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Phytochemical screening of *Moringa oleifera* Lam. and histo-protective evaluation of cadmium chloride toxicity in rats.

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ABSTRACT

The aim of this experiment was to determine the phytochemical screening and histopathological property of Moringa oleifera Lam. root ethanol extract in Wistar rats. The qualitative phytochemical screening was experimented using standard protocol. The animals were randomly divided into groups seven (n=5). Cadmium chloride (3.6 mg/kg) was administered across the groups for 7 days to established bio-toxicity. There after graded doses of the treatment groups (100, 200 and 400 mg/kg) and the control groups were treated for another two (2) weeks to remediate the deposition of cadmium toxicity. The animals were sacrificed and the targeted visceral organs (heart, lungs, liver and kidney) were isolated and prepared for histopathological analysis. The phytochemical results showed the presence of alkaloids, tannins, saponnins, flavonoids, phenol, anthraquinone and carbohydrate. The histopathological study elicited from the treatment revealed an ameliorative effect from the damage organs triggered by cadmium chloride, enhance displayed cardio-protective, reno-protective, hepato-protective and brochio-protective effect when compared with untreated control. The study showed that graded doses (100, 200 and 400 mg/kg) of the extract had a significant effect in the targeted organs against cadmium chloride toxicity.

***Keywords*:** Cadmium Chloride, histopathological, *Moringa Oleifera*, phytochemistry, rats.

INTRODUCTION

Moringa oleifera Lam. is a small, fast-growing evergreen or deciduous tree, having soft and white wood with corky and gummy bark and grows up to a height of 9 m (Garima *et al.,* 2011; Khawaja *et al.,* 2010). It is one of the most studied and used species with various use categories stretching from food and medicinal uses to water purification, bio-pesticide and production of biodiesel. It is used and valorized through food fortification. For instance, in Nigeria, it is used to fortify food formulations of corn, soy and peanut and as food fortificant in amala (stiff dough), ogi (maize gruel), bread, biscuits, yoghurt, cheese and soup making (Shiriki *et al.*, 2015; Oyeyinka and Oyeyinka, 2016; Gandji *et al.,* 2018). *M. oleifera* possess antispasmodic, expectorant, diuretic and stimulant activities. Whole plant exhibited cardiac circulatory tonic and antiseptic. Pods possess anthelmintic; diabetes and antipyretic effect. The root juice is utilized as cardiac tonic, antiepileptic, nervous debility, treat hepatic and spleen enlargement, asthma, detox toxins, inflammation and as a good diuretic agent. It is also use as anti-paralytic, anti-cholesterolemic, mosquito larvicidal activity (Garima *et al.,* 2011; Okwari, 2013; Tom and Benny, 2017).

The immune system is a host defense system that comprises of biological structures and processes organisms that protects against diseases (Beck and Habitat, 1996). It protect the immune system by detecting a wide variety of agents, known as pathogens, from viruses to parasitic worms, and distinguish them from the organism's own healthy tissue. In humans, the blood-brain barrier, blood-

cerebrospinal fluid barrier, and similar fluid-brain barriers separate the peripheral immune system from the neuro-immune system which protects the brain (Thomas, 2010; O'Byrne and Dalgleish, 2001). The immune system could be affected by environmental and dietary habits and it is believed a rich diet in antioxidants and micronutrients can boost the immune system (Sudha *et al*., 2010). This study evaluates the phytochemical screening and histopathological properties of *Moringa oleifera* root ethanol extract.

MATERIALS AND METHODS

Collection and identification of plant materials

Fresh samples of *Moringa oleifera* plant was collected in the rain forest at Obe, Sapele road, Benin City, Edo State, Nigeria. The plant was collected in July, and was identified and authenticated with the code (X690) by Dr. H. Akinnibosun, in the Department of Plant Biology and Biotechnology, University of Benin, Benin City, Edo State, Nigeria. A voucher number was issued (UBX-M517).

Plant preparation

The samples of *Moringa oleifera* plant was washed and air-dried at room temperature for 3 weeks and further oven-dried at a temperature of 40^{o}C. It was then pulverized to powder using the British milling machine. 800 g of the plant was macerated using 1400 ml of absolute ethanol solvent (Mukherjee, 2002). The maceration was done for 72 hours with periodic shaking and stirring. The filtrate was then concentrated into paste using water bath to give % yield of 10.6 %. The extract was store for further use.

Phytochemical screening

Phytochemical screening was carried out to check the presence of bioactive agent in the *M. oleifera* root ethanol extract. This was carried out using the standard method described by Trease and Evans (1989). The confirmatory tests were on alkaloid, tannin, saponnin, flavonoid, phenol, anthraquinone, carbohydrate and cardiac glycoside, using the following test protocol; saponins frothing test; saponins fehling's test; test for anthraquinones; test for flavonoids; flavonoids shinoda test; phenolic compounds test for ferric chloride; test for tannins; tannin ferric chloride test; test for alkaloids Dragendorff's; Wagner's; Hager's; Mayer test; test for glycosides; glycosides fehling's solution test; glycosides ferric chloride test; terpenoids salkowski's test.

Experimental animals

Thirty five (35) *Wistar* rats of 12 weeks old, weighing (190-220) were obtained from the Department of Biochemistry, University of Benin and allowed to acclimatize in the Animal House, of the Department of Animal and Environmental Biology, University of Benin, Edo State, Nigeria, for a period of 14 days with 12 hours day and 12 hour night in a well ventilated cages. With constant feed and water *ad libidum*. The Animal House was well ventilated throughout the course of the experiment. The ethical community of Life Sciences, University of Benin certified the use of animals after evaluating the protocol, the ethical number issued was LS20383 (MacDonald *et al*., 2020).

Inducement of cadmium chloride

Cadmium Chloride ($CdCl_2$) (3.6 mg/kg) stock was prepared with the highest weight of the animals, weighed and dissolved in distilled water. The prepared $CdCl_2$ was administered across the groups for 7 days via intra-peritoneal route to induce toxicity prior to the commencement of treatment.

Experimental design

After 7 days of inducing toxicity via cadmium chloride, the treatments were administered to the various groups. Group 1 to 3 were administered with (100, 200 and 400 mg/kg *M. oleifera* extract root ethanol extract). Group 4 received only clean water and Group 5 was administered with 3.6 mg/kg Cadmium Chloride only. The treatment lasted for 14 days afterwards the rats were anesthetized in a mild chloroform to sedate the rats and was dissected to isolate the visceral organs. The kidney, liver, heart, and lung were isolated, rinse in normal saline and fixed in 10% buffered formal-saline in preparation for tissue processing.

Histopathological analysis

The isolated heart, liver, kidney and lungs of *Wistar* rats were fixed in neutral buffered formalin.

Affixed organs were utterly dehydrated with 99.9 % ethanol along with 70 % ethanol, and 96 % ethanol washed using distilled water. 4 µm sections were prepared, and stained in hematoxylin-eosin dye. Stained tissues were optical photomicroscope (Leica MC170 HD, Leica Biosystems, Germany) viewed at x 400 magnification. This involves the preparation of tissues and organs into a slide for easy interpretation.

RESULTS AND DISCUSSION

The results obtained for the phytochemical screening showed the presence of alkaloid, tannin, saponin and carbohydrate as the phytoconstituents found in *M. oleifera* root ethanol extract as shown in Table 1. Phytochemicals are secondary plants metabolites responsible for many bioactivity of plant extracts. They are known to possess antioxidant, anti-inflammatory, antibacterial, immunomodulatory and anti-sickling activities concurred with the work of Egba *et al.* (2012) and Fuglie, (2001). The phytochemicals observed in *M. oleifera* ethanolic and aqueous root extract in the present study undoubtedly, are responsible for the cardiovascular and immunomodulatory effects of the plant extracts. Flavonoids have been shown to have antibacterial, anti-inflammatory, antiallergic antiviral and antineoplastic activity. This agreed with the report of Suaib *et al.* (2012).

The amelioration of the perivascular infiltrates of chronic inflammatory cells (vasculitis) induced in the heart of experimental Wistar rats by cadmium chloride, undoubtedly, had been made possible by the presence of flavonoids present in the extract. Alkaloids in *M. oleifera* leaves have been found to possess analgesic, antimalarial, antibacterial and antihypertensive properties (Dangi *et al*., 2002; Lockett and Calvert, 2000). The present study which shows alkaloids as one of the phytochemical constituents of *M. oleifera* ethanolic root extract as seen in Table 1, and which upholds antihypertension as one of the properties of *M. oleifera* ethanolic root extract corroborates the claim by Dangi *et al.* (2002). Results obtained from an experiment conducted by Matsui *et al.* (2009) indicated tea-leaf saponnins to be the active components in Fr2-3 and that these saponnins exhibited anti-hypercholesterolemic activity by inhibiting cholesterol absorption in the intestines. The fact that saponnins is one of the phytochemicals of the ethanolic root extract of *M. oleifera* is certainly a reason for the hypotensive property of the extract in the present study (Seriki *et al.,* 2015).

The heart, liver, kidney and lungs showed the histoprotective effect of graded doses of the extract as shown in Figure 1-4. Cadmium chloride induced patchy vascular ulceration, congestion and perivascular infiltrates of chronic inflammatory cells (vasculitis) in the heart (Figure 1). This is in line with the study of Bowen (2003). Graded doses of *M. oleifera* extract protected the heart and dilated the blood vessels against toxicity, compared to the control. Cadmium chloride is capable of causing vascular injury toxicity (erosion) in the bronchial vessels of the lungs. However, there was no obvious damage to the alveoli in the control group compared to graded doses of the extract, to activate local immune system of the lungs (bronchiolo-alveolar lymphoid aggregates) to a mild degree as shown in Figure 2. The extract activated the local immune response, producing florid effect.

Cadmium chloride induced severe vascular ulceration and steatosis in the liver. The extract elicited a protective measure of the liver cells as well as dilated its blood vessels also triggered the activation of the local immune response (kupffer cell) in liver cells compared with untreated control (Figure 3) (Owolabi *et al.,* 2012).

Cadmium chloride induced patchy tubular necrosis in the kidney. Treatment administered with graded doses of the methanol extract, showed no defect compared to the control. The heart, lungs and liver revealed absent parenchymal damages (Figure 4). The renal cells triggered no tubular necrosis in the cortex of the treated groups compared with the control as shown by Owolabi and Ogunnaike, (2014) that worked on the histological evaluation of the effects of Moringa leaf extract treatment on vital organs of murine models.

According to Bowen (2003), kupffer cells are a type of fixed macrophage. Molawi and Sieweke (2015) in their findings stated that liver kupffer cells (KCs) are self-maintained tissue-resident macrophages, this is similar with this present study in graded doses. The activation of kupffer cells in the liver and bronchiolo-aveolar lymphoid aggregates in the lungs of the Wistar rats, in this

Table 1: The Phytochemical Screening of *Moringa oleifera* Root Ethanol Extract

Parameters	Degree
Alkaloids	+++
Tannins	+++
Saponins	+++
Flavonoids	+
Phenol	+
Anthraquinone	++
Carbohydrate	+++
Cardiac Glycoside	-

Key: +++ Very highly present; ++ highly present; + fairly Present; - Absent

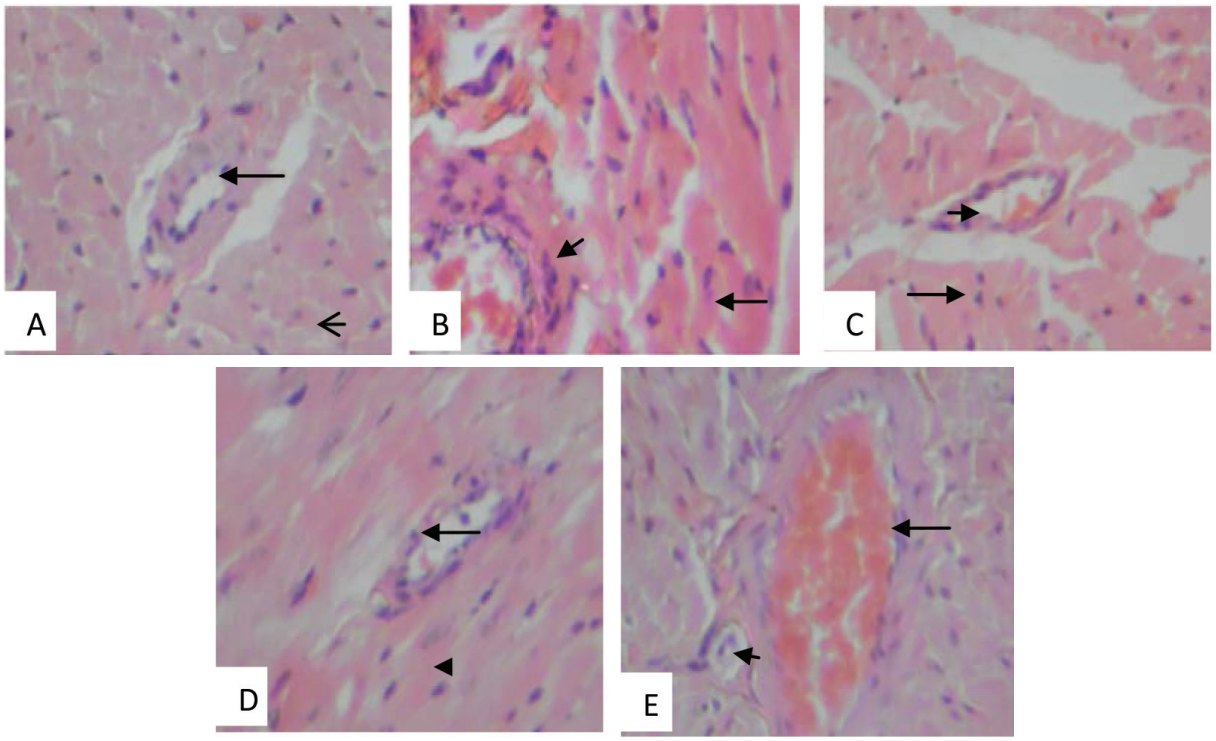

Fig. 1: Effects of *Moringa oleifera* root ethanol extract on cardiac cells

(A). Cadmium chloride 3.6 mg/kg showed long arrow, mild vascular congestion and short arrow, moderate vasodilatation (B). *M. oleifera* root ethanol extract 100 mg/kg: showed long arrow, normal coronary artery and short arrow, normal myocardium. (C). *M. oleifera* root ethanol extract 200 mg/kg: showed long arrow, mild vascular congestion and short arrow, normal myocardium. (D). *M. oleifera* root ethanol extract 400 mg/kg: showed long arrow, mild vascular congestion and short arrow, normal myocardium. (E). Normal control: showed long arrow, normal coronary artery and short arrow, normal myocardium (H&E x 100).

present study, elicited the effect of *M. oleifera* root ethanol extract with the capability to trigger the immune response. A similar study conducted by Edith *et al.* (2016) that showed the protective effect of the root ethanol extract of *M. oleifera* Lam., revealed on cardiac muscles and vessels in Wistar rats.

CONCLUSION

The present study showed the phytochemicals responsible for the histoprotective effect of *M. oleifera* root ethanol extract in dose dependent manner against toxicity in the targeted tissues had a progressive protection at graded doses. Hence

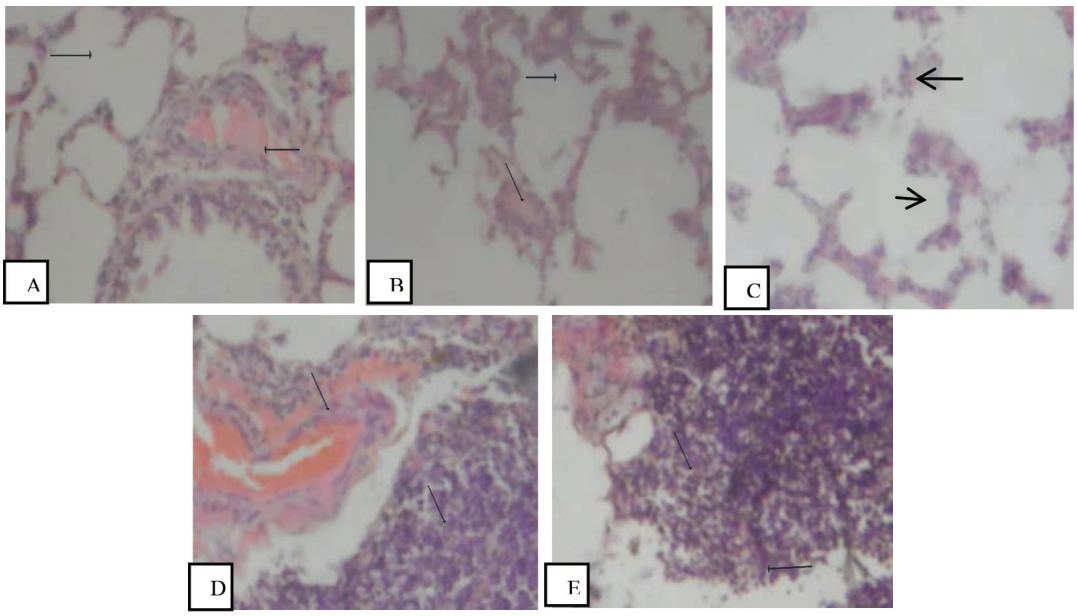

Fig. 2: Effects of *Moringa oleifera* root ethanol extract on the lungs cells
(A). Cadmium chloride 3.6 mg/kg showed: long arrow, normal alveoli and short arrow, patchy vascular intimal erosion. (B). *M. oleifera* root ethanol extract 100 mg/kg: showed long arrow, normal alveoli. (C). *M. oleifera* root ethanol extract 200 mg/kg: showed long arrow, moderate interstitial congestion and short arrow, moderate activation of bronchiolo-alveolar lymphoid aggregates. (D). *M. oleifera* root ethanol extract 400 mg/kg: showed long arrow, mild activation of bronchiolo-alveolar lymphoid aggregates (E). Normal control: showed long arrow, normal alveoli (H&E x 40).

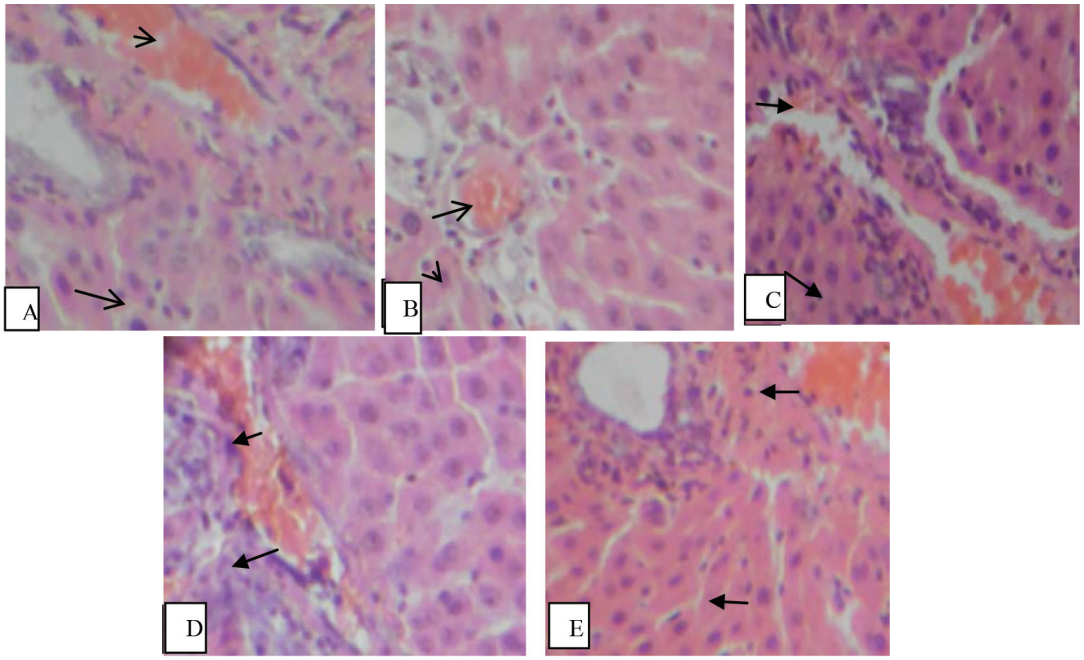

Fig. 3: Effects of *Moringa oleifera* root ethanol extract on the hepatic cells
(A). Cadmium chloride 3.6 mg/kg showed: long arrow, moderate vascular congestion and short arrow, moderate kupffer cell activation. (B). *M. oleifera* root ethanol extract 100 mg/kg: showed long arrow, mild vascular congestion and short arrow, moderate kupffer cell activation. (C). *M. oleifera* root ethanol extract 200 mg/kg: showed long arrow, mild vascular congestion and short arrow, mild kupffer cell activation. (D). *M. oleifera* root ethanol extract 400 mg/kg: showed long arrow, mild vascular congestion and short arrow, moderate kupffer cell activation. (E). Normal control: showed long arrow, mild vascular congestion and short arrow, mild kupffer cell activation (H&E x 100).

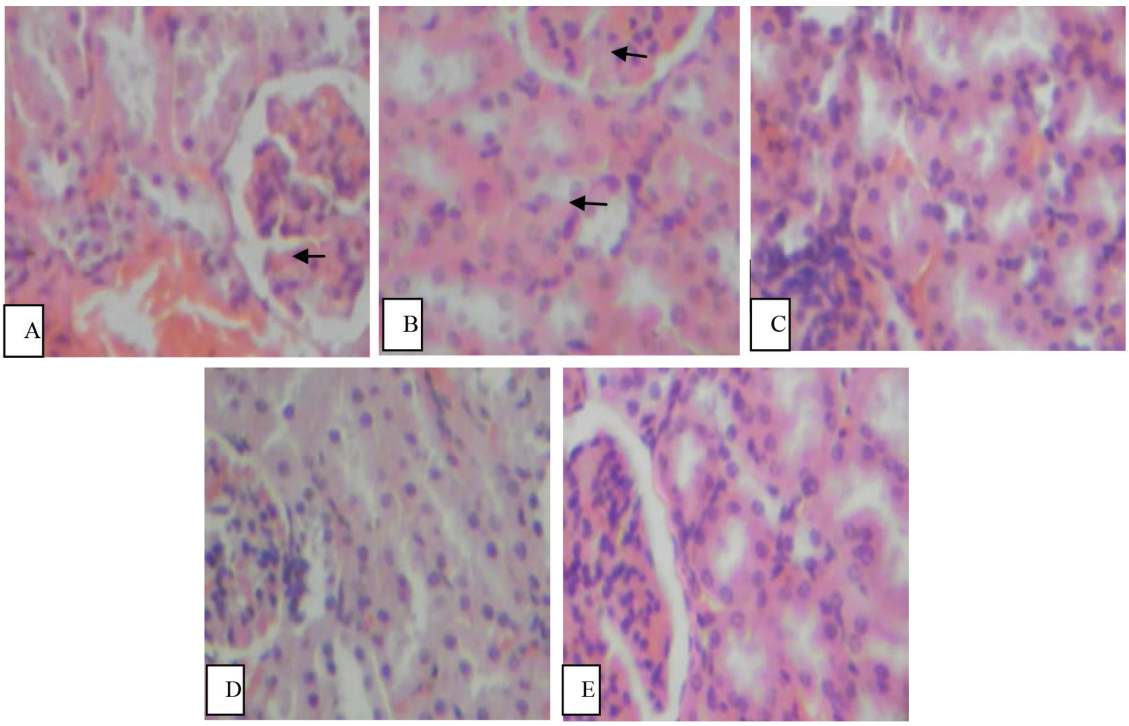

Fig. 4: Effects of *Moringa oleifera* root ethanol extract on the renal cells

(A). Cadmium chloride 3.6 mg/kg showed long arrow, focal tubular necrosis and short arrow, mild congestion. (B). *M. oleifera* root ethanol extract 100 mg/kg: showed long arrow, normal glomerulus and short arrow, tubules. (C). *M. oleifera* root ethanol extract 200 mg/kg: showed long arrow, normal tubules. (D). *M. oleifera* root ethanol extract 400 mg/kg: showed long arrow, normal tubules. (E). Normal control: showed long arrow, normal tubules (H&E x 100).

required further study to validate its ethnomedicinal benefits.

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