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Hepatoprotective Potentials of Methanolic Extract of the Leaf of Momordica charantia Linn on Cadmium-induced Hepatotoxicity in Rats

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Abstract
The present study was conducted to investigate the protective potentials of methanolic extract of the leaf of *Momordica charantia* (MC) against cadmium-induced hepatoxicity 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 total protein, albumin, alanine-amino transferase (ALT) and aspartate-amino transferase (AST) were evaluated. Histopathology of the liver was also studied. In cadmium challenged rats, compared to control, total protein and albumin levels were significantly reduced while ALT activity was significantly raised at the two-time intervals. Histological observations showed varying degree of hepatotoxicity. 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, hepatotoxicity, protective potentials.

1.0 Introduction

Liver is an abdominal organ which plays a vital role in detoxification and excretion of many endogenous and exogenous substances. The liver is a natural chemical factory which aids anabolism of complex molecules from simple substances absorbed from the gastro-intestinal tract (GIT). It neutralizes toxins, and manufactures bile which aids fat digestion and removes toxins through the bowels (Buraimoh *et al.,* 2011). Continuous exposure and intoxication of liver to different types of exogenous compounds on a daily basis may lead to hepatic dysfunction (Nithya *et al.,* 2012). Hepatic dysfunction due to exposure to environmental toxic agents is increasing worldwide. Among the various known hepatoxins, cadmium (Cd) preferentially localized in liver cells and causes liver injury. Cadmium is known for its deleterious effects on hepatocytes among which are enhancement of lipid peroxidation and oxidative damage through free radicals generation (Manca *et al.,* 1991; Alhazza, 2008).

Management of liver disease is still a challenge to the modern medicine and conventional medicine is now pursuing the use of natural products such as herbs to complement the liver needs (Gayatri *et al.,* 2011). Therefore, different plants are being evaluated on daily basis for their possible antioxidant and hepatoprotective effects against different chemical-induced liver damage in experimental animals.

Momordica charantia is a tendril bearing economically important medicinally vine belonging to the family cucurbitaceae (Paul and Raychaudhuri, 2010; Bakare et al., 2010). 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 (Kumar and Bhowmik, 2010). This study was designed to investigate the hepatoprotective potentials of the methanolic leaf extract of *Momordica charantia* on the serum protein, albumin, aspartate amino transferase (AST), alanine amino transferase (ALT), and histology of cadmium-induced liver damage.

2.0 materials and methods

2.1 Plant material

Fresh leaves of *Momordica charantia* (Bitter melon) were collected form 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.

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 (350g) 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 Virdii *et al.,* 2003. 500mls 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 4 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 220 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 1ml 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 300mg/kg body weight methanolic extract of *Momordica charantia* 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 six weeks. Five blood samples were collected by ocular puncture after 3 and 6 weeks of treatment and serum separated for various biochemical assays. Five rats were sacrificed by cervical decapitation at the two time intervals previously mentioned and liver tissues were removed, cleaned, and immediately fixed in 10% formalin for histological study.

2.4 Biochemical liver function estimations

The liver function parameters estimated from the serum were protein, albumin, aspartate amino transferase (AST), and alanine amino transferase (ALT). The activities of serum aspartate amino transferase (AST) and alanine amino transferase (ALT) were assessed by the methods of Reitman and Frankel (1957). The total protein in the serum was estimated using the method of Cheesbrough (1991). Serum albumin was determined by the dye bromocresol-green method of Doumas *et al.,* (1971).

2.5 Histopathological study

Five rats were sacrificed by cervical decapitation at the two time intervals previously mentioned and liver 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 ± 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.

3.0 Results

3.1 Biochemical estimations

Cadmium was metabolized in the liver by its microsomal enzymes. Cadmium intoxication could result into abnormal biochemical changes which were reflected in the serum. Table 1 showed the effect of cadmium and extract of *Momordica charantia* on serum total protein (TP) and albumin (ALB) levels at three and six weeks post-treatment. In cadmium challenged rats, compared to control, total protein and albumin levels were significantly reduced at the two-time intervals ($p < 0.05$). However, the toxic effect of cadmium was significantly controlled in the rats pre-treated with methanolic extract of Momordica charantia at the two time intervals ($p< 0.05$). Table 2 represents the effect of cadmium and extract of *Momordica charantia* on the activities of serum marker enzymes alanine amino transferase (ALT) and aspartate amino transferase (AST). In the cadmium only treated rats, administration of cadmium resulted into increase in the activity of ALT at three weeks post-treatment while the activity was significantly reduced at six weeks post-treatment ($p < 0.05$). In the rats pre-treated with extract of *Momordica charantia*, the activity of ALT was significantly reduced compared to both control and cadmium groups at the two-time intervals ($p < 0.05$). However, there was no significant difference in the activity of AST in all the groups at the two-time intervals.

3.2 Histological study

Figures 1 and 2 showed the histological studies of the liver tissues of the control, cadmium-only treated, and MC extracts pre-treated rats at the third week and sixth week post-treatment stages respectively. Cadmium caused marked damage of rat hepatocytes in the form of fatty degeneration, cytoplasmic vacuolations, and focal and diffused hepatocellular necrosis. There was deposition of cadmium around the portal vein causing portal tract fibrosis. The hepatocytes showed varying features of cytotoxicity ranging from a swollen nucleus and fragmented nucleolus to clumping of the nucleolus. Pre-treatment with methanolic extract of *Momordica charantia* leaves showed appreciable degree of recovery evidenced by numerous *Kupffer cells* in the liver tissues of rats pre-treated with the extract.

4.0 Discussion and conclusion

Cadmium is one of the most commonly used hepatotoxins in the experimental study of liver diseases. Cadmium is also one of the most toxic industrial and environmental metals that cause health hazard (Alhazza, 2008). One of the major functions of the liver is to detoxify xenobiotics and toxins, and administration of cadmium induces liver toxicity in animals (Andrade *et al.,* 2007; Arundel and Lewis, 2007; Nithya *et al.,* 2012). The results of our study showed that cadmium reduced total protein and albumin levels. The reduction in the concentrations of these metabolites is an indication of severe liver injury because liver is the principal organ involved in protein synthesis. Cadmium-induced hepatotoxicity is associated with production of reactive oxygen species which interact with membrane lipids of the liver cells to produce lipid peroxides (Dimitrova and Tishinova, 1989). These reactive oxygen species attack essential cell constituents such as proteins, lipids and nucleic acids (Stadman and Berlett, 1997). In this study the decrease in serum total protein levels in the rats exposed only to cadmium may be due to the attack of the reactive oxygen species on serum proteins. The normal serum levels of total protein and albumin in rats pre-treated with methanolic extract of *Momordica charantia* (MC) showed that the extract of MC has some degree of hepatoprotective ability.

The result of this study also showed increase in the activity of serum marker enzyme alanine amino transferase (ALT) at the end of three weeks treatment in cadmium challenged rats. This is a reflection of damage caused to liver by cadmium toxicity. Hepatopathy could lead to leakage of marker enzymes into the blood in conformity with the extent of liver damage (Nkosi *et al.,* 2005; Nithya *et al.,* 2012). Decreased level of ALT in cadmium treated rats at the end of six weeks treatment may be due to chronic and prolonged destruction of hepatocytes. The activity of ALT was found to be significantly reduced in the rats pre-treated with extract of *Momordica charantia* (MC) compared to that of the toxicant rats. This confirms the protective effect of extract of MC against cadmium-induced hepatic damage. A possible mechanism of the *Momordica charantia* extract as hepatoprotective may be due to its anti-oxidant effect as a result of its high flavonoids, ascorbic acids, phenols, triterpenes and alkaloids contents (Chaudhari *et al.,* 2009; Hossain *et al.,* 2011; Nithya *et al.,* 2012).

The results of histology of our study further showed that cadmium causes varying degree of liver damage ranging from fatty degeneration, cytoplasmic vacuolations, and focal and diffused hepatocellular necrosis to portal tract fibrosis. This supports previous studies (Chaudhari *et al.*, 2009). These pathological changes are as a result of oxidative damage by free radicals generation (Chaudhari *et al.*, 2009). These pathological changes were prevented to moderate extent in rats pre-treated with methanolic leaf extract of *Momordica charantia* in this study. This might be due to pronounced antioxidant properties and anti-lipid peroxidation activities of *Momordica charantia* (Chaudhari *et al.,* 2009; Hossain *et al.,* 2011; Nithya *et al.,* 2012).

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

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Table 1: Effect of cadmium and extract of *Momordica charantia* (MC) on serum total protein, TP, (g/L) and albumin, ALB, (g/L) levels of male rats.

Treatment Group	Control		Cadmium		Cadmium and Extract of MC	
Weeks	TP	ALB	TP	ALB	TP	ALB
3	63.45±2.19	27.67±0.97	52.45±3.23**	24.53±1.14	60.20±2.09	23.52±2.02
6	43.41±4.79	18.52±2.83	8.46±0.02**	3.19±0.01**	47.52±5.45¨	20.68±2.48¨

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

Table 2: Effect of cadmium and extract of *Momordica charantia* (MC) on serum aspartate amino transferase, AST, (U/L), and alanine amino transferase, ALT, (U/L) levels of male rats.

Treatment Group	Control		Cadmium		Cadmium and Extract of MC	
Weeks	AST	ALT	AST	ALT	AST	ALT
3	82.20±6.80	59.40±10.59	84.60±4.40	69.00±7.16	84.00±5.00	38.80±5.84**¨
6	68.00±12.37	46.75±11.08	69.50±0.65	13.50±0.65**	68.75±11.75	29.75±3.61**¨

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

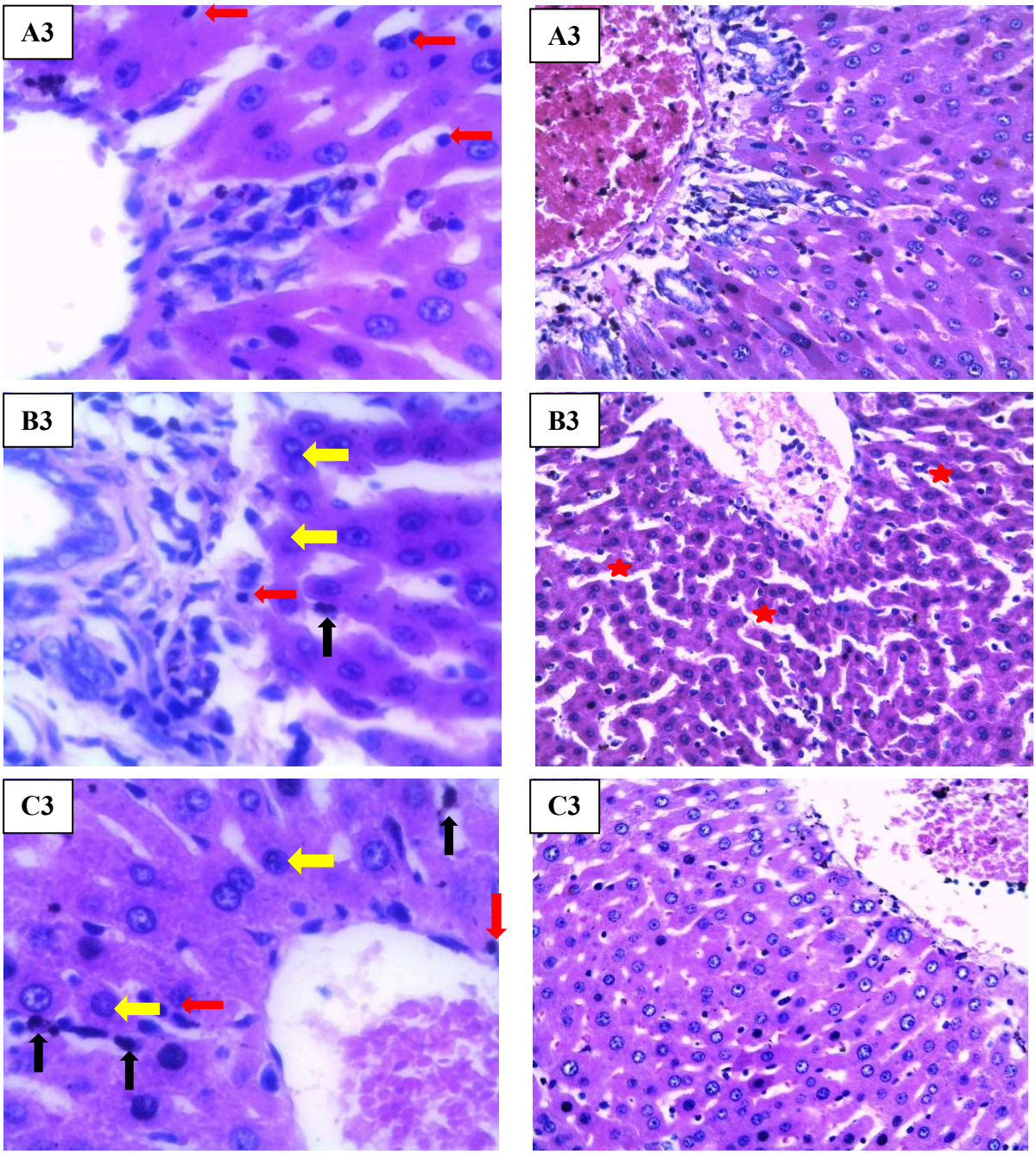

Figure 1: Photomicrographs of the liver tissues of A (control), B (cadmium only) and C (MC extract pre-treated) rats at 3rd week post-treatment stage showing a normally radiating hepatocyte plate in the Groups A and C. Group A showed a less sinusoidal space with darkly stained round nuclei of hematopoietic cells (red arrow) which is not so prominent in the group C. Groups B and C showed Kupffer cells (black arrow) in close relation to the hepatocytes and a larger sinusoidal space in Group B (red star). Degenerating hepatocyte are seen in the groups B and C (yellow arrow). General histo-architectural distortion is very obvious in Group B. Hepatocytes in Group B appear smaller in size compared to that of Groups A and C. Stain H&E. Mag. X1000 Left panel, X 400 Right panel.

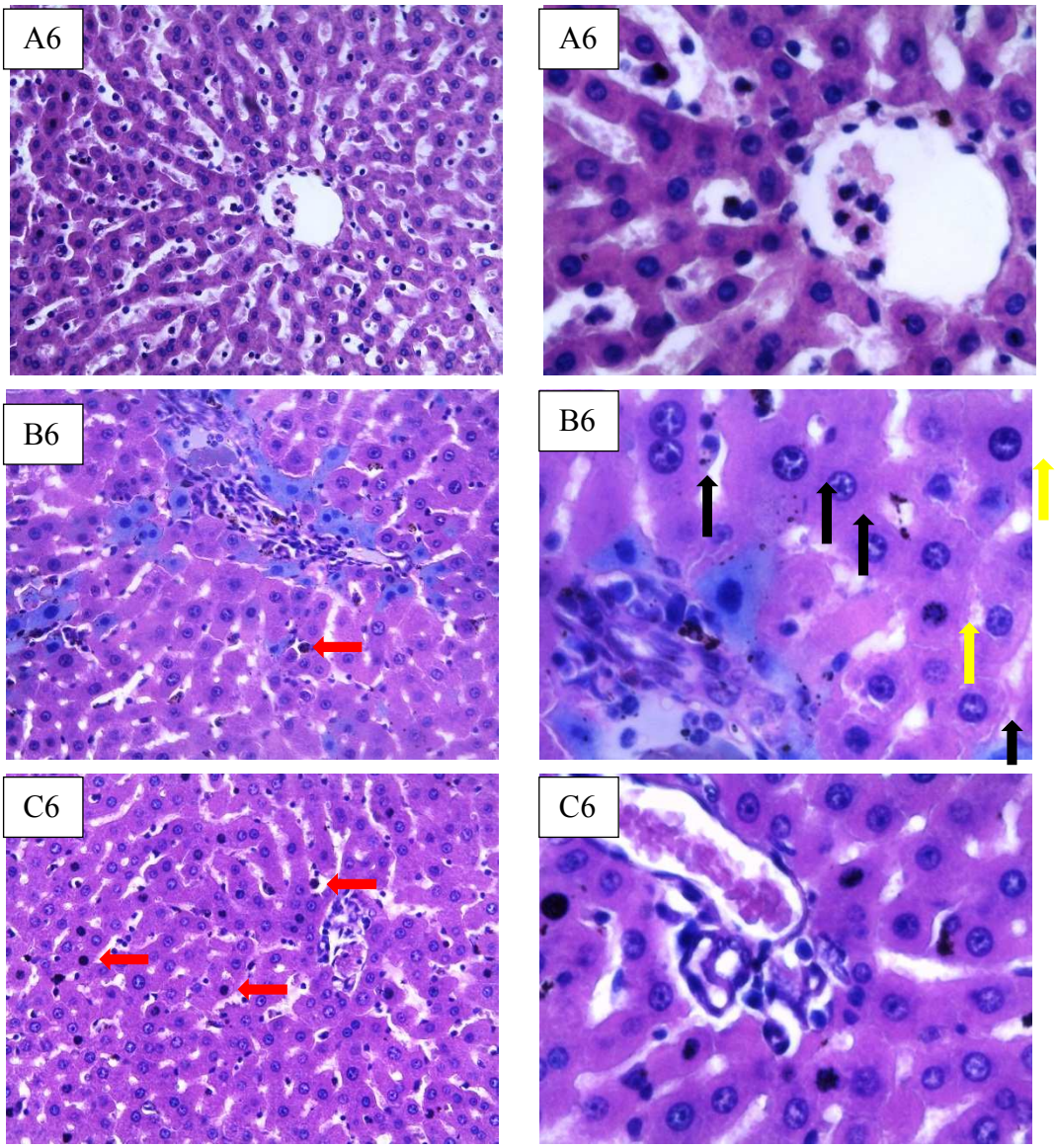

Figure 2: Photomicrographs of the liver tissues of A (control), B (cadmium only) and C (MC extract pre-treated) rats at 6th week post-treatment stage showing the hepatic plate around the central vein (A slides) and the portal veins (B and C slides). Left panels are of lower magnification than the right panel. A bluish stained deposit is noted around the portal vein of B while such deposit on C is minimal. The nuclei of the hepatocytes in B showed varying features of cytotoxicity ranging from a swollen nucleus and fragmented nucleolus (black arrow) to clumping of the nucleolus (yellow arrow). The ray of hepatic plate is well outlined in A while there is disruption in B, C showed a slight recovery of the hepatic plate arrangement. Numerous black stained dot-like deposits (Kupffer cells) (red arrow) are seen in the B and C slide, it is however more of the C slides. Stain H&E. Mag. X400 Left panels, X 1000 Right panel.