INTERNATIONAL JOURNAL OF PHYTOTHEARPY RESEARCH
ISSN 2278 – 5701

# Research Article

## Protective Effects of *Moringa oleifera* Lam. on Cadmium-induced Liver and Kidney Damage in Male Wistar Rats

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## Abstract:

The aim of this study was to induce liver and kidney damage in rats using cadmium and biochemically evaluate possible protective potentials of methanolic extract of *Moringa oleifera* (MO). Twenty-one adult male rats weighing 200-250g were randomly divided into three groups (A, B and C) of seven rats each. Group A rats served as 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 MO 500 mg/kg bwt before treating with cadmium chloride 2.5 mg/kg bwt subcutaneously. The rats were treated every other day regularly for three weeks. Blood samples were collected by ocular puncture and sera used for estimations of gamma-glutamyl transferase (GGT), protein, bilirubin, and urea levels. Animals were sacrificed by cervical decapitation and liver tissues were harvested and homogenized. The supernatant obtained was used to estimate GGT, protein and bilirubin levels. The liver GGT, and protein levels in rats treated only with cadmium were significantly ($p<0.05$) lower than in control rats while serum GGT and protein levels in cadmium treated rats were significantly ($p<0.05$) increased. Serum bilirubin and urea levels in rats challenged with cadmium were significantly ($p<0.05$) increased. However, administration of methanolic extract of MO one hour to cadmium exposure ameliorated the toxic effect of cadmium. The study concluded that methanolic extract of *Moringa oleifera* showed appreciable potentials in protecting against cadmium-induced liver and kidney dysfunction in rats.

**Keywords:** Cadmium, kidney, liver, *Moringa oleifera*, protective potentials.

## INTRODUCTION

Cadmium causes serious environmental and occupational hazards. Its exposure to humans and animals causes both acute and chronic tissue injury, and can damage various tissues and organs including liver, kidney, bone, gonads, and lungs [1]. The liver is a natural chemical factory which aids metabolism and detoxification of complex molecules. It neutralizes toxins, and manufactures bile which aids fat digestion and removes toxins through the bowels [2, 3, 4]. Continuous exposure and intoxication of liver to different types of environmental toxic agents on a daily basis may

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lead to hepatic dysfunction and decrease in its efficiency and functions [5]. Hepatic dysfunction due to exposure to environmental toxic agents is increasing worldwide. Cadmium is one of the most toxic industrial and environmental metals and possesses a continuing health hazard since it is rapidly distributed in tissues [6, 7]. Cadmium causes liver injury via production of reactive oxygen species, enhancement of lipid peroxidation and inhibition of antioxidant enzymes [7, 8].

Kidney is the major target organ of cadmium action. During chronic and sub-chronic exposure the metal may interfere with the metabolic process via renal cortex resulting in malfunctioning of kidneys [9, 10]. The proximal tubules are the main target of cadmium [11]. Nephrotoxicity caused by cadmium has been described in settings of industrial exposure and environmental pollution. Cadmium is used industrially in plating of steel, pigments, plastics, alloys, and nickel-cadmium batteries [12].

*Moringa oleifera* (also known as the horseradish tree, drumstick tree) is the most widely cultivated species of a monogeneric family, the Moringaceae, which is native to the sub-Himalayan tracts of India, Pakistan, Bangladesh and Afghanistan. It is a perennial softwood tree with timber of low quality, but which for centuries has been advocated for traditional medicinal and industrial uses [13]. Moringa trees have been used to combat malnutrition, especially among infants and nursing mothers. Moringa leaves contain more Vitamin A than carrots, more calcium than milk, more iron than spinach, more Vitamin C than oranges, and more potassium than bananas, and that the protein quality of Moringa leaves rivals that of milk and eggs [14, 15].

This study was designed to investigate the hepatoprotective and renoprotective potentials of methanolic leaf extract of *Moringa oleifera* on the serum bilirubin, serum and liver total protein, serum and liver gamma-glutamyl transferase (GGT), and serum urea of cadmium-induced tissue damage in rats.

## MATERIALS AND METHODS

### Plant material

Fresh leaves of *Moringa oleifera* were collected from the National Centre of Genetic Resources and Biotechnology (NCGRB), Moor Plantation, Ibadan, Nigeria. The plant was identified at the Botany unit of NCGRB.

### Preparation of Methanolic extract of plant material

The leaves of *Moringa oleifera* were shade dried under laboratory conditions for twenty-one (21) days and ground into fine powder by using an electrical mill. Two hundred and fifty gram (250g) of the dried powder was subjected to soxhlet extraction with 2.5 litres of 70% (v/v) methanol for 12 hours using modified method of Virdii *et al.,* 2003[16]. The extraction was done at the Department of Chemistry, College of Science and Technology, Osun State University, Osogbo, Nigeria. The filtrate obtained was concentrated under reduced pressure using Buchi Rotary evaporator and then lyophilized by freeze-drying at Central Laboratory Unit, Obafemi Awolowo University, Ile-Ife, Nigeria.

### Experimental animals

Twenty-one (21) adult male wistar rats weighing 200 g to 250 g were used for this study. The

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animals were purchased at the Animal House of the College of Health Sciences, Osun State University, Osogbo, Nigeria where they were kept
in well ventilated wooden cages and had free access to standard pellets (Mosodun Feeds, Osogbo, Nigeria) and clean water.

**Experimental design**

Twenty-one (21) adult male wistar rats weighing between 200 g and 250 g were randomly divided into 3 groups, A, B and C of 7 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 *Moringa oleifera* one hour before treating with cadmium. The rats were treated every other day regularly for three weeks as follows:

Group A (Normal saline) - Each rat in this group received 2ml per day of 0.9% w/v of Normal saline. Route of administration was per oral.

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

Group C (Extract + Cadmium) – Each animal in this group was pre-treated with 500mg/kg body weight methanolic extract of *Moringa oleifera* per oral one hour before subcutaneous 2.5mg/kg body weight Cadmium was given.

All the rats in the three groups were treated every other day regularly for three weeks. The weight of each rat was recorded at procurement, at the commencement of the study, then every other day during the course of the study. Blood samples were collected by ocular puncture at the end of 3rd week of treatment and sera separated for biochemical assays. The rats were then sacrificed by cervical decapitation and liver tissues were immediately harvested, homogenized in freshly prepared 0.25M sucrose solution. The homogenate was further centrifuged at 6000 rpm for 5 minutes at $4^0$C using a refrigerated centrifuge (TGL-16G, B-Bran Scientific and Instrument Company, England) and supernatant obtained was used for biochemical assays.

**Biochemical estimations**

The following biochemical parameters were estimated: serum total bilirubin, serum and liver total protein, serum and liver gamma-glutamyl transferase (GGT), and serum urea. The activities of serum and liver GGT were assessed by the method of Teitz (1987) [17]. Serum total bilirubin was assessed using the method of Jendrassik and Grof (1938) [18]. Serum and liver protein concentrations were determined using the Biuretic methods while serum urea level was determined by method of Faweett and Scott (1960) [19].

**Statistical analysis**

The data were expressed as the mean ± SEM. 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.

## RESULTS

Figure 1 showed the growth curve of experimental animals. The control rats showed significant ($p<0.05$) increase in mean body weights from 181 g at the commencement of the study to 225 g observed at the end of the study. The cadmium-only treated rats significantly ($p<0.05$) lost weight from 205 g to 200 g while those rats that were pre-treated with 500 mg

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extract of *Moringa oleifera* significantly ($p<0.05$) gained weight from 210 g to 230 g.

Figure 2 showed effect of cadmium and extract of *Moringa oleifera* on the gamma-glutamyl transferase (GGT) activities in liver tissues and sera of rats. The activity (0.15 U/L) of GGT in liver of rats treated-only with cadmium was significantly ($p<0.05$) reduced when compared with the enzyme activity (0.21 U/L) in control rats and activity (0.19 U/L) in rats pre-treated with extract of *Moringa oleifera.* Whereas, serum GGT activity (0.14 U/L) in cadmium-only treated rats was significantly ($p<0.05$) increased when compared to the activities in control rats and in those pre-treated with extract of *Moringa oleifera*.

**Figure 1:** Growth curve of the experimental animals

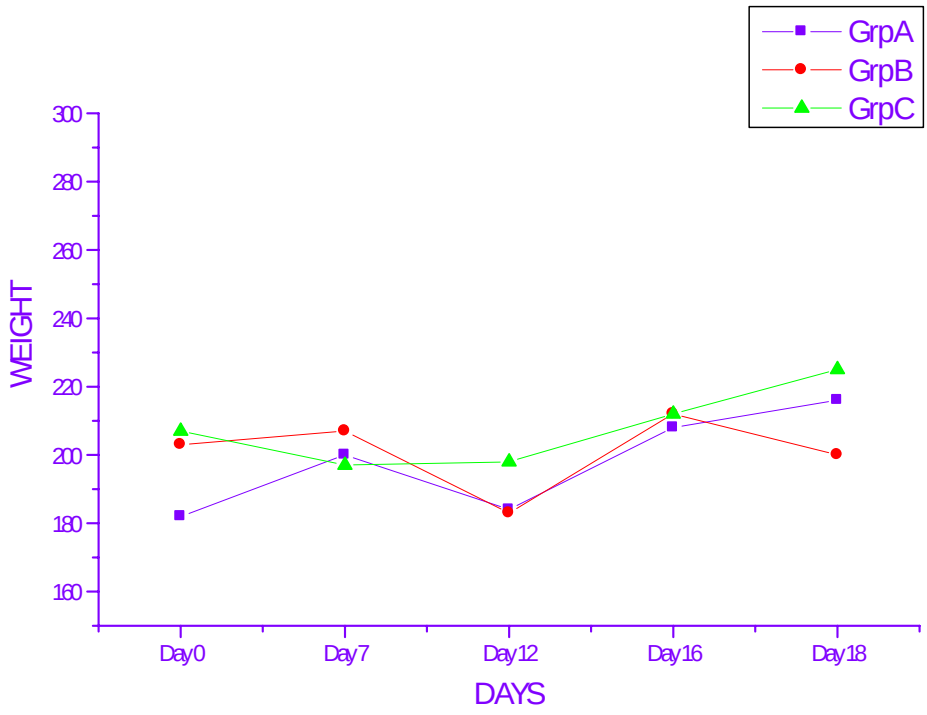

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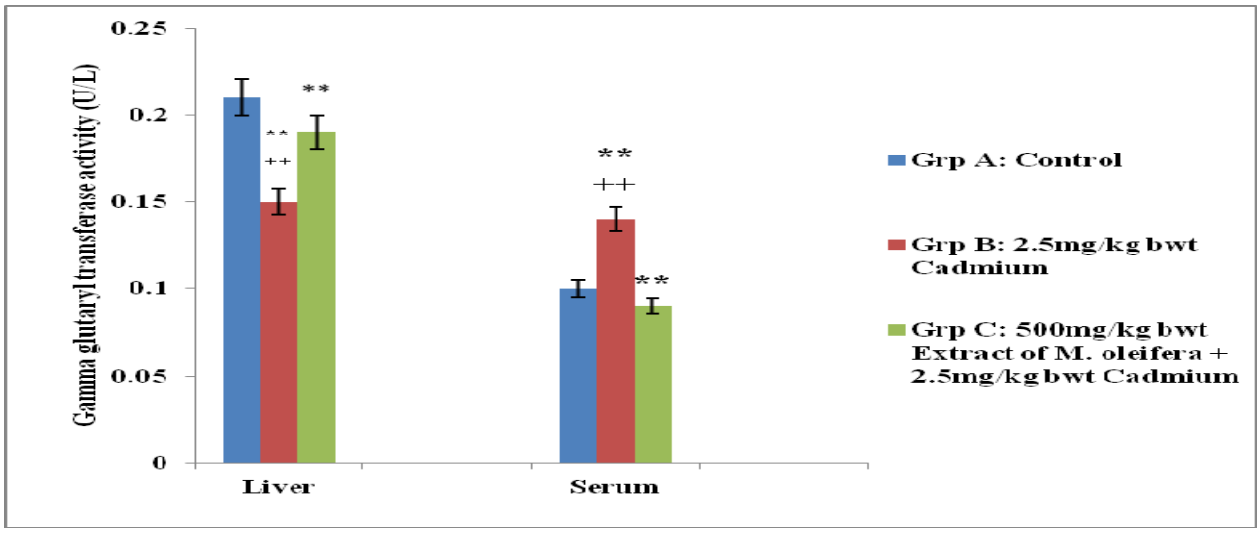
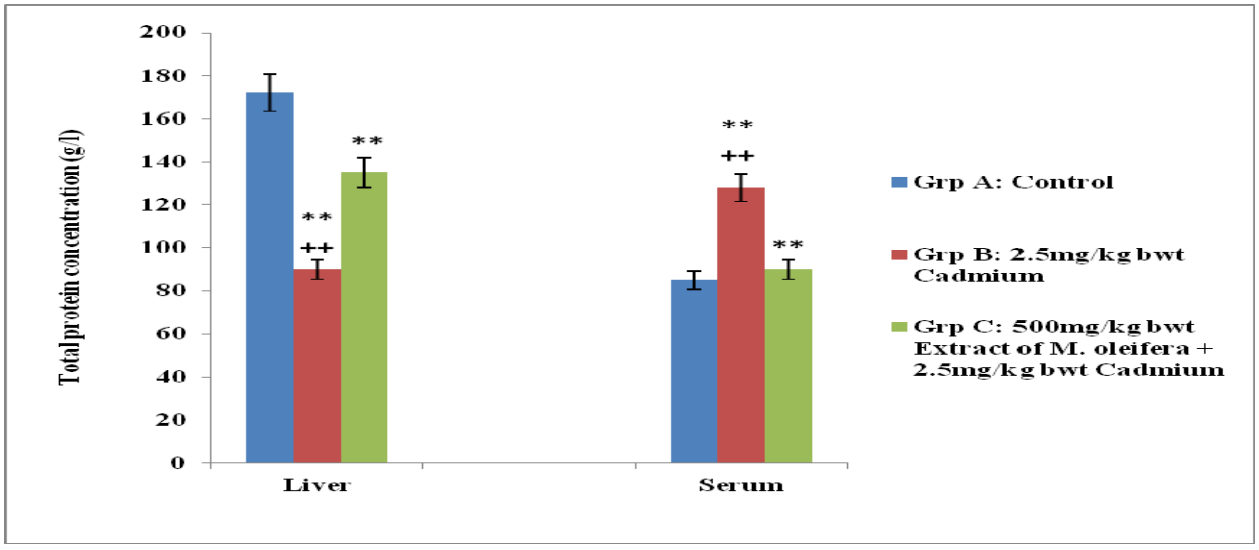
**Figure 2:** Effect of cadmium and extract of *Moringa oleifera* on the gamma-glutamyl transferase activities in liver tissues and sera of rats.




Each value is the mean + SEM (n= 7)
**Significantly different from control group, $p<0.05$.
++Significantly different from extract of *M. oleifera* group, $p<0.05$.

**Figure 3:** Effect of cadmium and extract of *Moringa oleifera* on protein concentrations in liver tissues and sera of rats.




Each value is the mean + SEM (n= 7)

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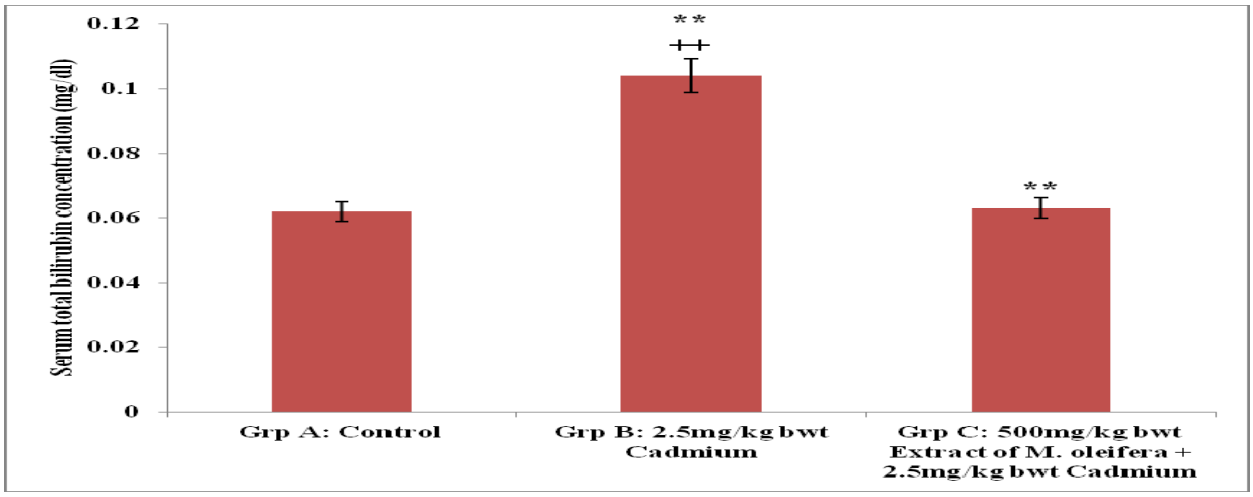
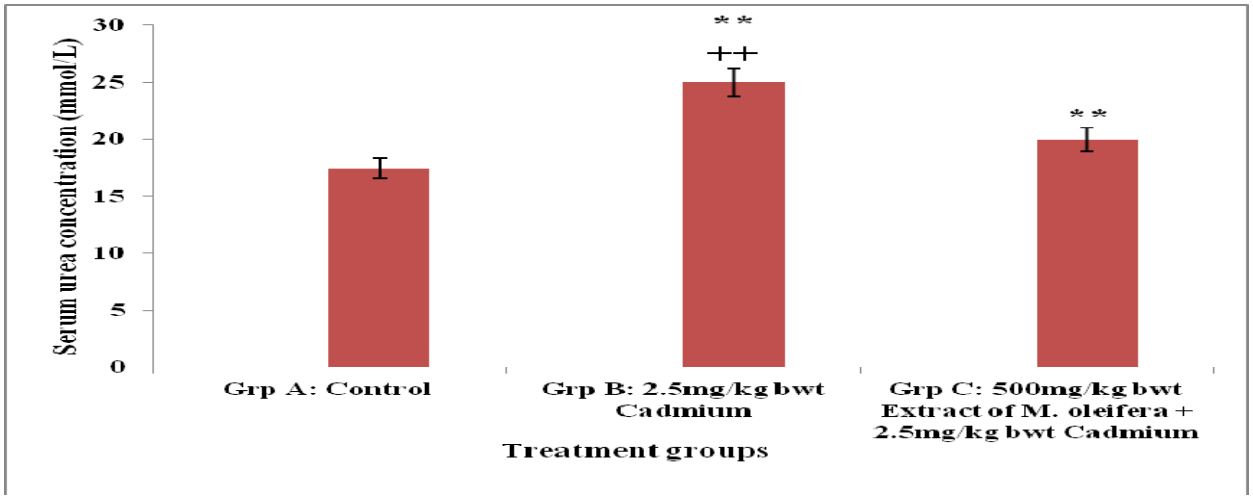
**Significantly different from control group, p<0.05.
++Significantly different from extract of *M. oleifera* group.
**Figure 4:** Effect of cadmium and extract of *Moringa oleifera* on serum bilirubin concentrations in rats.




Each value is the mean + SEM (n= 7)
**Significantly different from control group, p<0.05.
++Significantly different from extract of *M. oleifera* group.

**Figure 5:** Effect of cadmium and extract of *Moringa oleifera* on serum urea concentrations in rats.




Each value is the mean + SEM (n= 7)
**Significantly different from control group, p<0.05.

[++]Significantly different from extract of *M. oleifera* group.

Figure 3 showed effect of cadmium and extract of *Moringa oleifera* on protein concentrations in liver tissues and sera of rats. The protein level (90 g/L) in liver of rats treated only with cadmium was significantly ($p<0.05$) lowered when compared with protein level (172g/L) in control rats and protein level (135 g/L) in rats pre-treated with extract of *Moringa oleifera*. Whereas, serum protein level (130 g/L) in rats treated only with cadmium was significantly ($p<0.05$) increased when compared to the levels in control rats and in rats pre-treated with extract of *Moringa oleifera*.

Figure 4 showed effect of cadmium and extract of *Moringa oleifera* on serum bilirubin concentration. The bilirubin level (0.104 mg/dL) in rats treated only with cadmium was significantly ($p<0.05$) higher than the levels in control rats and rats pre-treated with extract of *Moringa oleifera*.

Figure 5 showed effect of cadmium and extract of *Moringa oleifera* on serum urea concentration. The urea level (25 mmol/L) in rats challenged with cadmium alone was significantly ($p<0.05$) higher than the levels (17.5 mmol/L) in control rats and (20 mmol/L) in rats pre-treated with extract of *Moringa oleifera*.

## DISCUSSION AND CONCLUSION

The results of our study showed that cadmium exposure causes significant body weight loss. This result supports previous work where the same dose of 2.5 mg/kg bwt cadmium was administered to rats five times a week for the period of four weeks resulted to significant decrease in body weights of male rats [20]. Cadmium is one of the most dangerous occupational and environmental toxins. It accumulates mainly in liver and kidneys, where it causes functional changes and then interstitial fibrosis [21, 22]. Inflammation and subsequent fibrosis of major organs may be responsible for weight loss observed in rats treated with cadmium in our study.

The results of this study also showed significant increase in serum GGT activity and significant decrease in liver GGT activity in rats challenged with cadmium. GGT is predominantly located in the cell membrane of the hepatocytes and may act to transport amino acids and peptides into the cell in the form of γ- glutamyl peptides. It may also be involved in some aspects of glutathione metabolism [23]. The release of liver GGT into the circulation is a clear indication of the loss of functional integrity of the cell membrane and a reflection of damage caused to liver by cadmium toxicity [4]. The near normal levels of GGT activities in liver and serum of rats pre-treated with methanolic extract of *Moringa oleifera* showed that the plant is hepatoprotective.

Cadmium-induced hepatotoxicity is associated with production of reactive oxygen species which attack essential cell constituents such as proteins, lipids and nucleic acids [4, 24, 25]. In this study, the significant decrease of liver total protein and increase of same in the serum of rats treated with cadmium may be due to the attack of reactive oxygen species on cell membrane and consequent release of intracellular contents into the circulation. Likewise, the increase in the level of serum total bilirubin reflects the depth of jaundice and increase in the release of cytosolic liver marker enzymes into the circulation [26].

The study further showed significant increase in the level of serum urea in rats treated with cadmium when compared to serum urea levels in control rats and those rats pre-treated with extract of *Moringa oleifera*. Urea level is used to monitor renal function and its level will not rise until at least half of the kidney nephrons are destroyed [27]. The near normal urea

concentration observed in the rats pre-treated with extract of *Moringa oleifera* in this study may be as a result of antioxidant properties of the plant by reducing oxidative damage to the microstructure of the kidney.

The study concluded that 500 mg/kg body weight methanolic extract of *Moringa oleifera* showed appreciable potentials in protecting against cadmium-induced liver and kidney dysfunction in rats.

**ACKNOWLEDGEMENT**

Authors acknowledge the support provided by Mr. Adeleke I.A. of the Department of Biochemistry, College of Health Sciences, Osun State University, Osogbo. We also appreciate the management of National Centre of Genetic Resources and Biotechnology (NCGRB), Moor Plantation, Ibadan, Nigeria. Finally, we acknowledge the help received from the scholars whose articles are cited and included in references of the manuscript.

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