EFFECTS OF AQUEOUS EXTRACT OF *MANGIFERA INDICA* STEM BARK ON CADMIUM INDUCED RENAL DAMAGE IN ADULT WISTAR RATS

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ABSTRACT
Background: Cadmium is one of the metallic substances that are not needed by the body but instead are very toxic to the body cells. Prolong .exposure to toxicant such as cadmium can lead to biochemical or pathological alterations in any organs of the body such as the kidney. .
Aim: The aim of the study is to investigate the effects of aqueous extract of stem back of *Mangifera indica* extract on cadmium induced renal damage in adult Wistar rats.
Methodology: Thirty (30) adult Wistar rats were recruited for the study. They were randomly assigned into six (6) groups of five (5) animals each, Group A received food and water only, group B received 300mg/kg BW of aqueous stem bark of *Mangifera indica* group C received 1200mg/kg BW of aqueous stem bark of *Mangifera indica*, group D received 0.1ml/kg body weight of cadmium only, group E received 0.1ml/kg BW of cadmium + 300mg/kg BW of aqueous stem bark of *Mangifera indica*, group F received 0.1ml/kg BW of cadmium + 1200mg/kg BW of aqueous stem bark of *Mangifera indica*. Daily administration of *Mangifera indica* bark and cadmium were given through an orogastric tube for 30days.
Results: It **s**howed that the orogastric administration of cadmium revealed significantly ($p < 0.05$) increased in the levels of urea and decreased in the levels of catalase (CAT) and superoxide dismutase (SOD) statistically. While, *Mangifera indica* groups at both high and low doses confirmed the reversal of these result to those comparable with the control. Histologically, group D showed focal tubular necrosis and heavy interstitial infiltrates of inflammatory cells. While the group that was given cadmium along with *M. indica* showed features of normal renal architecture at both lower and high dose.
Conclusion: It can be concluded that aqueous stem bark of *Mangifera indica* extract at both low and high dosage were able to ameliorate the renal tissue damage caused by cadmium on adult Wistar rats.
Keywords: *Mangifera indica* stem bark, Wister rat, SOD, Catalase and Renal tissue

INTRODUCTION
It has been found that the toxicity of long-term, low-dose cadmium exposure is related to cell apoptosis (Swiergosz, *et al*., 1998), Fujishiro, *et al.*, 2018), necrosis (Diaz *et al.*, 2017), autophagy (Chen *et al*., 2018), destruction of cell-cell junctions (So, *et al*., 2018), and disordered cell signaling pathways (Zhang, et al., 2017). There is evidence (Chen, *et al*., 2018, Ha, *et al*., 2016) showing that Cd may initially cause the imbalance of some ions, which subsequently cause further damage to the renal tissue.
Mangiferin is a naturally occurring glucoxilxanthone (Matkowski, *et al*., 2013, Sekar, 2015) derived from the numerous parts of *Mangifera indica* (Mango), such as the leaves, fruits, roots, flowers, seeds, and stem bark (Jyotshna, 2016).

Studies have showed *Mangifera indica* fruit (mango) possess anti-diabetic, hypotensive, antioxidant, anti-viral, cardiotonic and antiinflammatory properties (Barreto, *et al*., 2008). The stem bark of *Mangifera indica* has been reported to exert numerous pharmacological activities such as anti-oxidant, anti-tumor, anti-viral, anti-diabetic, anti-bone resorption, antispasmodic, analgesic, antipyretic and immunomodulatory effects (Kumar, *et al*., 2009).

The most active constituent of the stem bark is mangiferin and it has been reported to have lipolytic, antibone resorption, radioprotective, anti-inflammatory, antitumour, antibacterial, antifungal, antiparasitic, immunomodulatory, anti-allergic, and monoamine oxidase-inhibition activity (Wauthoz, *et al*., 2007).

In Nigeria, various parts of *Mangifera indica* trees are used in the management of different human and animal diseases, such as malaria (Ene, *et al*., 2010), dysentery, typhoid fever infection (Alo, *et al*., 2012).

The aim of the study is to investigate the effect of aqueous stem bark extract of *Mangifera indica* in cadmium chloride induced kidney damage in adult Wistar rat.

MATERIALS AND METHODS

Plant collection

Stem bark of Mangifera indica was collected from a *Mangifera indica* tree at anatomy back gate of the Ugbowo campus of the University of Benin, Benin city, Edo state, Nigeria. It was authenticated at the herbarium in the Department of Plant Biology and Biotechnology, University of Benin (UNIBEN), Benin City, Edo state, Nigeria. The stem bark was shade dry for 1 (one) week. It was then oven-dried at a temperature of 40^0C for about 30 minutes and then pulverized into powder form using the British Milling Machine. The powdered sample was weight to be 120g.

The powdered material was macerated by soaking the120g powdered *M. indica* bark sample in 2.1L of water for 24 hours with constant shaking and stirring every nine hours (9). Filtration was carried out to separate the residue from the filtrate and the filtrate was concentrated over hot water bath using crucibles to obtain a gel like extraction which was then preserved in a bottle that was kept inside a freezer.

The animals were kept in the cages for two weeks before the experiment for effective acclimatization. They had access to standard livestock feed and clean drinking water. Animal was housed in a clean plastic cage under natural light, ventilation and free from pests.

Animal grouping and treatment

Adult Wistar rats weighing 180g-230g was obtained from animal house of the Department of Anatomy, Faculty of Basic Medical sciences, University of Benin was used for this research. The rats were arranged into six groups of five rats per group (Group A,B,C,D,E, and F).

The following is the analysis of the treatment method employed for this study: Group A: Control rats, received food and water only.

Group B: received 300mg/kg body weight of aqeous stem bark of *mangifera indica* [low dose]

Group C: received 1200mg/kg body weight of aqeous stem bark of *Mangifera indica* (High does)

Group D: received 0.1ml/kg body weight of cadmium only

Group E: received 0.1ml/kg body weight of cadmium + 300mg/kg body weight of aqueous stem bark of *Mangifera indica* (Low dose)

Group F: received 0.1ml/kg body weight of cadmium + 1200mg/kg body weight of aqueous stem bark of *Mangifera indica* (High dose)

Daily administration of *Mangifera indica* bark and cadmium were given through an orogastric tube for 30days.

Sacrifice, tissue collection, processing and staining

During the sacrifice, the rats were kept in an enclosed container with cotton wool socked with about 50ml of chloroform for anesthesia. After about 2 minutes, the rats were removed from the enclosed container and placed in a supine position on a dissection table. Adomino- thoracic incision was made to expose the abdominal viscera using dissection scissors and surgical blades. After opening the thoracic and abdominal region, 5ml syringe was used to collect blood via cardiac puncture for kidney function test and anti- oxidant stimation. The kidney was harvested at the retroperitoneal region of the abdomen and fixed immediately using 10% of formal saline for histological analysis.

Staining of tissues was done by established methods (Drury, *et al.,* 1976).

Photomicrograph*y:* Stained slides were viewed using an optical photomicroscope (Leica MC170 HD, Leica Biosystems, Germany) and photomicrographs were taken at x100 magnification using an attached Eakins 14MP digital microscopic camera, model 2307su, manufactured by Eakins Microscope Store, UK.

Urea was assayed according to Tietz (1995). The activity of CAT was assayed by the method of Aebi (1984). SOD activity was determined by the method of Nishikimi *et al*., 1972.

Statistical analyses

All data were subjected to statistical analysis using the IBM SPSS statistics software (Statistical Package for Social Science) Version 25 (SPSS, Inc., Chicago, Illinois, USA) and relevant statistical values were obtained. The values of the treated groups were compared with those of non-treated group using the one-way analysis of variance (ANOVA) and the T-test method. Values of $P < 0.05$ were considered significant. LSD was used as the post-hoc test

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RESULTS

Table 1: Showing the biochemical and antioxidant effect of Mangiferin indica on cadmium

	Control	300mg/kg *Mangifera .indica*	1200mg/kg *Mangifera Indica*	Cadmium only	300mg/kg *Mangifera indica* plus cadmium	1200 mg/kg *Mangifera.indica* plus cadmium
Urea (mg/dl)	20.60 ± 1.00	34.91 ± 0.10	30.80 ± 0.34	98.10± 0.18*	35.80 ± 0.91	32.56 ± 0.87
CAT(unit/mg protein)	31.75± 0.05	33.20± 0.10	37.82± 0.20	91.37± 0.30	37.10± 0.60	38.42± 0.80
SOD(unit/mg protein)	12.12 ± 0.43	14.16 ± 0.28	16.03 ± 0.81	89.03 ± 0.34	17.31 ± 0.69	19.01 ± 0.26

* $P < 0.05$ indicates significant difference when other groups are compared with the control (group A).

HISTOLOGICAL SLIDES

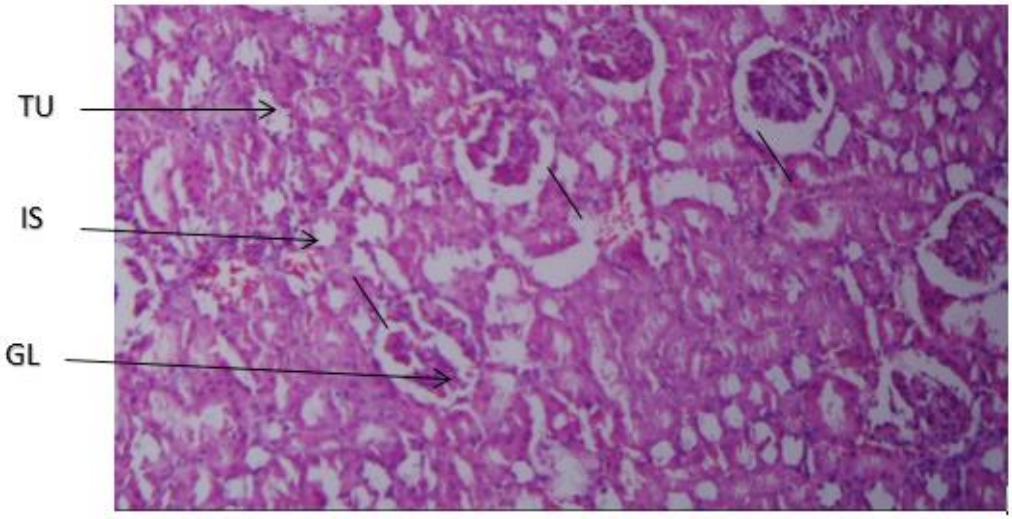

Plate 1: Rat kidney. Control. Composed of normal tissue architecture: tubules (TU), interstitial space (IS), glomeruli (GL) : H&E x 100

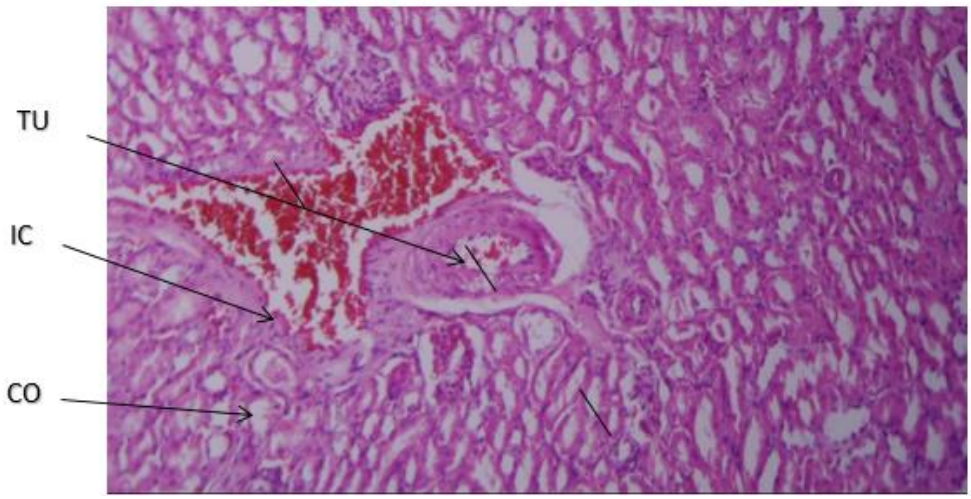

Plate 2: Rat kidney given Cadmium only showing: patchy tubular necrosis (TN), interstitial infiltrates of inflammatory cells (IC), interstitial congestion (C) : H&E x 100

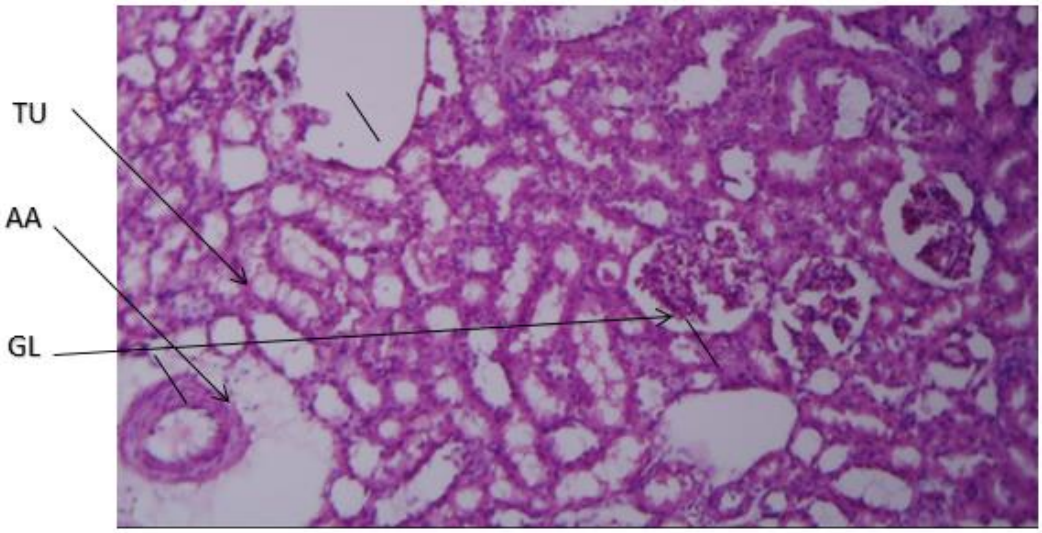

Plate 3: Rat kidney given low dose extract only showing normal architecture: tubules (TU), arcuate artery (AA), glomeruli (GL) : H&E x 100

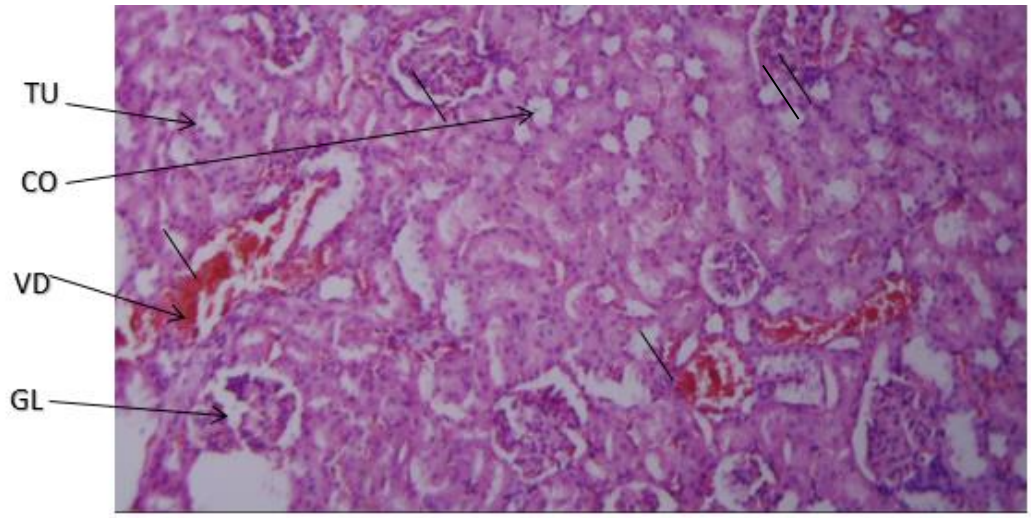

Plate 4: Rat kidney given high dose extract only showing normal architecture: tubules (TU), active interstitial congestion (CO), vasodilatation (VD), glomeruli (GL) : H&E x 100

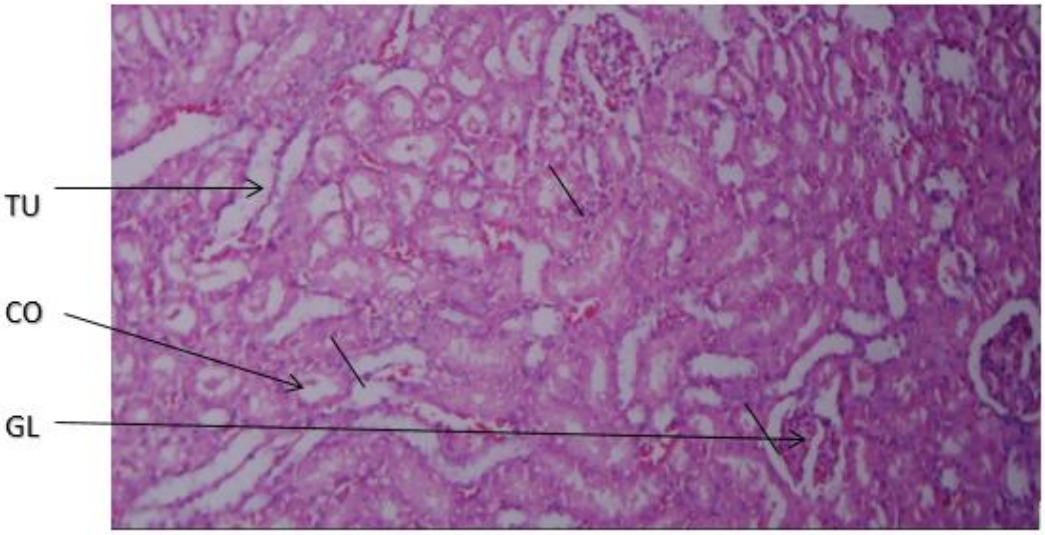

Plate 5: Rat kidney given low dose Extract + Cadmium showing normal architecture: tubules (TU), active interstitial congestion (C), glomeruli (GL) : H&E x 100

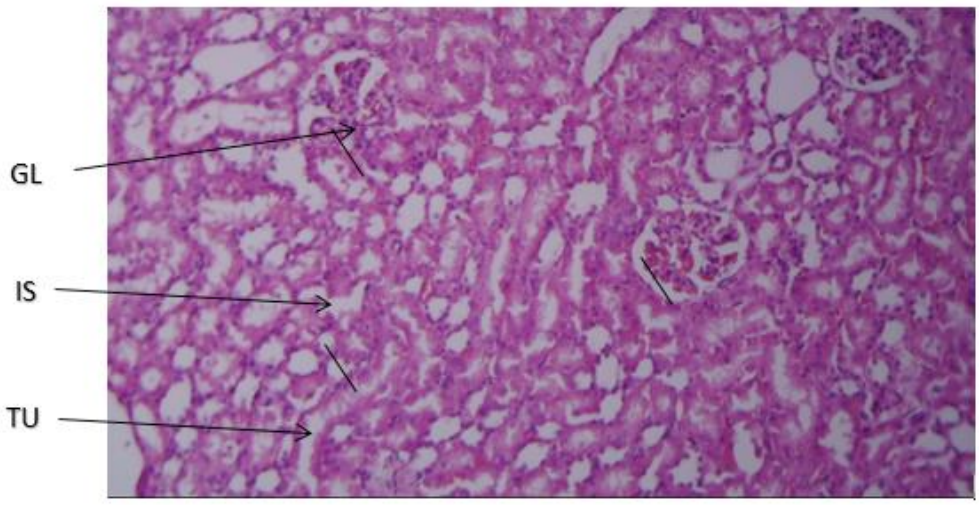

Plate 6.:Rat kidney given high dose Extract + Cadmium showing normal architecture: (GL), interstitial space (IS), tubules (TU) : H&E x 100

DISCUSSION

Serum creatinine and urea are considered as the index of nephrotoxicity (Afzal *et al*., 2004). Cadmium (Cd) enters the renal tissue where it deposits in the proximal tubules (Olubunmi et al 2017). Cd causes a decline in renal activity by increasing blood urea and creatinine levels, which leads to further renal damage by exposure to leaf extracts of P. amarus. It was suggested by Olubunmi and colleagues (Olubunmi, *et al*., 2017) that P. amarus extracts have no prophylactic or ameliorative effects on cadmiun-mediated kidney damage and rather that continuous exposure to these extracts are deleterious to the kidney. This result is in accordance with our research work (figure D), where we discovered that this group that was given cadmium chloride only showed focal tubular necrosis and heavy interstitial infiltrates of inflammatory cells.

Furthermore, Olubunmi *et al.*, 2017 studies on renal tissue toxicity by cadmium also support our biochemical analysis where we discovered that the group that we gave only cadmium showed biochemical derangement of urea level thereby making the group to be statistically significant when compared to the control group of our studies in table 1.

Importantly, mangiferin treatment reduces the kidney oxidative stress levels, and enhanced the antioxidant enzyme activities such as SOD, catalase, glutathione s-transferase (GST), glutathione peroxidase (Sadhukhan *et al*.,2018, Sahu *et al.*,2019). Also, He *et al.*, (2014) confirmed the reno-protective effect of mangiferin by virtue of its antioxidant, anti-inflammatory and anti-apoptotic properties in sepsis.

The decoction of stem bark of *M. indica* caused an increase in SOD level in 12-otetradecaecanoylphorbol- 13-acetate - induced oxidative damage in mice (Sanchez *et al*., 2000).

This is in accordance with our research findings where we discovered that *M. indica* was able to ameliorate the damage caused by cadmium chloride as shown in combined group and other groups like A, B, C, E and F in table 1 in which, the extract with the aid of one of its phytochemical component called mangiferin, which is a strong antioxidant agent caused the SOD and CAT level to be statistically increased when compared with the cadmium group.

A decoction of stem bark of *M. indica* preparation was proved to be antioxidant in nature, due to its ability to scavenge free radicals involved in microsome lipid peroxidation (Martinez *et al*., 2001). This study is in support of our work where we discovered that group that was given only aqueous stem bark extract of *Mangifera indica* showed values of SOD, CAT that are statistically insignificant when compared with the control group as revealed in Table 1. Bibu *et al*., 2011 assessed the therapeutic effect of aqueous extract of *Mangifera indica* stem bark on gentamicin-induced nephrotoxicity. They concluded that the aqueous extract of stem bark of *M. indica* prevented the gentamicin -induced free radical toxicity on the kidney

The histological result of our work depicted that cadmium only group (figure D) showed features of patchy tubular necrosis, interstitial infiltrates of inflammatory cells, interstitial congestion. This our experimental result is in collaboration with previous work done by Ehimigbai, and Nwosu, 2022, where they revealed that cadmium was able to caused renal tissue damage in the kidney of adult Wistar rat.

A recent study by Samadarsi and Dutta (2020) also supported the antioxidant efficacy of mangiferin against NaF-induced nephrotoxicity in kidney epithelial cells. Also, aqueous extract of *Mangifera indica* stem bark was reported to offer protection against carbon tetra chloride -induced liver toxicity in Wistar rats (Adeneye *et al*., 2015). Interestingly, a report by Ghosh *et al.*, (2012) confirmed the therapeutic positivity of mangiferin (15 mg/kg, ip) against galactosamine induced renal damage in rats. Also, acute oral toxicity assessment done by Ogbe *et al*., (2012) stated that *Mangifera indica* was documented to be non-toxic in animals at doses of 5000 mg/kg body weight.

This study is in support of our work where we discovered that group that was given only aqueous stem bark extract of *Mangifera indica* showed normal histological features of the kidney as revealed in figure B and C.

Awodele *et al* 2015 evaluated the reno-modulatory actions of the aqueous stem bark of *Mangifera indica extract* against carbon tetra chloride -induced renal damage in rats, they concluded that the extract was able to reversed the damage caused by the carbon tetra chloride on the renal tissue, This result is in tandem with our research work where we discovered that Mango tree back was able to ameliorate the damage caused by cadmium chloride on the kidney of adult Wister rat as depicted in figure E and F and table 1

CONCLUSION

It can be concluded that aqueous stem bark of *mangifera indica* at both low and high dosage were able to ameliorate the renal tissue damage caused by cadmium on adult Wistar rats.

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