

Effects of *Moringa oleifera* Biochemical Constituents on Kidney, Liver and Brain of Wister Rats

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Abstract: Medicinal plant have evolved over the centuries as essential parts of African civilization and are widely recognized today as representing its rich cultural and scientific heritage. The increasing demand for Medicinal plant products has renewed interest in the pharmaceutical industry in the production of herbal health care formulations, herbal-based cosmetic products, and herbal nutritional supplements. Thus, in addition to serving medical and cultural functions, Medicinal plants in Africa have economic importance. Global and national markets have been growing for medicinal herbs, and significant economic gains are being realized through the sale of medicinal plant products. The aim of this particular investigation was to observe the effects of *Moringa oleifera* 80% methanol leaf extract on the histological architecture of kidney, liver and brain tissues. Fifteen (15) rats were randomly divided into three (3) with five rats per group. Rats were exposed to 2000 mg/kg, 1000 mg/kg and 500 mg/kg of *Moringa oleifera* extract per os (p.o) along with the control group that was placed on commercial diet. There was no observed mortality in all experimental rats but there is deleterious effect in brain, liver and kidney in those that were exposed to higher doses especially the 2000 mg/kg. It's therefore concluded that higher dose of *Moringa oleifera* is toxic while moderate doses is safe to most vital organs especially brain, liver and kidney. There is need for further investigation to identify the phytochemical constituents that are responsible for the toxic effects.

Keywords: Brain, Extract, Kidney, Liver, *Moringa oleifera*, Phytochemical Constituents, Rats

1. Introduction

There are several publications, reporting the various benefits of *Moringa oleifera* leaf and other parts of the plant. Few publications have reported some of the specific effects of *Moringa oleifera* leaf extract on some body tissues or organs such as cerebro protective effect of *Moringa oleifera* against focal ischemic stroke [1]. Mumtaz et al., reported that *Moringa* leaf extract protect neuronal cells in animal models

with age-related dementia as observed in the hippocampus [2]. It was also reported that Lyophilized hydro-alcoholic extract of *M. oleifera* showed myocardial infarction preservative effect in isoproterenol (ISP)-induced rat model [3]. The report of Ouédraogo (2013) in their efforts to evaluate the protective effect of *Moringa oleifera* leaf extract against gentamicin-induced nephro-toxicity in rabbits shows reparative effect on affected kidneys. *Moringa oleifera* ethanolic leaf extract was reported to have hepato-protective

abilities in diclofenac induced toxicity [4]; acetaminophen induced toxicity [5], antitubercular drug [6] and carbon tetrachloride induced toxicity [7, 8]. Gbankoto et al., reported sub-lethal LD₅₀ effect of *Moringa* leaf aqueous extract on sperm, haematological and biochemical parameters as well as histo-pathological finding following oral [9]. Hence, toxicological research on biochemical constituents would do well to first establish the safety dose of the plant products. This will also help in the establishment of the use of the plant's products as standard nutritional supplements and natural or bio-medicinal products. Histo-pathological finding would provide more reliable and consistent picture of the effects produced by the interactions of the phytochemicals constituents with the body cells and tissue better than in vitro tests [10]. It will also confirmed toxic effect than analysis of biochemical activities as contained in extracted tissue fluids. Also, the use of histopathological methods of assessment of crude extract effects on body tissues is important because literatures are comparatively scarce on such methods of investigation. The aim of this research is to evaluate the histopathological effects of *Moringa oleifera* 80% methanol extract in some vital organs (kidney, liver and brain) of wister rats.

2. Material and Method

2.1. Plants Collection and Identification

Moringa oleifera leaf was purchase from Sokoto market on 15 May 2019. The leaf was identified and voucher number was assign by botanist at Faculty of Biological science Usmanu Danfodiyo University sokoto.

2.2. Plant Extraction

Plant leaf was separated from the stem, cleaned and cut into small pieces with anvil pruner, (UK). The leaf was allowed to air dried for two weeks at room temperature (26±1°C) in lab and crushed to smaller sizes. Two hundred (200) g of sample (leaf) was soaked for 3 days in 1000 mL 80% methanol in flat bottom flasks (Sigma Aldrich, USA). Crude mixture was shaken every day at 26°C to ensure adequate dilution and extraction. The process was repeated three times to extract most of the bioactive compound present in the leaf. The extract was then filtered with Whatman filter paper (1.5 Sigma Aldrich, USA) and then concentrated to semi-solid form at 42°C with a rotary evaporator (IKA® RV 10, USA). Percentage yield was calculated as the weight of the filtrate divided by the total weight of the ground powder in percentage. Yield (%)=[wt of extract (g)/wt of plant material (g)] x 100.

The resultant crude extract obtained was then weighed and transferred into sample bottles and stored at 4°C until required.

2.3. Plants Sample Dilution and Dose Preparation

Stock solution was prepared by dissolving 100 mg of the crude extract into 1 mL of 100% DMSO (100 mg/mL).

DMSO was use to solubilized the crude extract in aqua solvent. Preparation of sub-stocks in microliter (µg/mL) was carried out by diluting the stock solution with distilled water to the concentration of interest using two-fold serial dilution at eight concentrations (7.81-1000) µg/mL in 96-well microFigure (Sigma Aldrich, USA). DMSO (vehicle) was maintained at 0.1% in all concentration of extract.

2.4. Animals

Healthy adult male and female, aged 2-3 month, weighing 128-233g, were purchase from animals house at Faculty of pharmaceutical science Usmanu Danfodiyo University Sokoto. The animals were acclimatized for 2 weeks at Biochemistry lab Faculty of Veterinary Medicine (26 ± 2°C; 12: 12 hour dark/light cycle), fed with commercial feed ad libitum.

2.5. Study Design

Group-I –Five rats were given 2000 mg/kg of *Moringa oleifera* leaf extract orally.

Group-II –Five rats were given 1000 mg/kg of *Moringa oleifera* leaf extract orally.

Group-III –Five rats were given 500 mg/kg of *Moringa oleifera* leaf extract orally.

Group-IV –Five normal control rats were given commercial feed and distilled water.

Animals were sacrificed with aid of chloroform in aqueous solution. Each rat was placed on dorsal recumbence, midline skin incision was made using scalpel blade to open abdominal cavity. Liver and kidneys were removed and fixed in formol-saline. The skull was dissected and brain was gentle removed and fixed in formal-saline. The tissues were submitted to histopathology laboratory for tissue processing. Hematoxylin and eosin (H and E) and X 40 Magnification were used for analysis.

3. Result

Result of Group-I (2000 mg/kg) shows focal vacuolation, cellular infiltration lymphoid nodular formation in the molecular layer and cellular infiltration of the Brain (Figure 1). There is congestion of central vein and sinusoid with perivascular cuffing, hepatic nuclear vacuolation and hemorrhages with cellular infiltration in liver (Figure 2). Diffuse hemorrhages in the tubular structures, focal hyaline degeneration in both cortex and medulla were observed in Kidney (Figure 3).

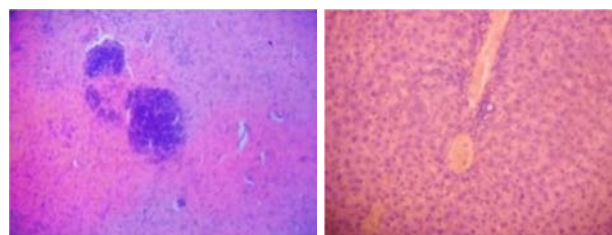


Figure 1. Brain (2000 mg/kg).

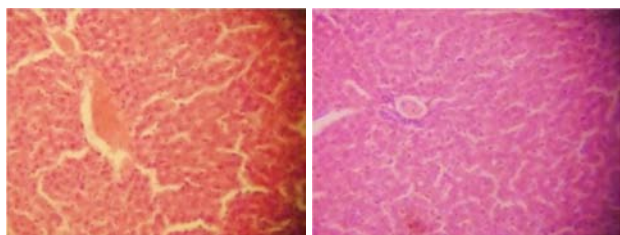


Figure 2. Liver (2000 mg/kg).

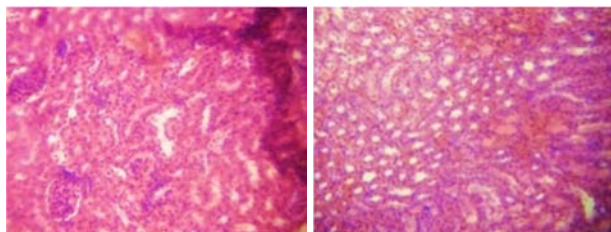


Figure 3. Kidney (2000 mg/kg).

Result of Group-2 (1000 mg/kg) shows vacuolation in the molecular layer with cellular infiltration of the Brain (Figure 4). There is congestion with perivascular cuffing in liver (Figure 5). Focal hyaline degeneration in the cortex and medulla were observed in Kidney (Figure 6).

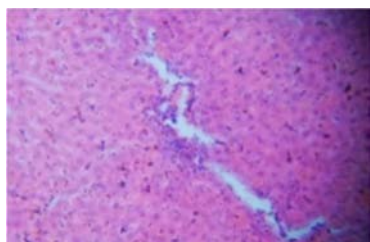


Figure 4. Brain (1000 mg/kg).

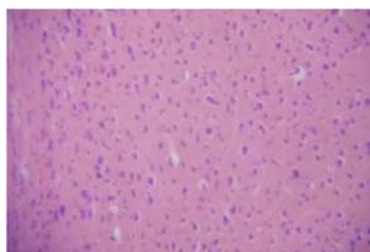


Figure 5. Liver (1000 mg/kg).

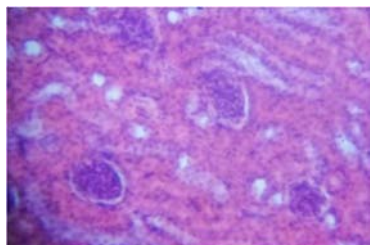


Figure 6. Kidney 1000 (mg/kg).

Result of Group-3 (500 mg/kg) shows low cellular infiltration and vacuolation in the molecular layer, mild congestion and hyaline degeneration of the Brain (Figure 7). There is mild congestion with perivascular cuffing and

hepatic micro-vacuolation in liver (Figure 8). Cortical vacuolation in the paranchyma and hyaline degeneration in the cortex and medullar were observed in Kidney (Figure 9).

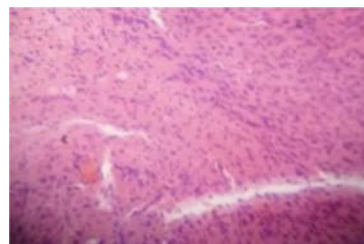


Figure 7. Brain (500 mg/kg).

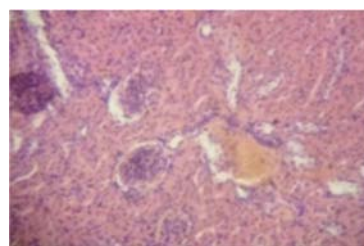


Figure 8. Liver (500 mg/kg).

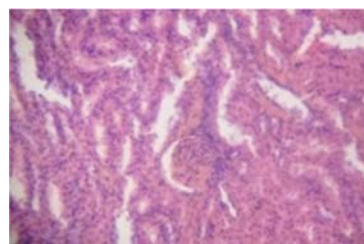


Figure 9. Kidney (500 mg/kg).

Result of Group-4 (control) shows normal appearance of brain tissues with no pathological changes (Figure 10). There is no observed change in architecture of the liver tissue as well as hepatic cells (Figure 11). Mild focal hyaline deposition in the kidney was observed in Kidney (Figure 12).

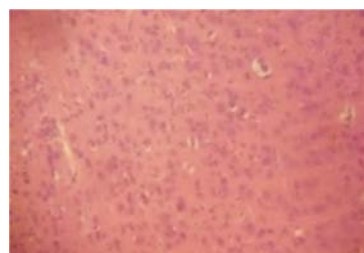


Figure 10. Brain (control).

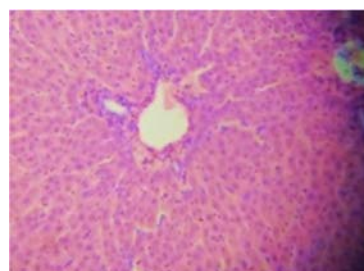


Figure 11. Liver (control).

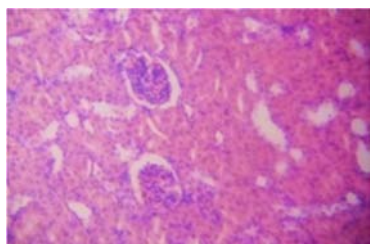


Figure 12. Kidney (control).

4. Discussion

Medicinal plants are still a reliable 18% rats that were exposed to Organohalogen Contaminants [11]. Hepatocytic microvesicular lipid accumulation (foamy cytoplasm), sharply demarcated macrovesicular lipid vacuoles in mainly periportal (zones 2–3) hepatocytes was also reported [12], source of active molecules (polyphenols, flavonoids) known for their antioxidant properties [13]. It is however important to note that there is need for more specific reports, especially considering the scope of the research activities leading to the present results. While the plant shows huge potentials to alleviate hunger and provide herbal and plant-derived medicinal products, especially for the developing nations; it is important to establish the primary effects of the leaf phytochemical activities and interactions with the body tissues and organs [14]. Cytoplasmic vacuolation is characterized by the present of clear vacuoles in both brain and hepatic tissues. This is due to adverse effect of the plant extract on the tissues. Vacuoles can be seen in histological sections of brain and other neural tissue for a number of reasons. Although they may be seen in neuronal bodies and glial cells, most vacuoles typically occur in the white matter of the brain and myelinated peripheral nerves, mostly as a result of alterations to myelin cause by toxic effect of compounds or drugs [15]. This agree with the finding reported by [16] which shows increase focal vacuolation with increase doses of *Punica granatum* peels powder. In addition to vacuolation in liver, mononuclear cell infiltrations (lymphocytes, macrophages, and neutrophils).

Congestion of central vein and sinusoid with perivascular cuffing, hepatic nuclear vacuolation and hemorrhages with cellular infiltration in liver may also be due to the effects of the crude extract on hepatic cells. Several studies reported the pathogenesis of liver congestion to be due to metabolic alteration caused by drugs or toxicant. The pathogenesis of most chemical-induced liver injuries is initiated by the metabolic conversion of chemicals into reactive intermediate species, such as electrophilic compounds or free radicals, which can potentially alter the structure and function of cellular macromolecules [17]. Many reactive intermediate species can produce oxidative stress, which can be equally detrimental to the cells. When protective defenses are overwhelmed by excess toxicant insult, the effects of reactive intermediate species lead to deregulation of cell signaling pathways and dysfunction of biomolecules, leading to failure of target organelles and eventual cell death [18]. This is

similar to the finding reported by [19], whose discovered biotransformation of certain xenobiotics to be among the causes of short-lived, unstable, highly reactive chemical species that can interact with functional biomolecules and potentially lead to adverse effects. The functional groups or structural motifs in xenobiotics that can produce harmful reactive intermediates have been defined as ‘toxicophores’ [20].

Diffuse hemorrhages in the tubular structures, focal hyaline degeneration in both cortex and medulla observed in the kidney may also result from the toxic effect of crude extract component. Most of the compounds administered in the crude extract were excreted through the kidney. Hence, high dose or concentration of the compound may trigger degenerative changes or abnormal function of the urinary system. This is the same with the discovery which observed high Focal osseous metaplasia in the kidney, inclusion bodies, eosinophilic intranuclear and cytoplasmic inclusion in a rat exposed to ethanol plant extract [21].

5. Conclusion and Recommendation

It can be concluded that high dose of the crude extract 2000 mg/kg causes deleterious effect in the brain, liver and kidney. 1000 mg/kg causes moderate effect compared to 500 mg/kg which cause mild to no toxic effect in both the 3 organs. Hence, moderate to low doses of *M. oleifera* if administered orally may be safe and not cause cytotoxic effect in brain, liver and kidney. Phytochemical studies to identify the lead compound responsible for the toxic effect are highly recommended.

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