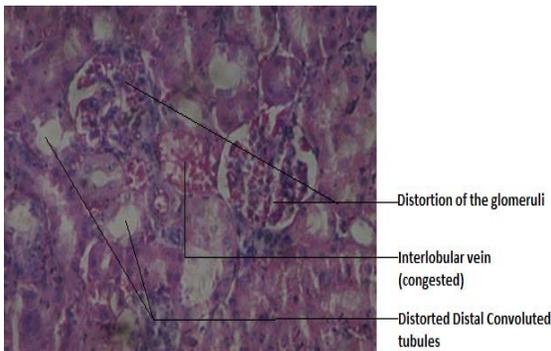


Fig. 5: A photomicrograph of guinea pigs kidney treated with 4.6 mg/kg of extract showing tubular and severe glomerular damages and inflammation (glomerulo-nephritis). Magnification X400 H & E

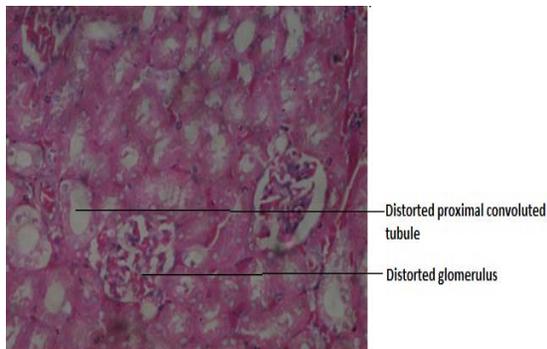


Fig. 6: A photomicrograph of guinea pig kidney treated with 7.0 mg/kg of extract showing severe glomerula damage and interstitial inflammation. Mag. X400. H & E

and venous congestion. Figure 4 and 5 were collected from guinea pigs treated with 4.6 mg/kg of plant extract. Figure 4; the sample was collected early in the study and it revealed distortion of glomeruli as well as venous congestion. Figure 5 represents the later part of the study and it showed glomerular and tubular inflammation. Figure 6 Represent the group that was treated with 7.0

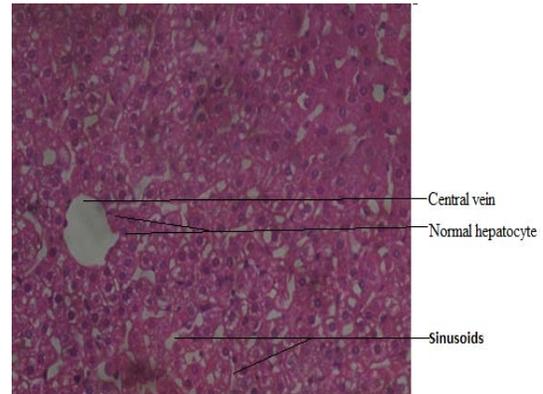


Fig. 7: A photomicrograph of Guinea Pigs liver from the control group (0 mg/kg of plant extract) Magnification X 400 H & E

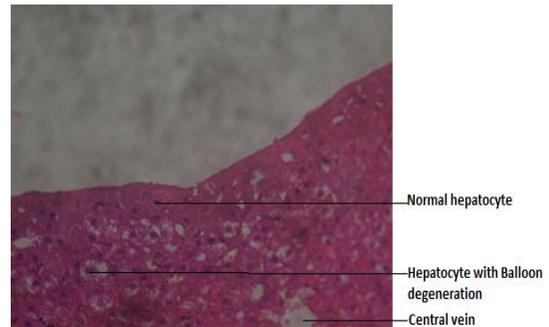


Fig. 8: A photomicrograph of guinea pig liver treated with 3.5 mg/kg of plant extracts showing normal hepatocytes with contiguous zone of cells with ballooning-degeneration. Magnification X400 H&E

mg/kg of plant extract, the findings are; glomerular, tubular and interstitial damages in both early and later parts of the study. The pattern of distortion is such that lower doses (3.5 and 4.6 mg/kg) affect mainly the glomeruli, while the higher dose (7.0 mg/kg) has a global effect on the kidney tissues (affecting; glomeruli, tubules, and interstitial spaces surrounding the tubules). These findings mean that the toxicity of methanolic extract of *Moringa oleifera* roots to the guinea pig's kidneys is both time-dependent and dose-dependent. The reversal group retained features of distortion of histo-architecture of kidney tissues, which means that injuries inflicted on the kidney tissues are irreversible. The findings are in keeping with Woodard *et al.* (2007), but contradict some previous studies (Mazumder *et al.*, 1999; Nikkon *et al.*, 2009).

Concerning the liver, Fig. 8 represents liver tissues collected from guinea pigs treated with 3.5 mg/kg of plant extract in the later stage, it revealed normal hepatocytes with contiguous zones of ballooning degeneration (microvesicular steatosis). Photomicrographs made from liver tissues treated with 3.5 mg/kg in the early stage of the study were found to be essentially normal

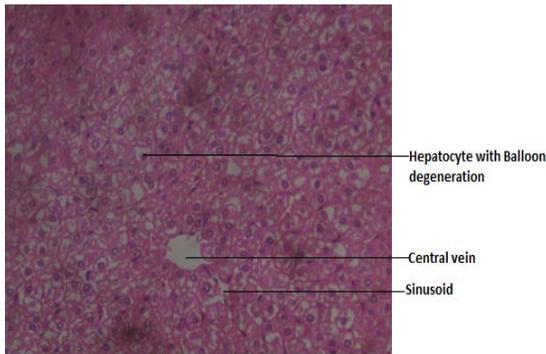


Fig. 9: A photomicrograph of guinea pigs liver treated with 4.6 mg/kg of extract showing ballooning degeneration of hepatocytes. Mag. X400.H & E

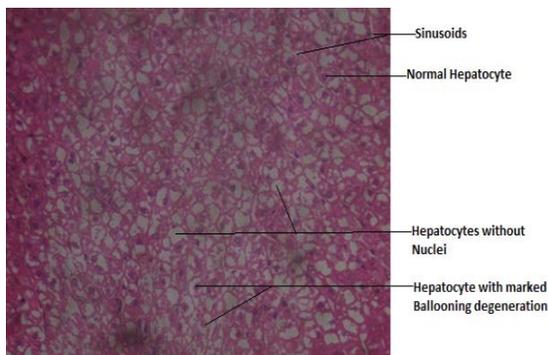


Fig. 10: A photomicrograph of guinea pig liver treated with 7.0 mg/kg of extract showing ballooning degeneration (macrovesicular steatosis). Magnification X400 H & E

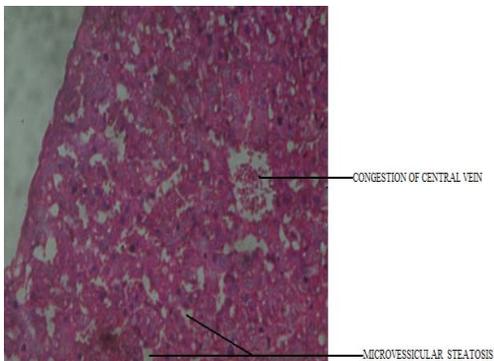


Fig. 11: A photomicrograph of guinea pigs liver treated with 4.6 mg/kg showing congestion of central vein and microvesicular steatosis. Mag. X400 H & E

(comparable to the control group, Fig. 7). Figure 9 and 11 were collected from the guinea pig group that was exposed to 4.6 mg/kg of plant extract; Fig. 9 was prepared from the early part of the study and it revealed congestion of the central vein, while Fig. 11 was collected in the later stage of the study, it revealed balloon degeneration of

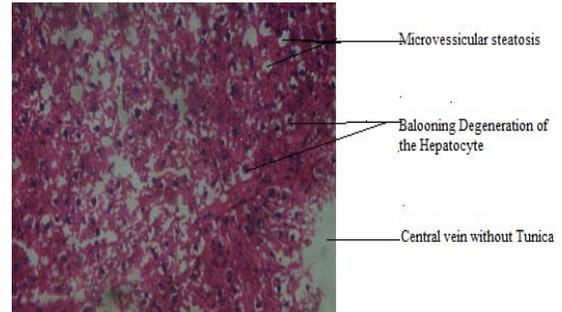


Fig. 12: A photomicrograph of guinea pig liver treated with 7.0 mg/kg showing macrovesicular steatosis. Magnification X400. H & E

the hepatocyte (microvesicular steatosis). Figure 10 and 12 were collected from guinea group treated with 7.0 mg/kg of plant extract. Figure 10 was prepared early in the study and it revealed ballooning degeneration of the hepatocytes (macrovesicular steatosis), while, Fig. 12 was prepared in the later stage of the study, it showed ballooning degeneration of the hepatocyte (macrovesicular steatosis) and distortion of the central vein. Microvesicular steatosis (also called fatty change, fatty degeneration or adipose degeneration) which is abnormal retention of lipids within a cell in vesicles that displace the cytoplasm. This is synonymous with liver tissues exposed to lower doses (3.5 and 4.6 mg/kg) of the plant extract. Whereas, Macrovesicular steatosis (which is development of vesicles large enough to distort the nucleus in addition to features of microvesicular steatosis) is characteristic of liver tissues treated with 7.0 mg/kg of plant extract. The fatty degeneration is made possible because liver is the primary organ of lipid metabolism, so is mostly associated with steatosis. These spaces were occupied by lipids in life but histological fixation caused the lipids to be dissolved, hence only empty/clear spaces are seen in the photomicrographs. The reversal group showed feature that are similar to the control group, which means that, the destructive effects of the plant extract on the histo-architecture of the liver is reversible. The result of our study contradicts Mazumder *et al.* (1999) and Nikkon *et al.* (2009).

CONCLUSION

Although the consumption of different parts of *Moringa oleifera* lam including the roots for various purposes has been widely accepted, Methanolic extracts of *Moringa oleifera* lam roots was found to distort the histo-architecture of both liver and kidneys of guinea pigs. These effects are time-dependent and dose-dependent. The liver and kidney of guinea pigs in the reversal group retained histo-architectural distortions.

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.
- 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 djalensis*. Ind. J. Pharmacol., 41(6): 258-261.
- Bhattacharya, J., G. Guha and B. Bhattacharya, 1978. Powder microscopy of bark-poison used for abortion: *Moringa pterygosperma* gaertn. J. Indian Forensic Sci., 17: 47-50.
- Chopra, R.N. and I.C. Chopra, 1957. Ind. J. Med. Res. Med., 15: 123.
- Duke James, A., 1982. Handbook of energy crops: *Moringa oleifera*. From the produce center for new crops Website.
- Faizi, S., B.S. Siddiqui, R. Saleem, K. Aftab and F. Shaheen, 1988. Bioactive compounds from the leaves and pods of *Moringa oleifera*. New Trends in Natural Products Chemistry, pp: 175-183.
- Fuglie, L.J., 1999. The Miracle Tree: *Moringa Oleifera*: Natural Nutrition for the Tropic. Church world service, Dakar. Revised in 2001 and Published as Miracle Tree: The Multiple Attributes of Moringa, pp: 68, 172. Retrieved from: <http://echotech.org/bookstore/advanced-search-result.php>
- Garima, M., S. Pradeep, V. Ramesh, K. Sunil, S. Saurabh, K.K. Jha and R.L. Khosa, 2011. Traditional Uses, phytochemistry and pharmacological properties of *Moringa oleifera* plant: An overview. Der Pharmacia Lettre, 3(2): 141-164.
- Kumar, C.S., B. Balanaurugam, S. Murugeswaran, P. Natarajan, S.P. Sharavanan, S. Petchimuthu and T.S. Murugan, 2010. Hepatoprotective activity of leaves and roots extracts of *Moringa oleifera* Lam. Int. J. Med. Res., 1(2): 90-93.
- 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. Indian J. Exp. Biol., 37: 612-614.
- Menhaz, K., 2009-2011. Nutrition Information. John Hopkin, Ratty Donovan.
- Morton, J.F., 1991. The horseradish tree, *Moringa pterygosperma* (Moringaceae)-A boon to arid lands? Econ. Bot., 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 teratogenic effects in Rats. J. Ethnopharmacol., 36: 147-154.
- Nikkon, F., S. Hassan, K.A. Salam, M.A. Mosaddik, P. Khondkar, M.E. Hague and M. Rahman, 2009. Benzylcarbamothioethionate from root bark of *Moringa oleifera* Lam. and its toxicological evaluation Boletin Latinoamericano y del caribe de plantas medicinales y Aromaticas, 8(2): 130-138.
- Shukla, S., R. Mathur and A.O. Prakash, 1988. Biochemical and physiological alteration in female reproductive organs of cyclic rats treated with aqueous extract of *Moringa oleifera* lam. Acta europaea fertilis, 19: 225-232.
- Tarafder, C.R., 1983. Ethnognaeology in relation to Plants: 2 plants used for abortion. J. Econ. Taxon. Bot. 4(2): 507-516.
- Woodard, D. and R. Stuart, 2007. The Vermont Safe Information Resources, Inc. Material Safety Data Sheet Collection. Retrieved from: <http://hazard.com/msds/tox/f/q77/q479.html>.