

Acute Toxicity and Effect of Aqueous Extract of *Moringa oleifera* Leaves on Organs and Tissues in Rats

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ABSTRACT

This research is conducted on the aqueous leaf extract to evaluate the acute toxicity and the prolonged effect of extract administration on organs and tissues of rats. The revised limit dose test of Up and Down procedure was used to determine the acute oral toxicity of the plant. The administration of the extract to rats at 5000 mg/kg body weight did not produce death at 24 hours. For sub acute toxicity, graded oral doses of 100, 200, 300 and 400 mg/kg body weight of the extract were administered daily for 21 days to rats in the treatment groups (5 rats per group) there was no extract administration to the rats in the control group. At the end of treatment, standard procedures were used to euthanize the rats and were examined both grossly and histopathologically. Extract administration for 21 days at various doses did not result in death of the rats; however, histological lesions occurred in the liver, kidneys, heart, lungs and intestine. It is concluded that the extract had a wide safety margin while the histopathological lesions found in the various organs suggested that the extract should be administered with caution when used for prolonged period.

Keywords: *Moringa oleifera*, aqueous extract, rats, histopathology

INTRODUCTION

Moringa oleifera is the most widely known and utilized plant of the family Moringaceae especially because of its medicinal qualities. It is a fast growing, deciduous and drought resistant tree reaching up to 3 meters in height just after 10 months of cultivation (Valia Patil, Patel and Kapadia, 1993). It is the most widely known and utilized of the *Moringaceae* with 14 known species (Ram, 1994). The tree has sparse foliage and often planted in compounds or used as fence in Northern

Nigeria, and grows up to 8 meters in height (Keay, 1989). *Moringa oleifera* tree has vast medicinal qualities and every part of the plant is said to have beneficial properties (Garima *et al.*, 2011). Indigenous people have found much success in using various parts of the tree to cure many physical ailments. For example, the juice from the leaves is believed to stabilize blood pressure, the flowers are used to cure inflammations, the pods are used for joint pain, the roots are used to treat rheumatism, and the bark can be chewed to enhance digestion (Ram, 1994). Oludoro and Aderiye (2007) report that the seed has the ability to cause biological coagulation in drinking water, and can be employed for water purification (Olsen, 1987; Jahn, 1988). The plant exhibits anti-inflammatory, antiulcer and anti-hypertensive properties (Pal, Mukherjee and Saham, 1995; Ezeamuzie, Amberkedeme, Shode and Ekwebelem, 1996), it was also known to possess anti-bacterial activity (Caceres, Cabrera, Morales, Mollinedo and Mendia, 1991; Sofowora, 1993) and in traditional therapy to control abortion and infertility (Watt and Breyer-Brandwijk, 1962; Oommachan and Khan, 1981).

Phytochemical investigation on the aqueous leaf extract of *Moringa oleifera* revealed the presence of carbohydrates, tannins, phlobatannins, saponins, cardiac glycoside, flavonoids and alkaloids (Ojo, Chibuzo, Onyeyili and Sandabe, 2010). The acute toxicity study has revealed its potential as herbal product with a wide safety margin (Ojo and Mahre, 2010; Kwaghe and Ambali, 2011), but few work has been done on the long term effect or sub-acute toxicity of the aqueous leaf extract of the plant on vital organs and tissues. This study is carried out to investigate the acute toxicity and effect of prolonged administration of aqueous leaf extract of *Moringa Oleifera* on organs and tissues of albino rats.

MATERIALS AND METHOD

Collection, Identification and Processing of plant material: Fresh leaves of *Moringa Oleifera* were obtained from its natural habitat in Mairi village, Jere Local Government Area of Borno State, Nigeria. The plant was identified and authenticated by a taxonomist in the Department of Biological Sciences, University of Maiduguri. One kilogram of the leaf was washed in distilled water to remove dust particles and then air-dried in shade. After drying, the leaves were pulverized into powder form using a blender and then stored in a clean bag until required.

Plant Extraction: The powdered sample, weighing 525g was mixed with 2 litres of distilled water in a conical flask. The mixture was homogenously mixed, allowed to stay overnight at room temperature and then filtered using Whatman filter paper (No. 1). The filtrate was then evaporated to dryness using an electric oven pre-set at 50°C, yielding 189.1g. The dried powder was kept at 4°C until needed (Trease and Evans, 1989).

Acute toxicity test: The acute oral toxicity test of the extract was determined by using the limit dose test, up and down method as described by Dixon and Mood (1948) and modified by Dixon (1965, 1991). Five adult male rats were randomly selected for the experiment. They were marked and housed individually in cages in the laboratory for 7 days to allow for acclimatization to the laboratory conditions. The rats were fasted overnight but allowed free access to water prior to dosing on each occasion. A rat from group A was picked, weighed and dosed orally with a limit dose 5000 mg/kg body weight of the freshly prepared aqueous extract. Another rat from the same group was administered the same dose of the extract until all the rats in the group received the same dose of the extract. Each rat was observed each time for instant death and then watched for successive 24 hours for the short-term outcome and finally for the next 12 days for any delayed toxic effects.

Experimental Animal and Treatment: Albino rats of both sexes bred in the laboratory of the Department of Veterinary Physiology, Pharmacology and Biochemistry, University of Maiduguri and weighing between 152 and 280grams were used. They were fed standard pelletized commercial grower ration (Vital feed, Nigeria Limited), containing 14.5% crude protein, 7% fat, 7.2% crude fibre, 1.2%, mineral and vitamin premix supplement. Drinking water was provided ad libitum. The animals were handled in accordance with International guiding principles for biochemical research involving animals (C.I.O.M.S., 1985).

Twenty-five rats weighing between 152 and 280grams were randomly selected and divided into five groups of five rats each. Groups A, B, C, and D were treated orally with 100, 200, 300 and 400 mg/kg body weight respectively, of aqueous leaf extract of *Moringa oleifera* daily, for 21 days, while the control (group E) received only water and feed for the same period. At the end of the experiment (21 days), the rats were necropsied and examined for gross lesions. The liver, intestine, heart, lungs, and kidneys were harvested and fixed in Bouin's fluid for 24 hours. The tissues were dehydrated through graded concentration of ethanol (70%, 95% and absolute), cleared in xylene and embedded in paraffin wax. The embedded tissues were sectioned at 7µm thickness and tissues were stained with Haematoxylin and Eosin (H and E) for light microscopic examination (Drury, Wallingthorn and Camerron, 1976). Photomicrographs of the sections were taken using cyberpix (S – 55IV) digital camera.

RESULTS AND DISCUSSION

Acute toxicity test: The acute toxicity test carried out reveals that the aqueous extract of *Moringa oleifera* was Non toxic and have a high safety margin with 5,000 mg/kg body weight not being lethal to rats.

Gross lesions: No gross lesions were observed in all the rats throughout the period of the treatment. The active principle(s) in the extract may have been metabolised

in the liver and excreted through the kidneys. The lesions observed in the liver and kidneys may be expected since the liver is the major organ of biotransformation in the body while the kidney is the primary organ of excretion.

Histopathological changes: Histopathological samples were obtained from the liver, intestine, heart, lungs and kidneys. The result of the effects of *Moringa oleifera* leaf extract on various organs and tissues following treatment for 21 days is presented in Fig. 1a to Fig. 5b. Fig. 1a shows the histology of the normal heart of a rat from the control group while Fig. 1b showed the heart of a rat treated with 200mg/kg of the extract showing myocardial necrosis, mononuclear cell infiltration (MN) and hyaline degeneration of walls of vasa vasori (VV).

Fig. 2a shows the histology of normal lung of a rat from the control group while Fig. 2b shows the lung of a rat treated with 300mg/kg of the extract showing moderate pulmonary congestion and oedema, thickened inter alveolar septa, and hyaline degeneration of blood vascular wall. Fig. 3a shows the histology of the normal small intestine of a rat from the control group while Fig. 3b shows small intestine of rat treated with 100mg/kg of extract showing moderate villous collapse and sloughing of epithelium into lumen, also multifocal areas of glandular necrosis (GN). Fig. 4a shows the histology of the normal liver of a rat from the control group while Fig. 4b shows the liver of a rat given 100mg/kg of extract showing an apparently normal organ, with mild periportal mononuclear cell aggregations. Fig. 5a shows the histology of the normal kidney of a rat from the control group while Fig. 5b shows the kidney of a rat given 100mg/kg of extract showing moderate cortical congestion, pinkish casts in tubules (arrows) and hyaline degeneration and thrombosis of arterioles.

The oral administration of the aqueous leaf extract of *Moringa oleifera* at limit dose of 5000 mg/kg body weight did not produce any sign of acute toxicity or instant death in any of the five rats tested. This suggested that the extract has low acute toxicity when administered orally. According to Bruce (2006), any substance with LD₅₀ estimated to be greater than 2000-5000 mg/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 LD₅₀ values recommended by the organisation of Economic Co-operation and Development (OECD, Paris, France) (Walum, 1998) are as follow: very toxic, <5 mg/kg; toxic, >5<50 mg/kg; harmful, >50<500 mg/kg; no label, >500<2000 mg/kg.

Therefore, the high LD₅₀ (>5000 mg/kg body weight) of the aqueous extract obtained in this study indicated that the extract could be considered very safe especially when administered orally where absorption may not be complete due to inherent factors limiting absorption in the gastro intestinal tract (Dennis, 1984). The generalised focal areas of necrosis with mononuclear cellular infiltration observed in organs and tissues of the body were indicative of death of the cells and the body's attempt to get rid of the necrotic tissues. The administration of the extract to

rats for 21 days at various doses did not result in the death of the animals; however, histological lesions occurred in the liver, kidneys, heart, lungs and intestine. The lesions observed in the liver and kidneys may be expected since the liver is the major organ of biotransformation in the body (Kaplan, Szabo and Opheim, 1988), while the kidney is the primary organ of excretion. The active/toxic principle(s) in the extract may have been metabolised in the liver and excreted through the kidneys. The presence of lesions in the lungs and the heart may be an indication of the extensive distribution of the active/toxic principle(s) in the body. The extract also caused intestinal lesions in treated rats. The observed intestinal lesions may not be serious enough to prevent complete absorption of nutrients.

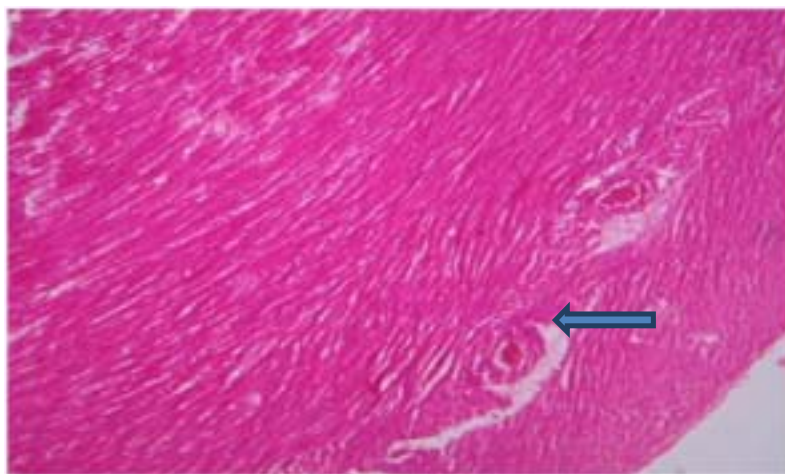


Figure 1a: Photomicrograph showing histology of the normal heart of a rat (H & E; x 350)

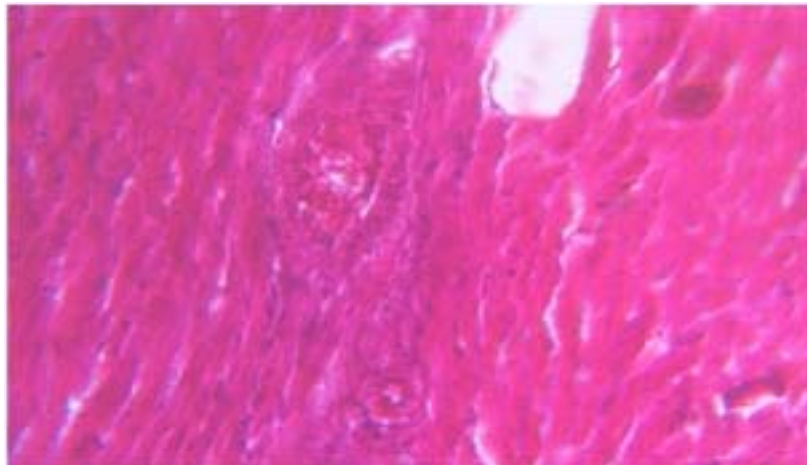


Figure 1b: Photomicrograph showing histopathology of the heart of a rat previously treated with 200mg/kg of the extract. There was a focus of myocardial necrosis, mononuclear cell infiltration (MN) (H &E; x 350)

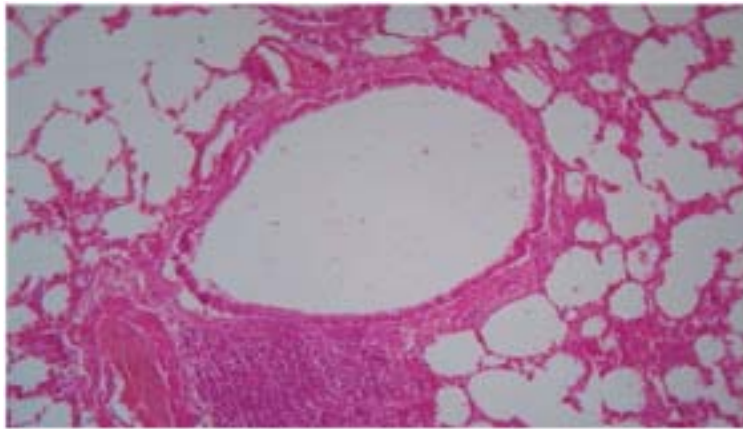


Figure 2a: Photomicrograph showing histology of the normal lung of a rat (H & E; x 350)

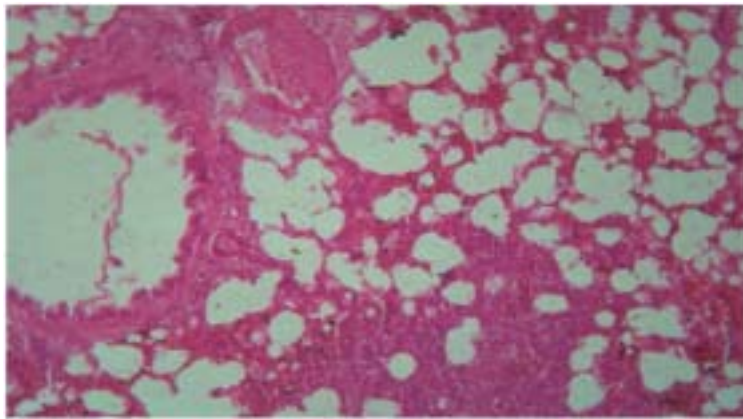


Figure 2b: Photomicrograph showing histopathology of the lung of a rat treated with 300mg/kg of the extract. There was moderate pulmonary congestion and oedema, thickened inter alveolar septa, and hyaline degeneration of blood vascular wall (black arrow) (H & E; x 350)

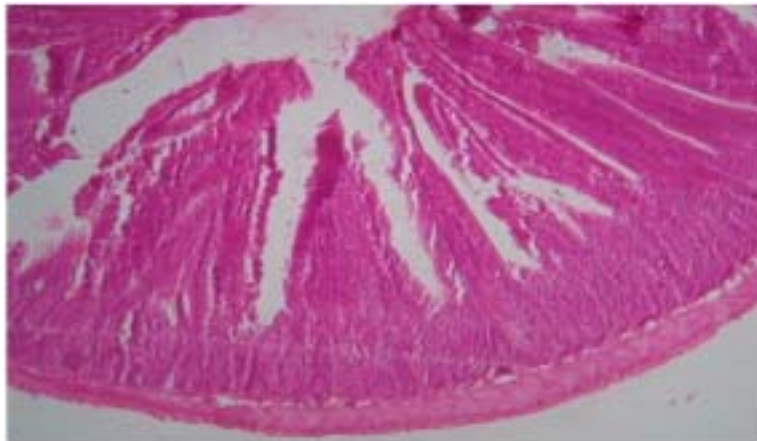


Figure 3a: Photomicrograph showing histology of the normal small intestine of a rat (H & E; x350)

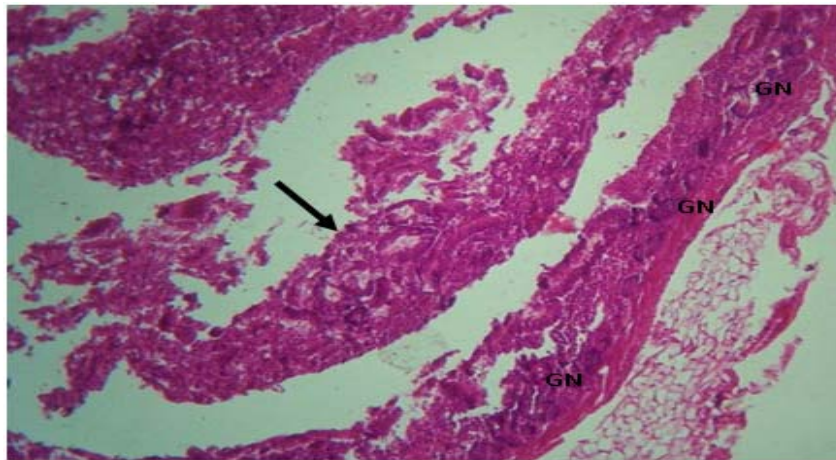


Figure 3b: Photomicrograph showing small intestine of a rat treated with 100 mg/kg of the extract. There was moderate villous collapse, glandular necrosis (GN) and sloughing of mucosal epithelium into the lumen (arrow) (H & E; x 350)

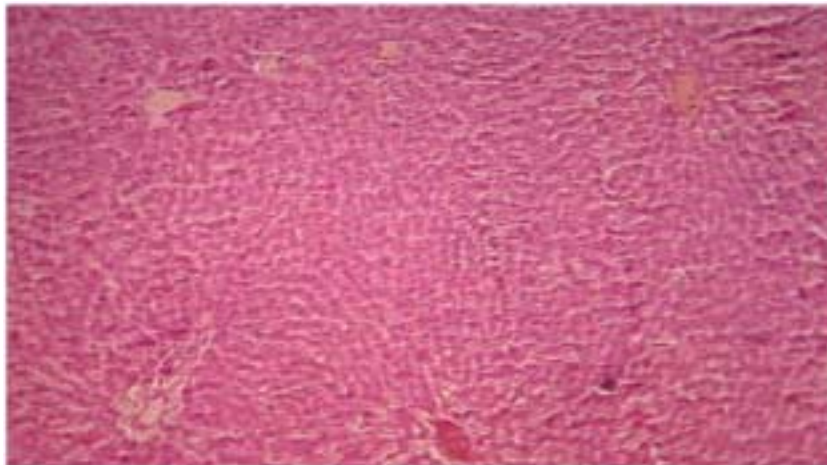


Figure 4a: Photomicrograph showing histology of normal liver of a rat (H & E; x 350)

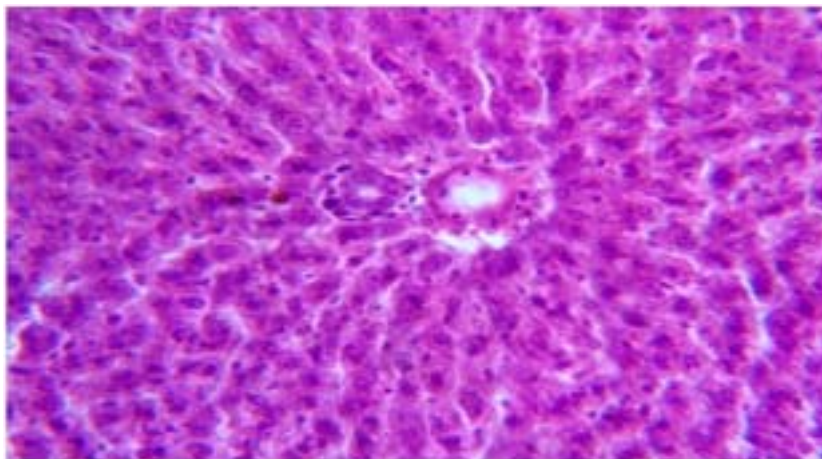


Figure 4b: Photomicrograph showing liver of a rat with a mild periportal mononuclear cell aggregation (arrow) (H & E; x 350)

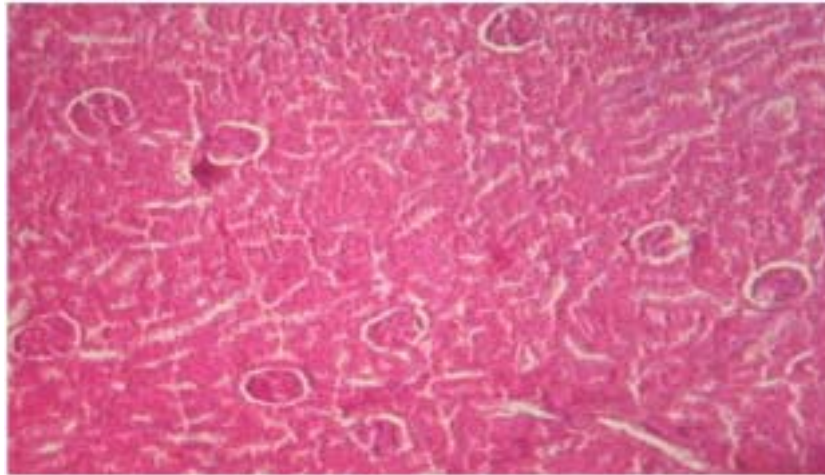


Figure 5a: Photomicrograph showing histology of the normal kidney of a rat (H & E; x 350)

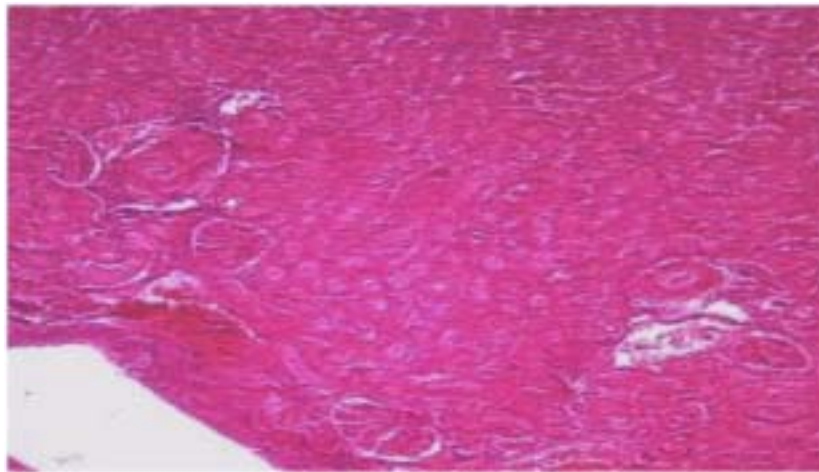


Figure 5b: Photomicrograph showing the kidney of a rat given 400mg/kg of the extract. There was moderate cortical congestion, pinkish casts in tubules (arrow) and hyaline degeneration and thrombosis of arterioles (short black arrow) (H & E; x 350)

CONCLUSION

This study was conducted on the aqueous leaf extract of *Moringa* to evaluate the acute toxicity and the prolonged effect of extract administration on organs and tissues in rats. The revised limit dose test of Up and Down procedure was used to determine the acute oral toxicity of the plant. Though the extract also caused intestinal lesions in treated rats, yet the observed intestinal lesions may not be serious enough to prevent complete absorption of nutrients. In conclusion, the lesions found in various organs suggest that the extract may have some toxic potential. However, no mortality was recorded; it should therefore be administered with caution when used.

REFERENCES

- Bruce R. D.** (2006). An Up and Down procedure for Acute toxicity testing. Entrez. Pubmed. [http://www. Ncbi.nih.gov/entrez/query](http://www.Ncbi.nih.gov/entrez/query).
- Caceres A., Cabrera O., Morales O., Mollinedo P. and Mendia P.** (1991). Pharmacological Properties of *Moringa oleifera*. Preliminary Screening for Anti-microbial Activity. *Journal of Ethnopharmacology*, 133 (3), 213 – 216.
- CIOMS** (1985). International Guiding Principles for Biomedical Research Involving Animals. Council for International Organisations of Medical sciences CIOMS. C/O World Health Organisation, 1211, Geneva 27, Switzerland.
- Dennis V. P.** (1984). *Mammalian metabolism of xenobiotic chemicals*. In Kacew, S. and Reasor, M. J. (eds.) *Toxicology and Newborn*. London: Chapman Hall, pp 1-32.
- Dixon W. J. and Mood A. M.** (1948). A method for obtaining and analysing sensitivity data. *Journal of American Statistics Association*, 43, 109 – 126.
- Dixon W. J.** (1965). The Up-and-Down method for small samples. *Journal of American Statistics Association*, 60, 967-978.
- Dixon, W. J.** (1991). Staircase Bioassay. The Up-and-Down method. *Neuroscience and Biobehavioural Review*, 15, 47-50.
- Drury R. A. B., Wallingthorn E. A. and Camerron S. R.** (1976). *Carleton Histological Techniques* (4th ed.) London: Oxford University Press.
- Ezeamuzie T. C., Amberkedeme A. W., Shode F. O. and Ekwebelem S. C.** (1996). Anti-inflammatory Effects of *Moringa oleifera* root. *International Journal of Pharmacognosy*, 34 (93), 207 – 212.
- Garima M., Pradeep S., Ramesh V., Sunil K., Saurabh S., Jha K. K. and Khosa R. L.** (2011). Traditional Uses, phytochemistry and pharmacological properties of *Moringa oleifera* plant: An overview. *Der Pharmacia Lettre*, 3(2), 141-164.
- Jahn S. A. A.** (1988). Using Moringa seeds as coagulants in developing countries. *Journal of American Water Works Association*, 80, 45-50.
- Kaplan L. A., Szabo L. L. and Opheim E. K.** (1988). *Clinical Chemistry Interpretation and Techniques*. (3rd ed.). Philadelphia: Lea Febiger.
- Keay R. W. J.** (1989). *Trees of Nigeria*. New York: Oxford University Press.
- Kwaghe A. V. and Ambali A. G.** (2011). Acute Toxicity Studies of the Ethyl Acetate Fraction of the Aqueous Extract of *Moringaoleifera* Leaf in Cockerels. *Sahel Journal of Veterinary Scienace*, 10(1),19-22.

- Ojo, N. A. and Mahre M. B.** (2010). Acute toxicity and effect of *Moringaoleifera* (horseradish) leaf extract on blood glucose of normal rats. *Moor Journal of Agricultural Research*, 11, 72-78.
- Ojo, N. A., Chibuzo G. A., Onyeyili P. A. and Sandabe U. K.** (2010). Preliminary elemental and qualitative phytochemical investigation of the leaves of *Moringa oleifera*. *Journal of Life and Environmental Sciences*, 11 (1), 626-629.
- Olsen, A.** (1987). Low technology water purification by bentonite clay and *Moringa oleifera* seeds flocculation as performed in Sudanese village: Effects of *Schistosomamansonicerariae*. *Water Resources*, 21, 81-92.
- Oluduro A. O. and Aderiye B. I.** (2007). Efficacy of *Moringa oleifera* seed extract on the Microflora or Surface and Underground Water. *Journal of Plant Science*, 2(4), 453-458.
- Oommachan, M. and Khan, S. S.** (1981). Plants in Aid of Family Planning. *Science Life*, 1, 64 – 66.
- Pal S. K., Mukherjee P. K. and Saham B. P.** (1995). Studies on the Anti-ulcer Activity of *Moringa oleifera* leaf Extracts on Gastric Uler Models in rats. *Phytotehr. Tes.* 6, 463 –465.
- Ram, J.** (1994). *Moringa* a highly nutritious vegetable tree .Tropical Rural and Island. A toll Development Experimental Station (TRADES) Technical Bulletin No. 2 .
- Sofowora A.** (1993). *Medicinal Plants and Traditional Medicine in Africa*. Ibadan: Spectrum Books Limited.
- Trease, G. E. and Evans, M. D.** (1989). *A textbook of Pharmacognosy* (13th ed.). London: Braillier, Tindal and Causse.
- Valia R. V., Patil V. K., Patel Z. N. and Kapadia P. K.** (1993). Physiological responses of drum stick (*Moringaoleifera*) land to varying levels of ESP. *Indian Journal of Plant Physiology*, 36(4), 261-26.
- Walum, E.** (1998). Acute oral toxicity. *Environmental Health Perspective*, 106, 497-503
- Watt J. M. and Breyer–Brandwijk, M. G.** (1962). *Medicinal and Poisonous Plants of Southern and Eastern Africa* (2nd ed.). London: Livingston Ltd..