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Original Article

Histopathological profiles of Albino rats induced with Viscum album leaf and stem aqueous extracts

Wasagwa John[1], Lawan B. Buratai[1], Abdullahi A. Biu[2], Samaila J. Badau[3], ThankGod E. Onyiche[2, 4]

[1]Department of Biochemistry, University of Maiduguri, P. M. B. 1069, Nigeria. [2]Department of Veterinary Parasitology and Entomology, University of Maiduguri, P. M. B. 1069, Nigeria. [3]Department of Veterinary Pathology, University of Maiduguri, P. M. B. 1069, Nigeria. [4]Department of Biological and Environmental Sciences, Faculty of Natural Sciences, Walter Sisulu University PBX1, Mthatha, South Africa.

Correspondences to: ThankGod E. Onyiche, Department of Biological and Environmental Sciences, Faculty of Natural Sciences, Walter Sisulu University PBX1, Mthatha, South Africa.

Email: et.onyiche@unimaid.edu.ng or tonyiche@wsu.ac.za

Abstract

Background: *Viscum album* is a hemiparasitic shrub that grows on various tree species and contains diverse active substances. **Objectives**: The histopathology of the toxic effects of *Viscum album* leaf and stem aqueous extracts from host plants *Azadirachta indica* (neem), *Psidium guajava* (guava), and *Acacia albida* on organs of albino rats was evaluated. **Methodology**: Thirty-five (35) albino rats divided into 7 groups (A-G) of 5 albino rats each was exposed to graded doses of 100, 200, 400, 800, 1600, 3200, and 6400mg/kg using the intraperitoneal route. The experimental rats were observed for 24 hours and subsequently sacrificed and their organs including liver, kidney, lungs, and heart were subjected to histopathology for any ultra-structural changes. **Results**: The histopathological findings indicate that the liver had vacuolar degeneration of hepatocytes and congestion of sinusoids for 800mg/kg of *Viscum album* (*Azadirachta indica*: neem) stem extract, and widespread vacuolar degeneration of hepatocytes at 3200mg/kg for *Viscum album* (*Acacia albida*) stem extract. The kidneys had congestion, tubular necrosis with deposition of hyaline materials in the intratubular lumen and capsular space of the glomerulus, and glomerular atrophy at 400mg/kg of *Viscum album* (*Psidium guajava*: guava) leaf extract, but indicated marked congestion, and tubular necrosis with 3200mg/kg of *Viscum album* (*Psidium guajava*: guava) stem. The lungs had marked thickening of the interstitium by edema fluid, red blood, and mononuclear cell infiltration at 3200mg/kg of *Viscum album* (*Acacia albida*) leaf extract. The heart had multifocal areas of mild necrosis of the myocytes with 3200mg/kg *Viscum album* (*Azadirachta indica*: neem) leaf extract, and multifocal areas of moderate haemorrhages with 3200mg/kg *Viscum album* (*Psidium guajava*: guava) leaf extract. **Conclusion**: Aqueous extracts of *Viscum album* in this study had bioactive components that could be toxic.

Keywords: *Acute toxicity, Albino rats, Histopathology, Viscum album.*

Introduction

Despite the use of phytotherapeutic preparations in treating health-related problems of both man and animals, acute and chronic phytotoxic effects of some plants have been reported.[1-3] The World Health Organization has strongly advised the conduct of scientific investigations into folkloric herbal remedies and included acute and chronic toxicological studies as safety assessment protocols for herbal products.[4]

Viscum album known as European mistletoe or common mistletoe is a plant that grows on a host tree

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and has a worldwide distribution. Mistletoe preparations are among the most widely used unconventional therapies[5-6] and European herbalists considered mistletoe to be a specific remedy for epilepsy and other convulsive nervous disorders.[7-8] Mistletoe has been used in Nigerian folk medicine for many generations.[9] However, *Viscum album* extracts contain mistletoe lectins, which are cytotoxic glycoproteins also known as viscumin or agglutinin, that damage cell membranes through cellular agglutination.[10-11] It is therefore worthwhile to scientifically investigate the plant for its toxicity on some essential organs.

Materials and Method

Viscum album Collection and Authentication

Fresh leaves and stems of *Viscum album* from three different host plants viz: *Azadirachta indica* (Neem), *Psidium guajava* (guava), and *Acacia albida* were collected from within the University of Maiduguri Campus and authenticated by a botanist from the Department of Biological Sciences, University of Maiduguri, Nigeria.

Preparation of Extracts

The leaf and stem of *Viscum album* from host plants *Azadirachta indica* (Neem), *Psidium guajava* (guava), and *Acacia albida* were rinsed with clean water and dried under shade (to avoid solar leaching) for one week. The dried leaves and stems were then ground, using mortar and pestle to obtain a 700g fine powder of each sample which was extracted with 1000mls of distilled water for 8 hours at 60^0C using a Soxhlet extractor (Quickfit, England^R). For the water extraction method, one liter of water was heated in a 2000mls round bottom flask fitted with a Soxhlet extractor on a heating mantle. The 700g of the fine powder was put in a thimble (a cloth jacket) which was then pushed into the Soxhlet extractor which was fitted to a condenser connected to an inlet for tap water. When the flask had been heated by the mantle, the water vaporizes passing through the Soxhlet extractor, where it was condensed and dropped back into the flask as water through the thimble containing the *Viscum album* samples. As the *Viscum album* materials got soaked in the thimble, its active component which had dissolved in the water then dropped into the flask. This procedure was continued until the thimble became very clear. The concentration of the *Viscum album* extract was done in an aluminium tray maintained overnight at 60^0C in an oven. The drying process removed the water, leaving only the extracts of *Viscum album* leaf and stem from *Azadirachta indica*, *Psidium guajava,* and A*cacia albida*. All the obtained extracts were stored at room temperature (27^0C) until required.

Acute Toxicity Studies

Details of the study design have been published previously.[21] Briefly, eight (8) groups (A-H) of 5 adult albino rats each from both sexes weighing between 110 and 220 grams were used for the acute toxicity studies. Following the intraperitoneal administration of 100, 200, 400, 800, 1600, 3200, and 6400mg/kg as graded extract doses to groups B-H with A as normal control all rats were observed for 24 hours for clinical signs of toxicity and death. Experimental albino rats were handled according to the International Guiding Principles for Biomedical Research Involving Animal Use and Care.[12] The experimental albino rats were kept within ambient conditions (temperature: 27 ± 1^0C., photoperiod: 12 hours natural light and 12 hours dark, humidity 40±5%). They were fed with standard feed (Grant Cereal Ltd, UAC Nigeria Plc, Jos, Nigeria) and portable water was provided *ad libitum.*

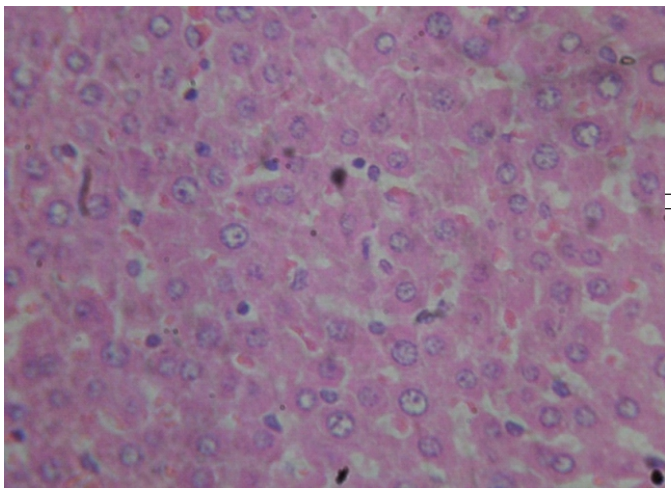
Histopathological Studies

Samples of the liver, kidney, heart, and lungs from dead albino rats were examined for histopathology. Sections of each organ were taken and fixed in 10% buffered formalin, embedded in paraffin wax, sectioned at 3-4µm thickness, and stained with haematoxylin and eosin (H&E) stain. Tissue slides were examined using an Olympus light microscope and photomicrographs were taken using Canon Power Shot A470 digital camera (Model PC1267® Canon Inc. New York, USA).

Results

Details of the acute toxicity findings and the LD_{50} have been published previously.[21] Briefly, the calculated median lethal dose (LD_{50}) for the leaf and stem of *Viscum album* from *A. indica* was 1440mg/kg/bw and 600mg/kg/bw respectively. Also, the calculated median lethal dose (LD50) value of *Viscum album* leaf and stem from *P. guajava* was 2400mg/kg. Finally, the LD50 values of *Viscum album* leaf and stem from *A. albida,* were computed to be 2400mg/kg/bw and 2880mg/kg/bw respectively. The histopathological lesions of

toxicity are shown in Plates 1-7. The Plates lying side by side are for normal organs. Plate 1 is the photomicrograph of a liver of an albino rat showing lesions suggestive of vacuolar degeneration of hepatocytes and congestion of sinusoids following the administration of 800mg/kg of *Viscum album* (*Azadirachta indica*: neem) stem extract (H&E, x400). Also, plate 2 shows lesions of widespread vacuolar degeneration of hepatocytes (arrows) following administration of 3200mg/kg *Viscum album* (*Acacia albida*) stem extract (H&E, x400). Plate 3 shows **a** photomicrograph of the kidney harvested from an albino rat showing congestion (short arrow), tubular necrosis with deposition of hyaline materials in the intratubular lumen and capsular space of the glomerulus (long arrows), and collapsing and segmentation of the glomerulus (arrowhead) following the administration of 400mg/kg of *Viscum album* (*Psidium guajava*: guava) leaf extract (H&E, x400). Furthermore, plate 4 shows the photomicrograph of a kidney of an albino rat with marked congestion (short arrow) and tubular necrosis (long arrow) following administration of 3200mg/kg of *Viscum album* (*Psidium guajava*: guava) stem (H&E, x400). On plate 5, lesions on the lungs from albino rats indicate marked thickening of the interstitium by edema fluid (short arrow), red blood cell (long arrow) due to haemorrhage and mononuclear cells (arrowhead) following the administration of 3200mg/kg of *Viscum album* (*Acacia albida*) leaf extract (H&E, x400). Plate 6 shows a photomicrograph of a heart of an albino rat with multifocal areas of mild necrosis of the myocytes (arrows) following treatment with 3200mg/kg *Viscum album* (*Azadirachta indica*: neem) leaf extract (H&E, x400). Lastly, plate 7 shows a photomicrograph of a heart of an albino rat with multifocal areas of moderate hemorrhages (arrows) following treatment with 3200mg/kg *Viscum album* (*Psidium guajava*: guava) leaf extract (H&E, x400).



Control Liver

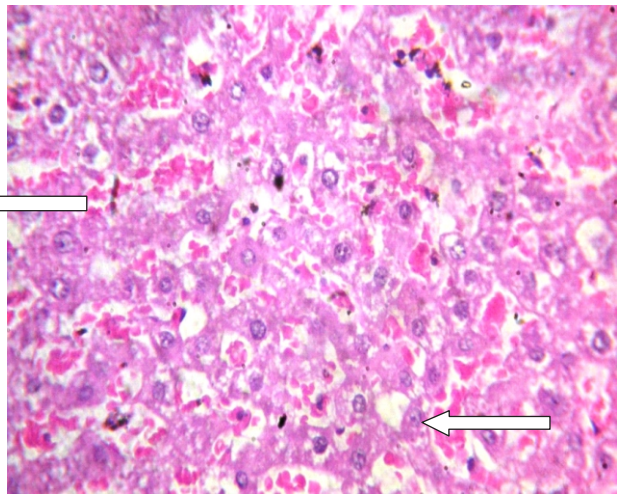


PLATE 1**:** Photomicrograph of a liver of a rat showing mild vacuolar degeneration of hepatocytes (short arrows) and congestion of sinusoids (long arrows) following the administration of 800mg/kg *Viscum album* (*Azadirachta indica*: neem) stem extract H&E, x400.

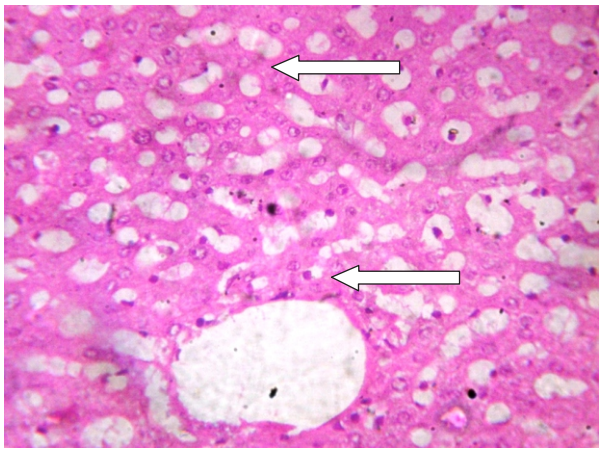
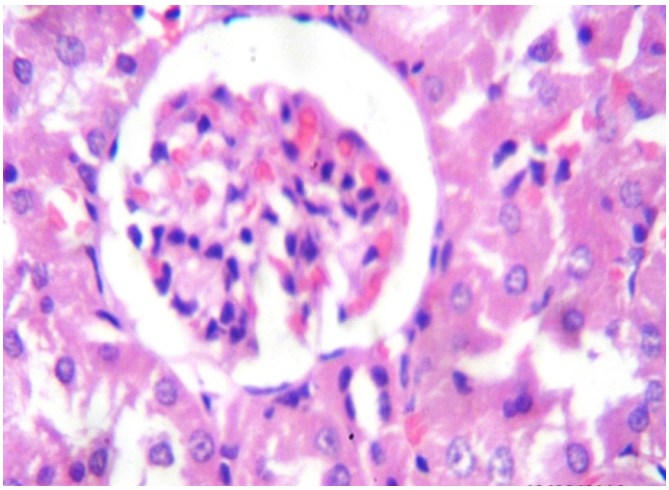


PLATE 2: Photomicrograph of a liver of a rat showing widespread vacuolar degeneration of hepatocytes (arrows) following the administration of 3200mg/kg *Viscum album* (*Acacia albida*) stem extract H&E, x400



Control Kidney

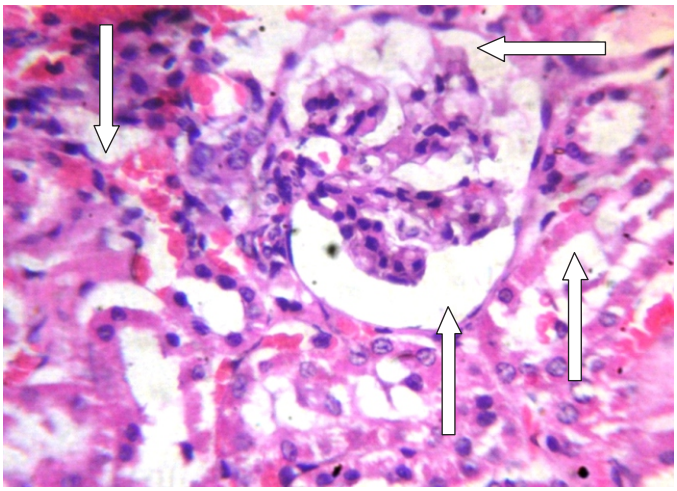


PLATE 3: Photomicrograph of a kidney of a rat showing congestion (short arrow), tubular necrosis with deposition of hyaline materials in the intratubular lumen and capsular space of the glomerulus (long arrows), and collapsing and segmentation of the glomerulus (arrowhead) following the administration of 400mg/kg of *Viscum album* (*Psidium guajava*: guava) leaf extract H&E x400.

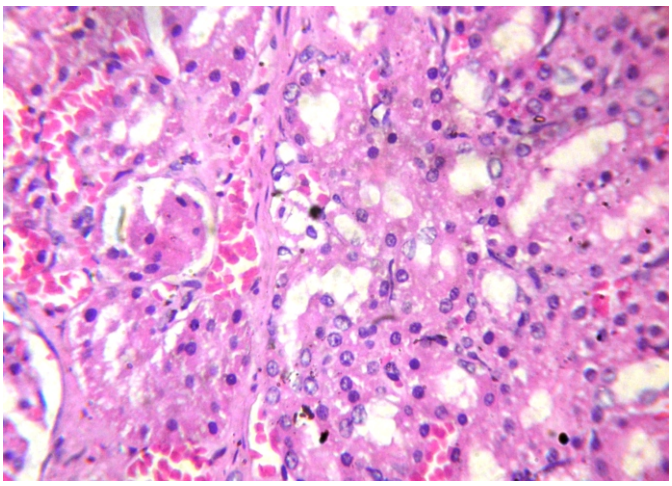
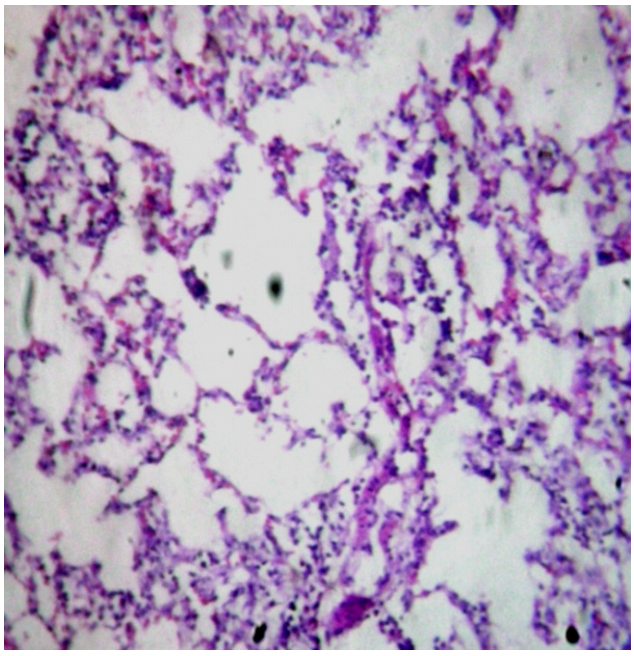


PLATE 4**:** Photomicrograph of a kidney of a rat showing marked congestion (short arrow) and tubular necrosis (long arrow) following administration of 3200mg/kg of *Viscum album* (*Psidium guajava*: guava) stem H&E, x400.



Control Lungs

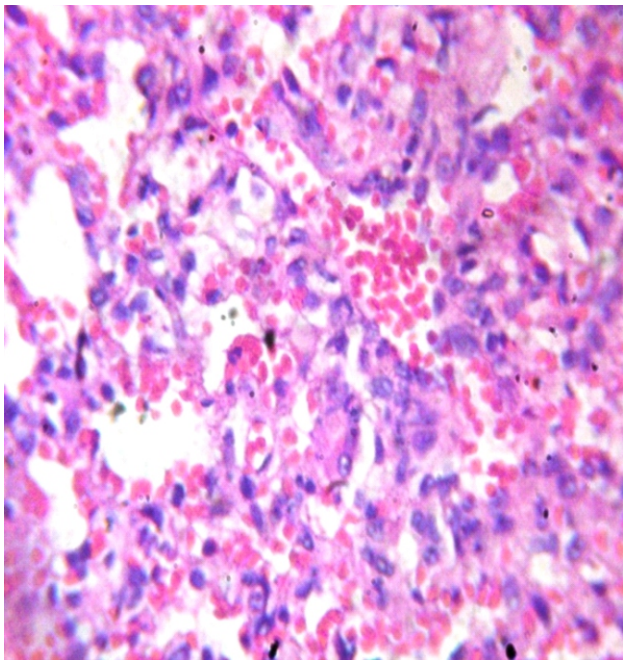
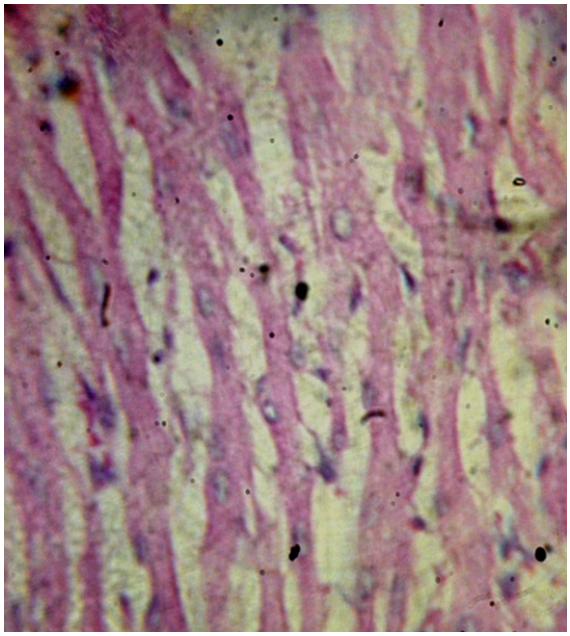


PLATE 5**:** Photomicrograph of a lung of a rat showing marked thickening of interstitium by edema fluid (short arrow), red blood cell (long arrow) due to haemorrhage and mononuclear cells (arrowhead) following the administration of 3200mg/kg of *Viscum album* (*Acacia albida*) leaf extract H&E, x400.



Control Heart

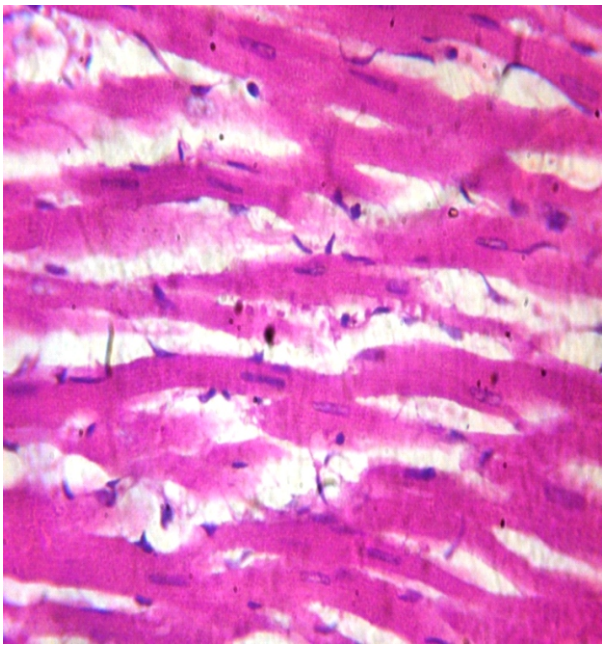


PLATE 6: Photomicrograph of a heart of a rat showing multifocal areas of mild necrosis of the myocytes (arrows) following treatment with 3200mg/kg *Viscum album* (*Azadirachta indica*: neem) leaf extract H&E, x400.

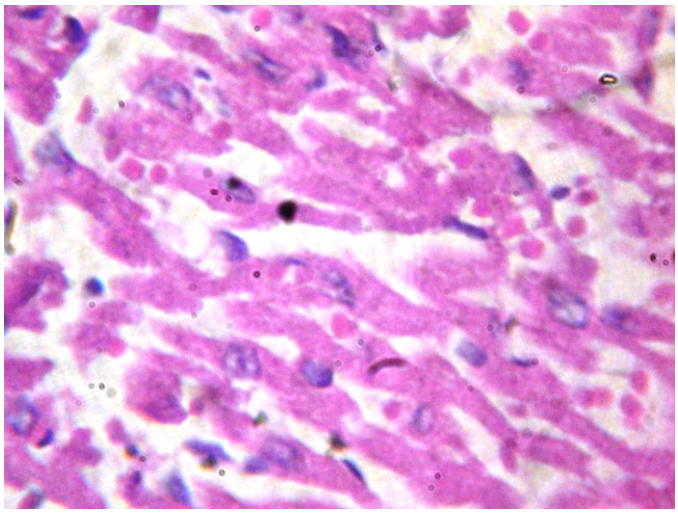


PLATE 7: Photomicrograph of a heart of a rat showing multifocal areas of moderate haemorrhages (arrows) following treatment with 3200mg/kg *Viscum album* (*Psidium guajava*: guava) leaf extract H&E, x400.

Discussion

The use of herbal medicine is gaining popularity and is widely perceived by the public as being healthy, natural, and free from side effects.[13] Many believe that these herbal medicines have no side effects or potential risks because of their natural origins. Medicinal herbs in many countries are not under strict control and are mostly self-prescribed by consumers with regard to dose and frequency of administration. One important point that has been disregarded by the users of herbal plants is that the phytochemicals present in plants may be natural to them but are not natural to the human body.

The liver of albino rats in this study had sinusoidal congestion, widespread vacuolar degeneration, and necrosis of hepatocytes. These changes have been documented in albino rats exposed to high doses of some herbal plants.[22-23] In the lungs, there was marked thickening of the interstitium by edema fluid, and red blood cells due to haemorrhage and mononuclear cell infiltration. The kidneys had marked congestion, intratubular hyaline deposition, coagulative tubular necrosis, and glomerular atrophy while the heart was hyperemic with myocardial vacuolation and multifocal necrosis. All

these lesions have been reported by several researchers [14-17] and they observed that these lesions are not specific as many plant toxins are known to produce them. In similar toxicological investigations Nigatu *et al*., [18] reported that liver sections of mice treated with 800 mg/kg body weight of aqueous root extract of *Gnidia stenophylla* showed some intrahepatic and extrahepatic bile retention indicating induction of cholestasis (biliary stasis). Inflammatory cellular infiltrations were also seen around the central vein and portal tract indicating mild hepatitis.

Mbaya *et al.,* [19] and Biu *et al.,* [20] reported that degenerative changes in the liver may probably arise from biotransformation of the active/toxic principles of plant extracts, while those observed in the kidneys may be associated with toxicity-related-excretory processes in albino rats. *Viscum album* extracts are reported to contain mistletoe lectins, which are cytotoxic glycoproteins also known as viscumin or agglutinin, that damage cell membranes through cellular agglutination.[10-11]

Conclusion

This study has demonstrated that *Viscum album* from three different host plants induces toxic effects on some organs such as the liver, kidney, lungs, and heart. Some of the changes observed include vacuolar degeneration of hepatocytes, congestion, and tubular necrosis of the kidney with deposition of hyaline material in the intra-tubular lumen. The lungs showed marked thickening of the interstitium by edema fluids with infiltration of mononuclear cells. Lastly, there was multifocal area of mild necrosis of the myocytes.

Conflict of Interest

The authors declared no conflicts of interest.

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