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Effect of Administration of Aqueous Extract of *Moringa Oleifera Lam* Leaves on the Histo-Architecture of Male Adult Wistar Rat's Kidney

***Gudaji, A.[1], Mustapha, S. D[1]**
[1]Department of Anatomy,
Faculty of Basic Medical Sciences,
Bayero University, Kano
gudajikzr@yahoo.com,
agudaji.ana@buk.edu.ng

Abstract
Moringa oleifera lam is the botanical name of Horseradish tree, Drumstick tree and Ben oil tree, being its English names. Moringa oleifera has various nutritional and health benefits which covered many fields including industries. However, it is important to note that most investigations on this plant are basic and the reports require proper and deep evaluation of the exact benefits to human health. The aim of the study is to investigate effect(s) of aqueous extract of Moringa oleifera lam leaves on the histo-architecture of male adult Wistar rat's kidney. The objective of the study is to determine if the effects of the extract is dose and time-dependent. Twelve (12) male adult Wistar rats were used for the study. They were acclimatized and randomly distributed into groups A-D. They were given daily administration of extract of Moringa oleifera lam leaves for three (3) weeks. Doses of 200, 400 and 600 mg/kg were given to groups B, C and D respectively while group A was given distilled water. Three Wistar rats; one from each group were sacrificed on 8th, 15th and 22nd days. Tissues collected were immediately processed histologically for hematoxylin and eosin stain. The photomicrographs were observed under the microscope with magnifications of x40 and x100. Histological sections of group A revealed that the kidneys section showed normal tissue constituents. For group B, histological sections of kidney sections showed mild glomerulus degeneration and inflammations. For group C, histological sections of kidney had severe glomeruli damage and degeneration with inflammatory cells as well as tubular lumina filled with amorphous eosinophilic materials. Histological sections of kidneys from group D showed severe glomeruli damages than the other groups. Histological sections of all treated groups showed that the effects were dose-dependent and time-dependent.

Keywords: ***Moringa oleifera lam, Kidney, Wistar rats, Male, Histo-architecture***

INTRODUCTION
Moringa oleifera, Lam, is a natural as well as cultivated variety of the genus *Moringa* belonging to family *Moringaceae*. *Moringa Oleifera* commonly called Drumstick, Horseradish or Miracle tree. The tree is a fast-growing, drought-resistant tree that is native to the southern foothills of the Himalayas in north-western India. The tree is grown mainly in semi-arid, tropical, and sub-tropical areas (Majhi, 2013). India is the largest producer of Moringa, with an annual production of20.61 lakh metric tonnes of tender fruits from an area of61,600 hectares. Among Indian states, Andhra Pradesh leads in both area and production followed by Karnataka, and Tamil Nadu (Pandey, 2013). The plant is grown for food and it is an exceptionally nutritious vegetable tree with varieties of potential value (Ozumba, 2011). The tree is valued mainly for its tender pods, which are esteemed as vegetable, tender leaves and flowers are also used as vegetable. There is considerable variation among nutritional values of *Moringa*, which

**Author for Correspondence*

depends on factors like genetic background, environment and cultivation methods (Brisibe *et al*., 2009). Nutritional composition of the plant plays a significant role in nutritional, medicinal and therapeutic values (Al-kharusi *et al.,* 2009). Green leaves and fruit pods of drumstick are rich sources of minerals like calcium, iron and good sources of vitamin A, B, C and protein including fair amounts of sulphur containing amino acids (Ram, 1994). Apart from providing nutrition, it also contributes to the appealing colour, texture and flavour of the food.

Moringa oleifera is an important food commodity which has had enormous attention as the 'Natural Nutrition of the Tropics'. The leaves, fruit, flowers and immature pods of this tree are used as a highly nutritive vegetable in many countries, particularly in India, Pakistan, Philippines, Hawaii and many parts of Africa (D'souza and Kulkarni, 1993; Anwar and Bhanger, 2003; Anwar *et al*., 2005, Anwar *et al*., 2007). It is known as 'Mother's Best Friend' because of its utilization to increase woman's milk production and is sometimes prescribed for anaemia (Estrella *et al*., 2000; Dawn *et al*., 2015).

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 (Paul and Didia, 2012).

MATERIALS AND METHODS

Twelve (12) healthy male Albino Wistar rats were allowed to acclimatize and adapt with the new environment for two (2) weeks before the administration of the *Moringa oleifera* leaf extract. These rats were randomly divided into group of four (4) with each group containing three (3) Wistar rats and the groups were labeled A, B, C and D.

Group A serves as the control group was given distilled water, group B was treated with *M.oleifera* leaf extracts of 200mg/kg body weight daily at the calculated concentration of 10 mg/ml, group C was treated with *M. oleifera* leaf extracts of 400 mg/kg body weight daily at the calculated concentration of 20 mg/ml, and group D was treated with *M.oleifera* leaf extracts of 600 mg/kg body weight daily at the calculated concentration of 30 mg/ml.

The grouped animals were placed in two separate steel cages with each group member painted with different permanent markers and numbered from 1-3 with the exception of group A. Group B was painted with blue permanent marker, number 1(200 mg/kg), 2 (400 mg/kg) and 3 (600 mg/kg) body weight had one, two, and three rounded ring on their tails respectively. Group C painted with green permanent marker had one (200 mg/kg), two(400mg/kg), and three (600 mg/kg) body weight, rounded ring on their tails respectively and group D painted with black permanent marker also had one (200 mg/kg), two (400 mg/kg), and three(600 mg/kg) body weight, rounded ring on their tails respectively. All these were done for easy identification of each member of the four (4) groups.

The leaf powder of *M.oleifera* were measured using weighing balance and macerated. 100 g of the powder was macerated with 1000ml of distilled water, shaken and allowed to soak completely for 24 hours. The plant derived aqueous extract tested in this study was prepared and the homogenous solution was filtered using number 1 Whitman filter paper and the filtrate was placed in a boiling water bath with temperature range of 70°-98°C. The process continued until all the water evaporated and the resulting extracts obtained were crude solid. After the extraction process, the resulting crude solid extracts of *M.oleifera* was dissolved in distilled water. The extract was stored in sub-zero temperature in the refrigerator.

Weight of each Wistar rat was measured with weighing balance before dissolving the extracts in distilled water and volume, number of volume administered/rat and concentration of *M.oleifera* leave were calculated.

Doses of 200 mg/kg, 400 mg/kg and 600 mg/kg were administered to groups B, C and D respectively at different concentrations. The extract was administered orally daily in the morning between 8:00 am 10:00 am by holding the smooth skin of the neck of the rats gently, carefully, slowly and properly until it cried and opened its mouth at the same time insulin syringe containing extracts was introduced into the mouth of the animal and the administration was done slowly and gently at time interval.

Four rats, one from each group were slaughtered at 8th, 15th, and 22nd day of the experiment. The rats were put in the desiccators containing cotton wool soaked in chloroform (anesthesia) for about 2-3 minutes, removed and a midline incision was done through the ventral abdominal wall. The kidneys were collected immediately and fixed in white rubber container containing 10% normal saline (fixative) for minimum of 24 hours.

After fixation of the kidneys in 10% normal saline, the tissue was dehydrated using alcohol of different strengths, then cleared using xylene, then embedded in molten paraffin wax, sectioned and stained using hematoxylin and eosin, placed on glass slide containing albumin as an adhesive, and then covered with coverslip and then mounted on microscope for viewing and snapping.

RESULTS

Plates IA & IIB show the photomicrographs of normal histo-architecture of the kidney of Wistar rats that were given distilled water. Glomerulus (G); Urinary Space (US); distal convoluted tubules (DCT) and proximal convoluted tubules (PCT).

Plates IIA & IIB:H&E photomicrograph of kidney (day 8) of male adult Wistar rat treated with 200mg/kg of the extract. The histo-architecture shows mild degenerating Glomerulus (DG); prominent distal convoluted tubules (DCT) and proximal convoluted tubules (PCT) at x100 (IIA). Prominent/enlarged nucleus, loss prominence of the urinary space/Bowman's capsule, blood vessel (BV).

Plates IIIA & IIIB:H&E photomicrograph of the kidney (day 8) of male adult Wistarrat treated with 400 mg/kg of*Moringa oleifera* leave extract. The histo-architecture shows mild degenerating and distortion of Glomerulus (DG and GD); prominent distal convoluted tubules (DCT) and proximal convoluted tubules (PCT).

Plates IVA & IVB:H&E photomicrographs of kidney (day 8) of male adult Wistar rat treated with 600mg/kg of the *Moringa oleifera* leave extract. The histo-architecture shows further increase Glomerulus distortion and inflammation (GD); degeneration and disappearance of urinary space (US).

Plates VA & VB:H&E photomicrograph of kidney (day 15) of male adult Wistar rat treated with 200 mg/kg of *Moringa oleifera* leave extract. The histo-architecture shows degenerating Glomerulus (DG); further increase Glomerulus distortion and inflammation (GD); disappearance of urinary space (US); hyperplasia of the epithelial cells and increase in PCT and DCT size.

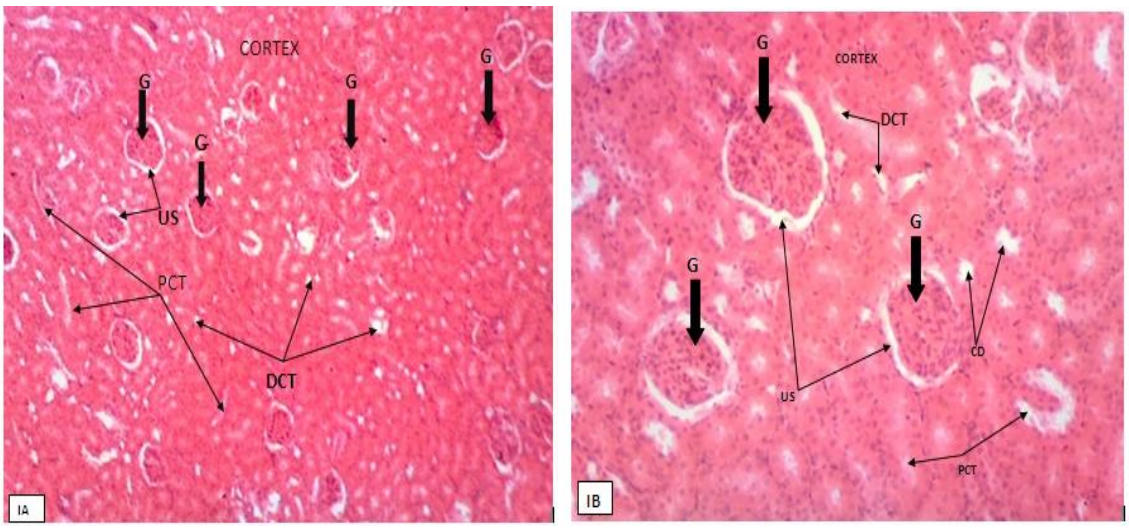
Plates VIA & VIB: H&E photomicrograph of the kidney (day 15) of male adult Wistar rat treated with 400mg/kg of the *Moringa oleifera* leave extract. The histo-architecture shows severe degenerating and degenerated Glomerulus (DG); Glomerulus distortion and inflammation (GD); disappearance of urinary space (US); hyperplasia of the epithelial cells and increase in PCT and DCT size.

Plates VIIA & VIIB:H&E photomicrograph of the kidney (day 15) of male adult Wistar rat treated with 600mg/kg of the *Moringa oleifera* leave extract. The histo-architecture shows severe degenerating and degenerated Glomerulus (DG); Glomerulus distortion and inflammation (GD); disappearance of urinary space (US); and mild PCT and DCT inflammation; blood vessel (BV).

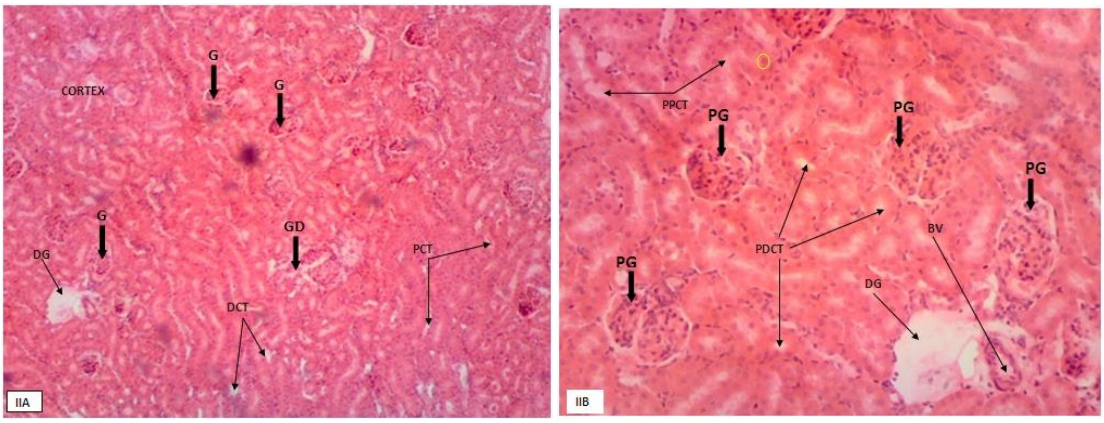
Plates VIIIA & VIIIB: H&E photomicrograph of the kidney (day 22) of male adult Wistar rat treated with 200 mg/kg of the extract. The histo-architecture shows moderate degenerating Glomerulus (dG); degenerated Glomerulus (DG); mild distal convoluted tubules (DCT) and proximal convoluted tubules (PCT) inflammation; vascular hypertrophy at X100 (VIIIB). Hypoplasia and degeneration of tubular epithelial cells.

Plates IXA & IXB: H&E photomicrographs of the kidney (day 15) of male adult Wistar rat treated with 400 mg/kg of the *Moringa oleifera* leave extract. The histo-architecture shows severe degeneration of Glomerulus (DG); Glomerulus distortion and (GD); severe distortion/occlusion of vascular lumen (DVB), hypertrophy and hemorrhage at both magnification; degeneration PCT and DCT.

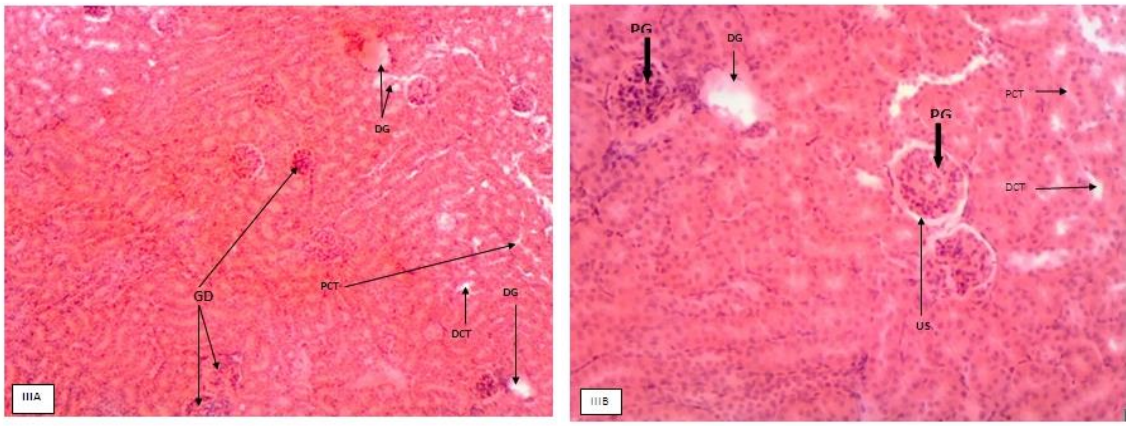
Plates XA & XB: H&E photomicrograph of the kidney (day 15) of male adult Wistar rat treated with 400 mg/kg of the *Moringa oleifera* leave extract. The histo-architecture shows severe degenerating (dG) and degenerated Glomerulus (DG); distortion of collecting duct (dCD); and increase in PCT and DCT lumen.



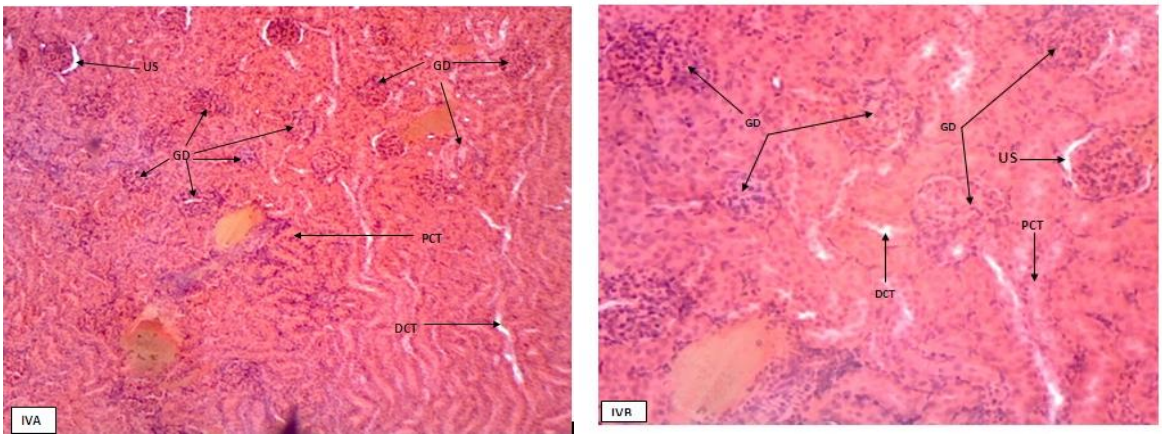
PlatesIA & IB: H&E photomicrographs of the kidney of male adult Wistar rat from the control group given distilled water body weight of the extracts. The photomicrograph shows normal histo-architecture of the kidney. Glomerulus (G);urinary space (us); distal convoluted tubules (DCT) and proximal convoluted tubules (PCT). Mag. IA (x40) and IB (x100)



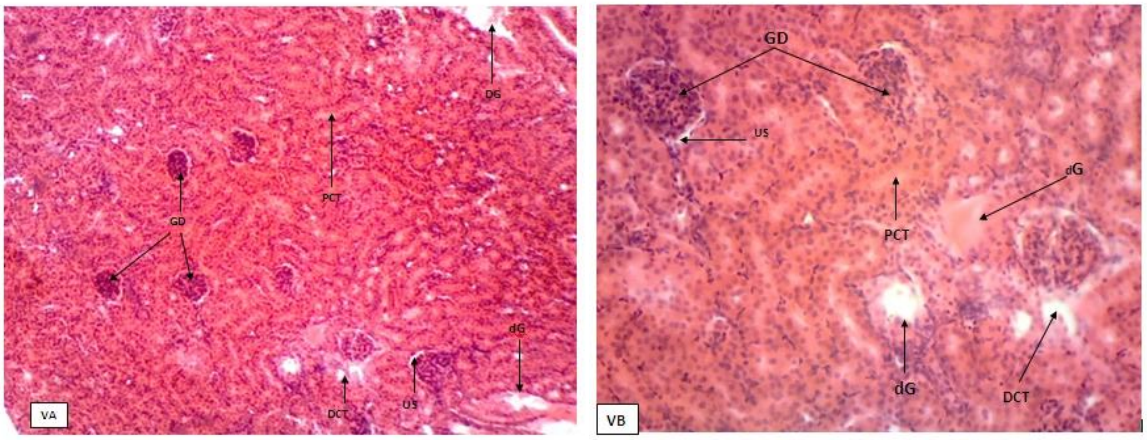
PlatesIIA & IIB:H&E photomicrograph of kidney (day 8) of male adult Wistar rat treated with 200mg/kg of the extract. The histo-architecture shows mild degenerating Glomerulus (DG); prominent distal convoluted tubules (DCT) and proximal convoluted tubules (PCT) at x100 (IIA). Prominent/enlarged nucleus, loss prominence of the urinary space/Bowman's capsule, blood vessel (BV) at X100 (IIB). Mag. IIA (x40) and IIB (x100)



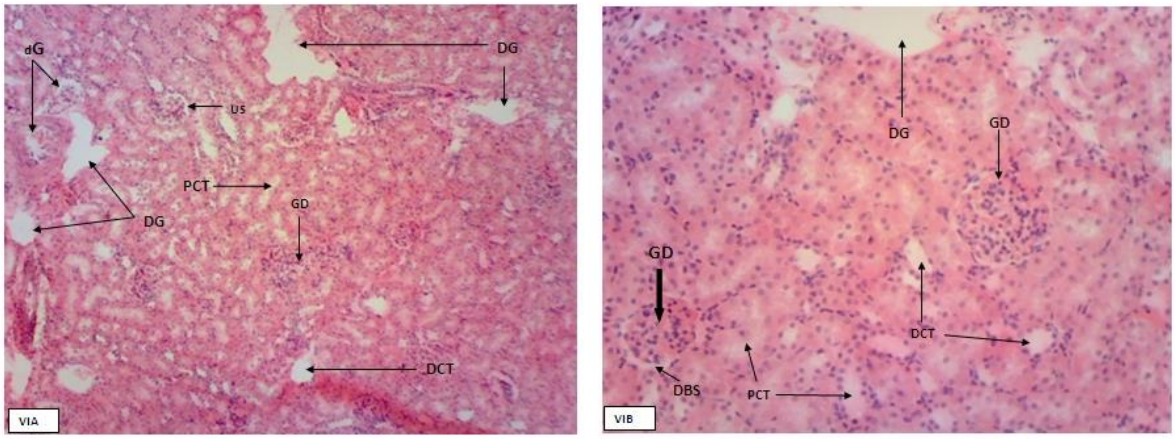
Plates IIIA & IIIB:H&E photomicrograph of the kidney (day 8) of male adult Wistarrat treated with 400 mg/kg of*Moringa oleifera* leave extract. The histo-architecture shows mild degenerating and distortion of Glomerulus(DG and GD); prominent distal convoluted tubules (DCT) and proximal convoluted tubules (PCT). Decrease urinary space. Mag.IIIA(x40) and IIIB(x100).



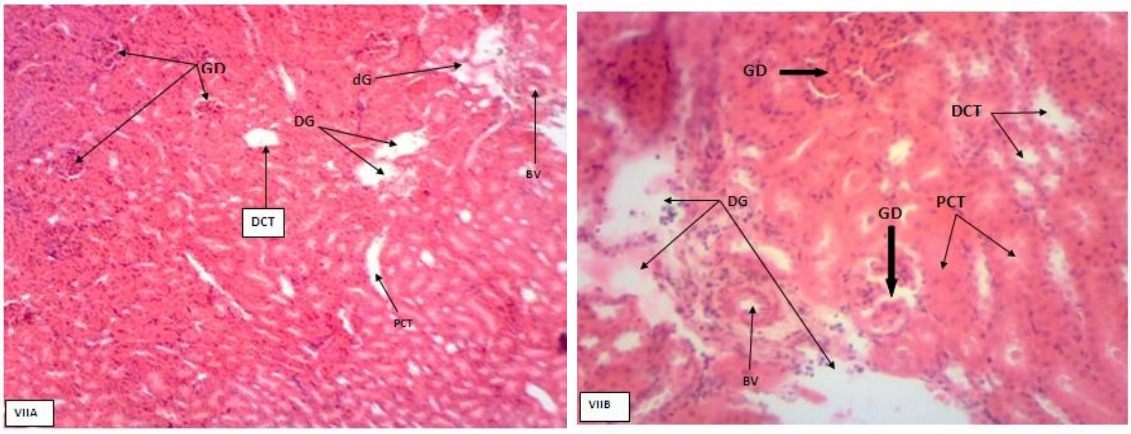
Plates IVA & IVB:H&E photomicrographs of kidney (day 8) of male adult Wistar rat treated with 600mg/kg of the *Moringa oleifera* leave extract. The histo-architecture shows further increase Glomerulus distortion and inflammation (GD);degeneration and disappearance of urinary space (US). Magnification x40 (IVA), x100 (IVB).



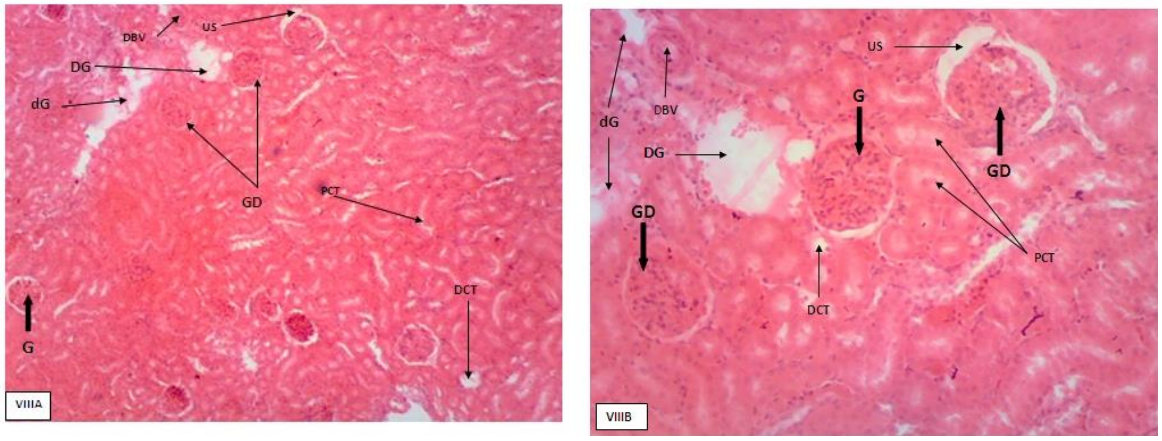
PlatesVA & VB:H&E photomicrograph of kidney (day 15) of male adult Wistar rat treated with 200 mg/kg of *Moringa oleifera* leave extract. The histo-architecture shows degenerating Glomerulus (DG); further increase Glomerulus distortion and inflammation (GD); disappearance of urinary space (US); hyperplasia of the epithelial cells and increase in PCT and DCT size. Magnification x40 (VA) and x100 (VB).



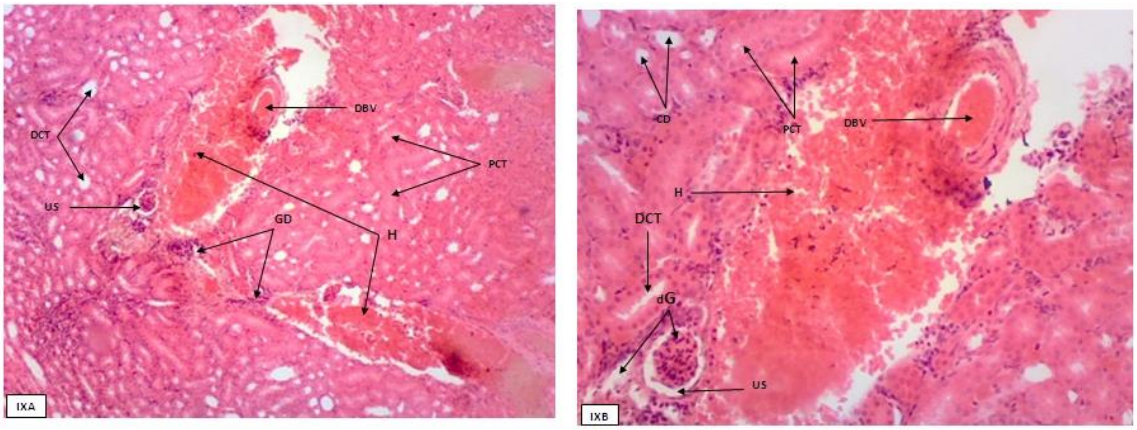
Plates VIA & VIB: H&E photomicrograph of the kidney (day 15)of male adult Wistar rat treated with 400mg/kg of the *Moringa oleifera* leave extract. The histo-architecture shows severe degenerating and degenerated Glomerulus (DG); Glomerulus distortion and inflammation (GD); disappearance of urinary space (US); hyperplasia of theepithelial cells and increase in PCT and DCT size. Magnification x40 (VIA) and x100 (VIB).



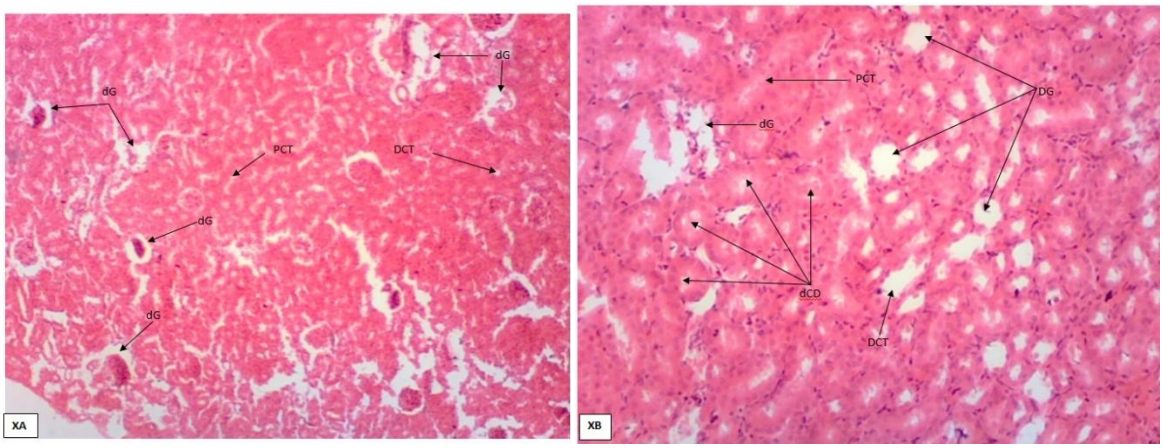
Plates VIIA & VIIB:H&E photomicrograph of the kidney (day 15) of male adult Wistar rat treated with 600mg/kg of the *Moringa oleifera* leave extract. The histo-architecture shows severe degenerating and degenerated Glomerulus (DG); Glomerulus distortion and inflammation (GD); disappearance of urinary space (US); and mild PCT and DCT inflammation; blood vessel (BV). Magnification: x40 (VIIA) and x100 (VIIB).



Plates VIIIA & VIIIB: H&E photomicrograph of the kidney (day 22) of male adult Wistar rat treated with 200 mg/kg of the extract. The histo-architecture shows moderate degenerating Glomerulus (dG); degenerated Glomerulus (DG); mild distal convoluted tubules (DCT) and proximal convoluted tubules (PCT) inflammation; vascular hypertrophy at X100 (VIIIB).Hypoplasia and degeneration of tubular epithelial cells. Mag.VIIIA (x40) and VIIIB(x100).



Plates IXA & IXB:H&E photomicrographs of the kidney (day 15) of male adult Wistar rat treated with 400 mg/kg of the *Moringa oleifera* leave extract. The histo-architecture shows severe degeneration of Glomerulus (DG); Glomerulus distortion and (GD); severe distortion/occlusion of vascular lumen (DVB), hypertrophy and hemorrhage at both magnification; degeneration PCT and DCT. Magnification x40 (IXA) and x100 (IXB).



Plates XA & XB:H&E photomicrograph of the kidney (day 15) of male adult Wistar rat treated with 400 mg/kg of the *Moringa oleifera* leave extract. The histo-architecture shows severe degenerating (dG) and degenerated Glomerulus (DG); distortion of collecting duct (dCD); and increase in PCT and DCT lumen. Magnification x40 (XA) and x100 (XB).

DISCUSSION

Kidney is the organ responsible for urine formation, removal of waste product such as urea, creatinine, uric acid bilirubin and product of metabolism and serve as the principal organ for homeostasis. Maintenance of water balance, electrolytes (Na^+, $K^+$and Cl) and acid-base balance are kidney functions (John, 2006; Webster, 2017). The study conducted on the effect of aqueous extract of *Moringaoleifera* leave showed glomeruli degeneration, distortion and disruption ranging from mild, moderate and severe as well as complete degeneration of glomerulus based on doses and time of administration. Glomerulus inflammation known as glomerulo-nephritis and congestion of blood vessel also became evident. This showed that prolonged administration of aqueous leave extract of *Moringa oleifera* leave on albino Wistar rat alters ultra-filtration and removal of waste product. Furthermore, serum level of electrolytes concentration in treated groups when taken may increase.

The histopathological examination of photomicrographs of the renal cortices in adult Wistar rats treated with graded doses of *Moringa oleifera* leave extract resulted in histological changes in the kidney including cell reduction, inflammation, degeneration and distortion of glomeruli tufts, hemorrhage and occlusion of blood vessel. The findings are supported by Oyagbemi *et al.*(2013) who suggested that chronic use could predispose animals to hepatic and kidney damage. However, studies by Paliwal *et al.,* (2011) reported the anti-nephrotoxic effect of *Moringa oleifera lam*; Ezejindu *et al.,* (2014) reported that *Moringa oleifera* leaf extract would not produce any deleterious effects on the kidney of experimental animals even in cases of chronic administration; Awodele *et al.*(2012) reported *Moringa* leaf consumption to be relatively safe at sub-lethal doses especially with respect to its effects on the kidney and liver tissues and Owolabi *et al.*(2014)reported that at moderate doses, *M. oleifera* leaf extract ingestion is safe for the renal tissues. In one study, animals (rats, mice, and rabbits) were given a higher than the recommended daily dose of *M. oleifera* leaf powder for adults, which was suggested to be around 1,600mg daily. This amount was found to be equivalent to a child consuming 375 grams of *M. oleifera* leaf daily, and no adverse side effects were seen on any of the study animals (Boven and Morohashi, 2002; Stohs and Hartman, 2015). In another study that used mice, the dosage level of 30mg/kg per day was found to elicit a beneficial effect while no toxicities were seen at this dosage (Faizi *et al*., 1998). Another study using rats, determined that there were no dangerous toxicity levels associated with *M. oleifera* at or below 1,000 mg/kg, while supertoxicity levels were seen above 3,000 mg/kg (Asare *et al*., 2012).

In another study by Ganatra *et al.* (2012) concluded that toxicity study of aqueous extracts of *Moringa oleifera* leave was 15.9g/kg body weight while that of ethanolic extract was 17.8g/kg. These were supported by the work done by Adedapo *et al*. (2009), that showed the high safety efficacy of *Moringa oleifera* leave.

Finally, this finding shows that even at moderate doses, aqueous extract of *Moringa oleifera lam* leave has effect on the renal tissues of experimental groups of Wistar rats, while at high doses the extract has deleterious effect on the renal tissues and subsequently kidney damage which finally leads to renal failure of the experimental animals.

CONCLUSION

The study showed that aqueous extract of *Moringa oleifera lam* leaves was found to distort the histo-architecture of male adult Wistar rat's kidney. These effects were found to be time-dependent and dose-dependent respectively.

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