

Cadmium-induced Renopathy in Wistar Male Rats: Protection by Methanolic Leaf Extract of *Momordica charantia* Linn.

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Abstract: The aim of present study was to evaluate the protective potentials of methanolic extract of the leaf of *Momordica charantia* (MC) against cadmium-induced renopathy in rats. Thirty male adult wistar rats were randomly divided into three groups (A, B and C) of ten rats each. Group A rats served as the control and received normal saline orally. Group B rats were treated with cadmium chloride 2.5 mg/kg bwt subcutaneously while group C rats were pre-treated orally with extract of MC 300 mg/kg bwt before treating with cadmium chloride 2.5 mg/kg bwt subcutaneously. The rats were treated every other day regularly for six weeks. Blood samples were collected by ocular puncture and five rats each per group were sacrificed at third week and six week post-treatment intervals. Serum creatinine and urea were evaluated. Histopathology of the kidney was also studied. In cadmium only-treated rats, serum creatinine and urea levels were significantly ($p < 0.05$) increased when compared to control rats at the two time intervals. Histological observations showed varying degree of renal toxicity. However, the toxic effect of cadmium was significantly controlled in the rats pre-treated with methanolic extract of MC at the two time intervals.

Keywords: *Momordica charantia*, cadmium chloride, renopathy, protective potentials.

I. Introduction

Cadmium is well-known heavy metal present in the environment and causes serious environmental and occupational hazards to humans [1, 2]. Both acute and chronic exposure to cadmium can damage various organs including kidney, liver, testes, lungs, and bone depending on the dose, route and duration of the exposure. In humans, the kidney is recognized as the most critical organ affected by chronic exposure to cadmium [3, 4], whereas acute exposure to cadmium mainly affects the liver [5]. Kidney damage is the initial site of damage in cases of low-level chronic inhalation exposure or oral exposure, and the lung is the initial site of damage in high-level inhalational exposure [6]. During the chronic exposure the metal may interfere with metabolic process via renal cortex resulting in renal dysfunction [2]. Chronic cadmium intoxication causes focal cortical damage in humans and apoptosis of the proximal tubular cells has been demonstrated in rats and dogs [7].

Momordica charantia is a tendril bearing economically important medicinally vine belonging to the family cucurbitaceae [8, 9]. It is a vegetable widely cultivated in tropical areas including West Africa, East Africa, Amazon, and the Carribean, both as medicine and fruit. All the plant parts including flower, leaves, stem, fruits and seeds have been used traditionally to treat array of conditions like diabetes, hypertension, cancers, ulcers, asthma, Gastrointestinal problems, fever, inflammation, erectile dysfunction, bacteria and viral infections [10]. This study was designed to investigate renoprotection of the methanolic leaf extract of *Momordica charantia* on the serum urea and creatinine, and histology of cadmium-induced kidney damage.

II. Materials And Methods

2.1 Plant Material

Fresh leaves of *Momordica charantia* (Bitter melon) were collected from a farm in Osu, Osun State, Nigeria. The plant was identified and authenticated at the Ife Herbarium, Department of Botany, Obafemi Awolowo University, Nigeria, where the specimen copy was deposited. The Herbarium identification number was 16591.

2.1.1 Preparation of Methanolic extract of Plant material

The leaves of *Momordica charantia* were shade dry under laboratory conditions for thirty (30) days and ground into fine powder by using an electrical mill. The powder was kept in air-tight container until use. Three hundred and fifty gram (350 g) of the dried powder was subjected to soxhlet extraction with 3.5 litres of 70% (v/v) methanol for two (2) consecutive days using modified method of Virdee *et al.*, 2003 [11]. 500 mls of warm water was added to the mixture to suspend the chlorophyll. The mixture was filtered to remove the suspended chlorophyll and filtrate was evaporated to dryness under reduced pressure using Buchi Rotary

Evaporator and the yield was calculated. The extraction was done at DRPU (Drug Research and Production Unit) in the Faculty of Pharmacy, Obafemi Awolowo University, Ile-ife, Nigeria.

2.2 Experimental Animals

Thirty (30) adult male wistar rats weighing 200 g to 250 g were used for this study. The animals were acclimatized for 2 weeks at the Animal House of the College of Health Sciences, Obafemi Awolowo University, Nigeria where they had free access to standard pellets (Mosodun Feeds, Osogbo, Nigeria) and clean water.

2.3 Experimental Design

Thirty (30) adult male wistar rats weighing between 200 g and 250 g were randomly divided into 3 groups, A, B and C of 10 rats each. A was the control group and received Normal saline, B was cadmium only treated group and group C was pre-treated with methanolic extract of *Momordica charantia* one hour before treating with cadmium. The rats were treated every other day regularly for six weeks as follows:

Group A (Normal saline) - Each rat in this group received 1 ml per day 0.9% w/v of Normal saline. Route of administration was per oral.

Group B (Cadmium only) – Each rat in this group received 2.5 mg/kg body weight Cadmium per day. Route of administration was subcutaneous.

Group C (Extract + Cadmium) – Each rat in this group was pre-treated with 300 mg/kg body weight methanolic extract of *Momordica charantia* per oral one hour before subcutaneous 2.5 mg/kg body weight Cadmium was given.

All the rats in the three groups were treated every other day regularly for six weeks. Five blood samples were collected by ocular puncture after 3 and 6 weeks of treatment and sera separated for various biochemical assays. Five rats were sacrificed by cervical decapitation at the two time intervals previously mentioned and kidney tissues were removed, cleaned, and immediately fixed in 10% formalin for histological study.

2.4 Biochemical Renal Function Estimations

The renal function parameters estimated from the serum were urea and creatinine. The urea and creatinine levels in the serum were determined by Randox diagnostic kits (Randox Laboratory Ltd., Crumlin, County Antrim, UK) by method of Faweett and Scott, 1960 [12].

2.5 Histopathological Studies

Five rats were sacrificed by cervical decapitation at the two time intervals previously mentioned and kidney tissues were removed, cleaned, and immediately fixed in 10% formalin. The tissues were transferred into an automatic processor where they went through a process of dehydration. The tissues were then cleared in Xylene and embedded in paraffin wax. Serial sections of 5 micron thick were obtained using a rotary microtome. The tissue sections were deparaffinised hydrated and stained using the routine haematoxylin and eosin staining method (H&E). The stained sections were examined under the light microscope.

2.6 Statistical Analysis

The data were expressed as the mean \pm SE. Statistical difference between groups were assessed by paired-samples T-test using SPSS package (version 16.0) and p values < 0.05 were considered significant.

III. Results

3.1 Serum creatinine and urea concentrations

Table 1 showed the effect of cadmium and extract of *Momordica charantia* (MC) on serum urea and creatinine levels at three and sixth week post-treatments. In cadmium only-treated rats serum creatinine levels were significantly ($p < 0.05$) increased when compared to control rats at the two time treatment intervals. However, serum creatinine levels in rats pre-treated with extract of MC were significantly ($p < 0.05$) reduced when compared with cadmium only-treated rats. There was no significant ($p < 0.05$) difference in serum creatinine levels of control rats and those pre-treated with extract of MC. Serum urea level of cadmium only-treated rats was increased at three week post-treatment (though not significant at $p < 0.05$). However, at sixth week post-treatment, there was significant ($p < 0.05$) increase in the level of serum urea of rats treated-only with cadmium when compared to control rats and those pre-treated with extract of *Momordica charantia*.

3.2 Histological studies

Figures 1 and 2 showed the histological studies of the renal tissues of the control, cadmium-only treated, and MC extracts pre-treated rats at the third week and sixth week post-treatment stages respectively. The histological analysis of the renal cortex revealed that control rats showed normal morphology. In cadmium-only treated rats, there was significant reduction of the glomerular spaces at the two-time treatment intervals.

There was recovery of glomerular spaces in rats pre-treated with methanolic extract of *Momordica charantia* (MC). There was also slight clumping of nucleus in rats pre-treated with extract of MC at the end of three weeks treatment. There was significant deposit of blue-stained materials (thought to be cadmium deposits) which further obscured the outline of renal corpuscles in cadmium-only treated rats. There was slight deposit of blue-stained materials in rats pre-treated with extract of MC. Macula densa was not obvious in all rats challenged with cadmium.

IV. Discussion And Conclusion

Cadmium is also one of the most toxic industrial and environmental metals that cause health hazard [13]. Nephrotoxicity caused by cadmium has been described in settings of industrial exposure and environmental pollution [14]. The current study was focused at determining the protective potential of methanolic extract of the leaf of *Momordica charantia* (MC) on cadmium-induced renal damage in rats. The result of our study supported the fact that cadmium induces renal toxicity [7, 15, 2, 1]. The results of this study also showed that cadmium-induced renal damage increases serum creatinine and urea levels. This is in agreement with previous studies (16, 17, 18, 19, 20). Pathogenesis of cadmium-induced renal damage has been attributed to the binding of cadmium to a low molecular weight binding protein called methalothionein. More than 80% of cadmium is bound to methalothionein. Methalothionein is a protective protein found largely in liver and kidney [16]. Urea and creatinine levels are used to monitor renal function and their levels will not rise until at least half of the kidney nephrons are destroyed [19]. The results of our study also showed that methanolic leaf extract of *Momordica charantia* (MC) improved renal function of the rats pre-treated with the extract. This activity may be due to pronounced antioxidant properties of the plant of study by reducing oxidative damage to the microstructure of the kidney [21].

Cadmium-induced kidney damage was also evaluated using histological approach. The results of our study further showed that cadmium causes renal damage. There was significant reduction of the glomerular spaces which was responsible for significant increase in the biochemical parameters such as creatinine and urea. There was also significant cadmium deposits in the cortex of the rats treated with cadmium alone. Deposition of cadmium in the cortex may be due to binding of cadmium to small molecular weight protein, metallothionein [22, 1]. However, cadmium-induced renal damage was restored to near normal morphology by methanolic leaf extract of *Momordica charantia* (MC). The ability of extract of MC to prevent oxidative damage caused by heavy metals like cadmium may be due to its anti-lipid peroxidation activity and antioxidant property [21, 23].

In conclusion, we therefore inferred that cadmium induces toxic renal damage, and methanolic leaf extract of *Momordica charantia* has appreciable potentials to prevent damage to the kidney.

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Table 1: Effect of Cadmium and Extract of *Momordica charantia* (MC) on Serum Urea (mmol/L) and Creatinine, CREA, (µmol/L) of Male Rats.

Treatment Group						
	Control		Cadmium		Cadmium and Extract of MC	
Weeks	UREA	CREA	UREA	CREA	UREA	CREA
3	14.13±0.64	71.99±7.63	16.31±1.01	104.35±13.12**	14.36±1.04	76.04±6.54**
6	8.61±0.01	68.74±0.02	10.81±0.77**	101.54±11.16**	8.09±2.06**	79.01±8.92**

Values are expressed as mean ± SEM (n= 5). **Significantly different from control, p<0.05; *Significantly different from cadmium group, p<0.05.

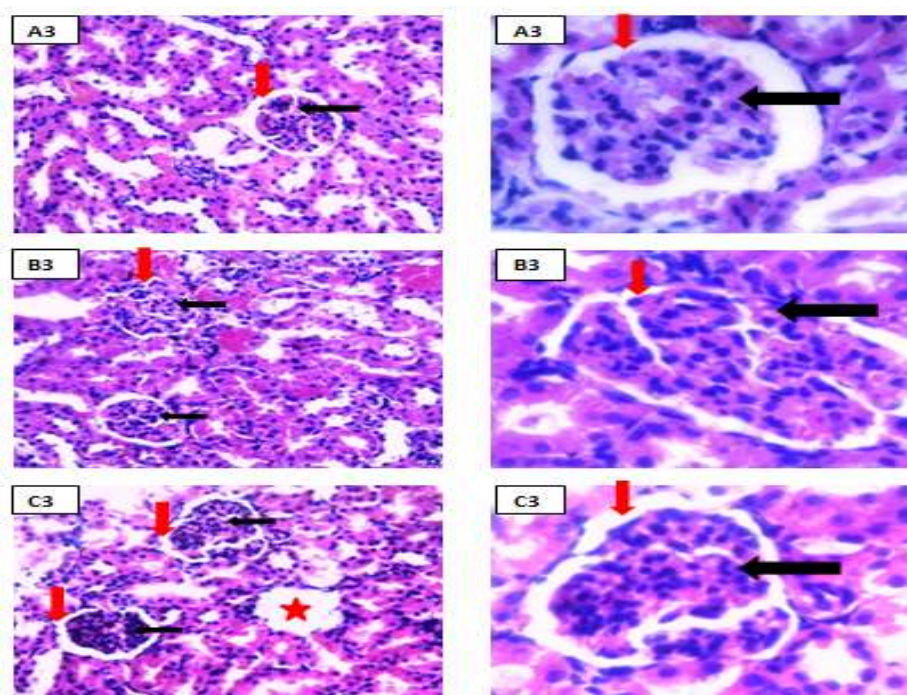


Figure 1: Photomicrographs of the renal cortex showing the renal corpuscle (black arrow). There is slight clumping of the glomerulus in C slides as well as an empty renal corpuscle. Glomerular space (red arrow) is markedly removed in B slides while there is recovery of the glomerular space in C slides.

Stain H&E. Mag. X400 Left Panel, X1000 Right Panel.

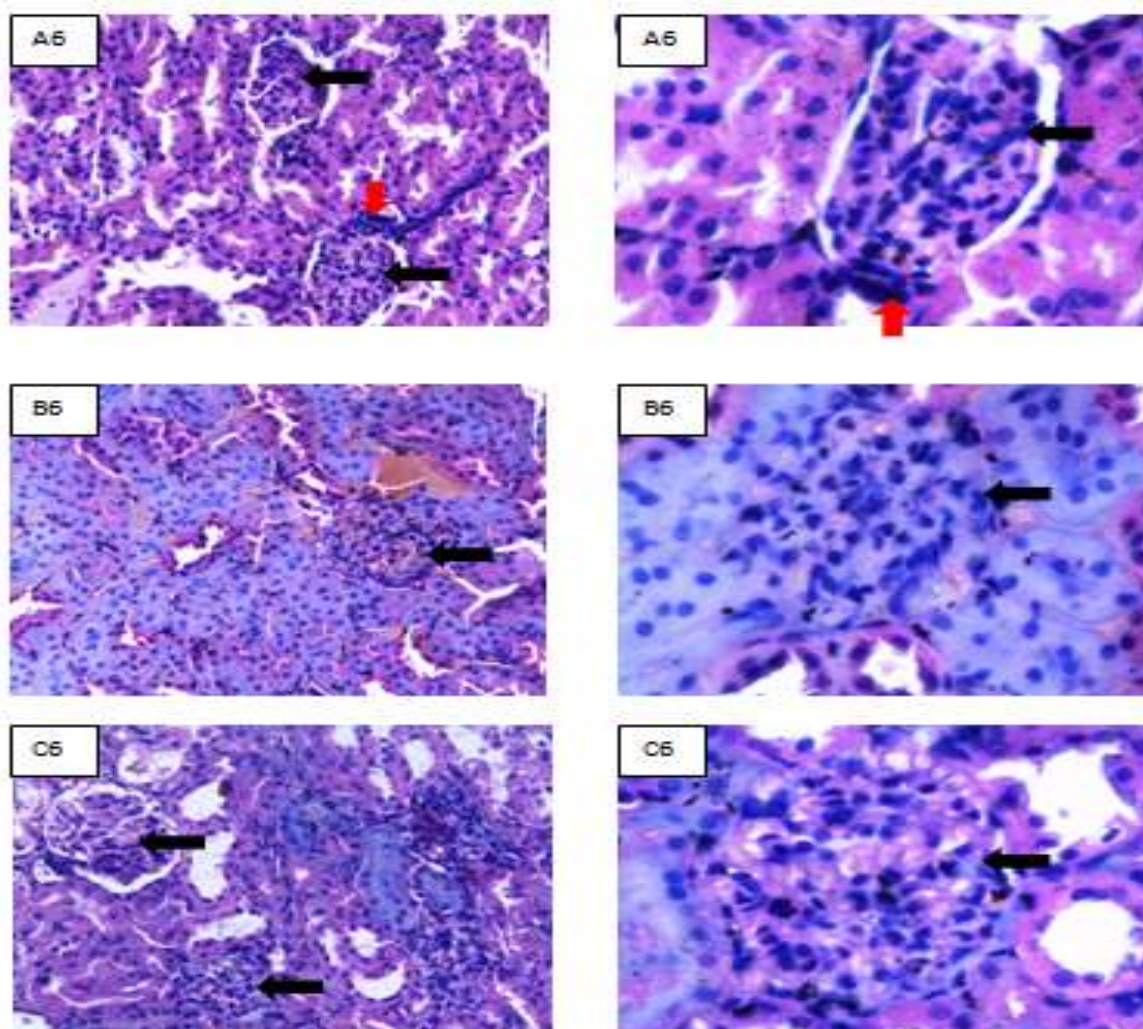


Figure 2: Photomicrographs of the renal cortex of A (control), B (cadmium only) and C (MC extract pre-treated) rats at 6th week post-treatment stage showing the renal glomeruli (black arrow), macula densa (red arrow). The cellular components of the renal corpuscle of B are closely packed with obliteration of the glomerular space. A bluish stained deposit further obscures the outline of the corpuscle in the B slides. A lesser obliteration of the glomerular space is seen in the C slides, with lesser deposit of the bluish stained material. Macula densa is not obvious in the B and C renal corpuscle. Stain H&E. Mag. X1000 Right panel, x400 Left panel.