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Original Research Article

Ameliorative Effects of D-3-O-Methyl-chiroinositol in Acute and Sub-Chronic Cadmium Chloride Induced Hepatotoxicity

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ABSTRACT

D-3-O-Methyl-chiroinositol (D3O), isolated from the stem bark of *Piliostigma thonningii* has a structural formula similar to phosphatidylinositol phosphate, which participates in the insulin signaling pathways that stimulate glucose transport, and is known to possess strong antioxidant activities. The purpose of the present study was to determine the ameliorative effects of D-3-O-Methyl-chiroinositol on acute and sub-chronic $CdCl_2$-induced hepatotoxicity. Twenty-four rats were assigned to three treatment groups (n = 8). Group A (2 mL distilled water), group B: (2.5 mg/kg b.w. $CdCl_2$ only) and group C: (2.5 mg/kg b.w. $CdCl_2$ and D-3- O- methyl-chiroinositol, 2 mg/kg b.w.). Treatment was daily. Blood was collected from 4 animals per group and analyzed for liver enzymes and a liver section was excised for histopathological analysis at the end of months 1 and 2. The results showed a significant decrease in body and liver weights of the cadmium chloride only challenged group and an increase in the body and liver weights in the D3O-treated and normal groups. D3O significantly ameliorated ($p<0.05$) the effects of $CdCl_2$ on liver enzymes as seen in the elevated levels of AST, ALP and ALT when compared to $CdCl_2$ only-challenged group. Histopathological studies revealed ameliorative and proliferative (revival) changes in the liver after 60 days of treatment. The administration of D-3-O-methyl-chiroinositol provided a significant protection against $CdCl_2$-induced hepatotoxicity and initiated proliferation of liver cells.

Keywords: D-3-O-Methyl-chiroinositol (D3O), *Piliostigma thonningii*, Cadmium chloride ($CdCl_2$), Hepatotoxicity, Liver enzymes.

Introduction

Cadmium is a transition metal possessing a very long biological half-life which is now considered a ubiquitous environmental pollutant in the past decades because of its extensive and continued use in production factories and agriculture.[1] Exposure of individuals to cadmium is associated with bone, lung, renal and hepatic damage,[2] and there is enough evidence in humans to classify cadmium and its related compounds as carcinogenic substances.[3]

Several studies using laboratory animals (mice, rats, and hamsters) have provided clear evidence that cadmium at higher doses is a potent developmental toxicant.[4-6] Cadmium causes oxidative modifications of DNA, such as the formation of 8-hydroxydeoxyguanosine, and the generation of strand breaks in different cell types, for example, liver and kidney cells.[7,8]

Oxidative DNA damage caused by cadmium has been associated with an increased production of free radicals especially reactive oxygen species (ROS),[9] and interactions between this metal and DNA repair enzymes.[10,11] Interestingly, there is evidence suggesting that Cd^{2+} binds covalently to N7 centers of adenine and guanine, and that it can form intra-strand bifunctional adenine-thymine (AT) adducts, suggesting a direct attack on the DNA molecule.[12]

Cadmium induced toxicity has been shown to be alleviated by antioxidants; L -ascorbic acid,[13] broccoli,[14] natural anti-oxidant garlic[15] and naringenin,[16] which is naturally occurring citrus flavonone.

Previous study demonstrated that aqueous extracts of onion and garlic could proffer a measure of protection against Cd-induced testicular oxidative damage and spermiotoxicity by possibly reducing lipid peroxidation and increasing the antioxidant defense mechanism in rats.[17] The hepatoprotective effect of onion and garlic extracts on cadmium (Cd)-induced oxidative damage in rats has also been reported.[18]

D-3-O-Methyl-chiroinositol (D3O) (Figure 1), isolated from the stem bark of *Piliostigma thonningii* has a structural formula similar to phosphatidylinositol phosphate, which participates in the insulin signaling pathways that stimulate glucose transport, and is known to possess strong antioxidant activities.[19] It has been observed that D-3-O-methyl-chiroinositol reduces urinary potency with impaired glucose tolerance, insulin resistance and type 2 diabetes mellitus in rhesus monkeys and human subjects.[20]

The present study was undertaken to assess the effects of D3O in ameliorating acute cadmium toxicity in Wistar rats.

Materials and Methods

Extraction and purification of D-3-O-methyl-chiroinositol

D-3-O-methylchiroinositol (D3O) was isolated from the stem bark of *Piliostigma thonningii*, as described by Asuzu *et al*.[21] The stem bark of the plant was exhaustively extracted with 80% methanol in a Soxhlet extractor at 40°C for 12 h. The pure compound was isolated using a column and TLC, lyophilized and stored in the fridge at 4°C until used for the experiments.[19]

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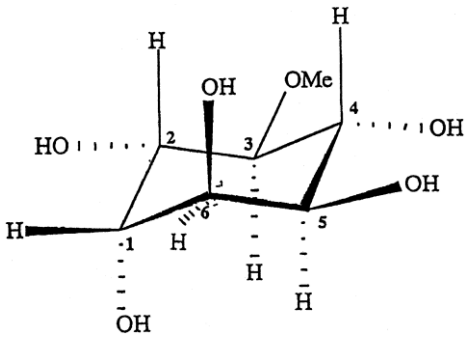

Figure 1: Chemical structure of D-3-O-Methyl-chiroinositol

Animals

The conduct of the research was approved and in accordance with the approved research guidelines on laboratory animal use of the Faculty of Basic Medical Sciences, University of Calabar (Approval Number: 019C20227), where the animal study was carried out. All animals were humanely handled and their welfare respected throughout this study as stipulated in the 1964 Helsinki Declaration, as amended.[22] Twenty-four 10-week- old male Wistar rats weighing 170 - 190 g were obtained from the laboratory unit of the Department of Biochemistry, College of Medical Sciences, University of Calabar, Calabar and were maintained at an ambient temperature between 28 - 30°C, with a humidity level of 55 ± 5 %, and a standard (natural) photoperiod of approximately 12 h of light (6:30 am - 18: 30 pm) alternating with approximately 12 hours of darkness (18:30 pm - 6:30 am). Prior to the experiment, the rats were acclimatized for 7 days with *ad libitum* feed and water. Afterwards, the rats were randomly assigned to 3 groups (n = 8): group A (control) was orally administered 2 mL distilled water; group B was administered 2.5 mg/kg b.w. $CdCl_2$ in drinking water;[23] group C was administered 2.5 mg/kg b.w. $CdCl_2$ in drinking water and D-3-O-methylchiroinositol (D3O) at 2 mg/kg b.w. daily dissolved in 0.5% Tween20 and administered *per os*.[19] The experiment lasted for 2 months. Four animals from each group were humanely euthanized at the end of each month.

Biochemical and liver enzyme analysis

All the animals were bled from the ocular retrobulbar plexus of the eye of anaesthetized rats 12 h after last treatment administration. Full anaesthesia was induced by placing each mouse in an inhalation chamber with 4 % isoflurane (IsoFlo, Abbott Laboratories, Berkshire, UK) regulated with a calibrated vaporizer,[24] and the animals humanely euthanized thereafter. Blood samples were collected into anti-coagulant-free sample bottles and allowed to clot.

The resultant serum was collected into pre-labelled Eppendorf tubes on ice after centrifugation at 3000 rpm for 10 min and used for determination of biochemical parameters. Alanine aminotransferase (ALT) activity was determined using the established method.[25] Aspartate aminotransferase (AST) activity was determined following the Tietz method.[26] Alkaline phosphatase (ALP) activity was done according to described methods.[27] Liver tissues were also harvested for histological examination

Statistical analysis

Data obtained were presented as mean ± SEM and analysed using one-way analysis of variance (ANOVA) and posthoc comparisons were carried out using either Dunnett's t-test or Tukey's test (where appropriate) on GraphPad Prism version 5.01. Values of $P < 0.05$ were considered significant in the study.

Results and Discussion

Effect of D3O on body and liver weights in cadmium chloride induced hepatotoxicity in albino rats

Environmental contamination by Cd is a worldwide problem. Cd is a highly toxic heavy metal and its toxicity occur by ingestion and inhalation.[28] Cadmium is a potent teratogen in laboratory animals, causing hepatotoxicity. Due to its heterogenicity with respect to molecular targets, the mechanisms behind cadmium toxicity are not well understood. The toxic effects of trace elements upon the liver, its hepatocytes and enzymes have been reported in recent years. It is generally considered that $CdCl_2$ poisoning causes histopathological changes in the liver of laboratory animals. Little attention had been drawn to the possible treatment with pure bioactive compounds from plant origin of the toxic effects of $CdCl_2$ on the liver and liver enzymes. Exposure to $CdCl_2$ significantly ($p \leq 0.05$) decreased body weight but body weight increased in control and D3O-treated groups (Table 1). Also, liver weights significantly ($p \leq 0.05$) decreased in $CdCl_2$ only challenged group. Rats treated with $CdCl_2$ plus D3O showed a significant increase in body and liver weights as compared to $CdCl_2$ treated group.

Effect of D3O on liver enzymes in cadmium chloride induced hepatotoxicity in albino rats

The effect of D3O on liver enzymes in cadmium chloride induced liver damage in Wistar rats is shown in Table 1. It was observed that after 60 days of treatment with $CdCl_2$, D3O and their combination to rats, $CdCl_2$-challenged group developed hepatocellular damage that is evident from significant elevation in serum activities of AST, ALP and ALT levels in comparison to control rats. D3O showed significant restoration of the altered biochemical parameters at a dose of 2 mg/kg when compared to $CdCl_2$-challenged group. The liver enzymes (AST, ALP and ALT) in the Control group within the 2 months showed no significant difference from the $CdCl_2$+D3O-treated group. These enzymes were observed to increase in the $CdCl_2$ only-challenged group. Elevated liver enzymes may indicate inflammation or damage to liver cells (hepatocytes). Inflamed or injured liver cells leak higher than normal amounts of certain chemicals, including liver enzymes, into the blood stream, which result in elevated liver enzymes during blood tests. Metabolism and accumulation of $CdCl_2$ occur in liver and kidney so they are prone to Cd toxicity.[29,30] In this study, D-3-O-methyl-chiroinositol 2 mg/kg b.w. was shown to ameliorate the $CdCl_2$ toxicity in liver of rats. The mean body weight of $CdCl_2$ only-challenged group significantly decreased with a significant increase in relative liver weight, which comes in agreement with the findings of El-demerdash *et al*.[31] Rahman *et al.* reported that long-time exposure to Cd is directly proportional to increased risk of diabetes mellitus which expounds the weight loss in rats.[32] Also, exposure to heavy metals is known to deteriorates the glucocorticoid system which in turn is associated with weight loss.[33] The rats treated with D-3-O-methyl-chiroinositol alongside $CdCl_2$ exposure showed significant improvement in liver functions. Significant restoration of hepatic enzymes was observed, so D-3-O-methyl-chiroinositol was hepato-protective against $CdCl_2$ toxicity. The observed protective effect of D-3-O-methyl-chiroinositol may be due to its functional groups which has been reported to possess a membrane stabilizing activity by inhibiting the generation of reactive oxygen species (ROS) and maintaining the cell membrane structural integrity in diabetes.[34] Our findings exhibited elevation in liver enzymes in Cd only-challenged group. These findings come in the same line with Toppo *et al*. who revealed that Cd toxicity causing hepatic cell damage and its enzymes AST, ALP and ALT release into circulation,[35] therefore the level of these enzymes in blood was higher than normal.[36]

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Table 1: Ameliorative effect of D3O on $CdCl_2$-induced hepato-toxicity on body weight, liver weight and enzymes

Group	Control		$CdCl_2$ only		$CdCl_2$+D3O	
Period (month)	**Month 1**	**Month 2**	**Month 1**	**Month 2**	**Month 1**	**Month 2**
Body weight (g)	185.53 ± 1.26^{a}	237.31 ± 5.70^{b}	171.91 ± 1.12^{a}	148.04 ± 12.22^{a}	$205.5 \pm 15.61^{a,b}$	231.02 ± 10.02^{b}
Liver weight (g)	7.63 ± 0.18^{a}	8.53 ± 0.27^{a}	4.87 ± 0.19^{b}	3.93 ± 0.24^{b}	7.83 ± 0.18^{a}	8.10 ± 0.15^{a}
AST (IU/µL)	63.41 ± 1.08^{a}	63.14 ± 0.99^{a}	125.01 ± 2.11^{b}	132.12 ± 0.49^{b}	118.82 ± 0.35^{b}	119.51 ± 1.11^{b}
ALP (IU/µL)	168.91 ± 0.74^{a}	167.80 ± 0.32^{a}	293.43 ± 2.75^{b}	297.61 ± 1.27^{b}	247.73 ± 1.96^{c}	238.33 ± 1.77^{d}
ALT (IU/µL)	38.73 ± 0.20^{a}	37.71 ± 0.35^{a}	193.53 ± 2.09^{b}	197.33 ± 0.66^{b}	135.51 ± 1.16^{c}	124.14 ± 2.09^{d}

Different superscript in a row indicate significant difference at (p≤0.05)

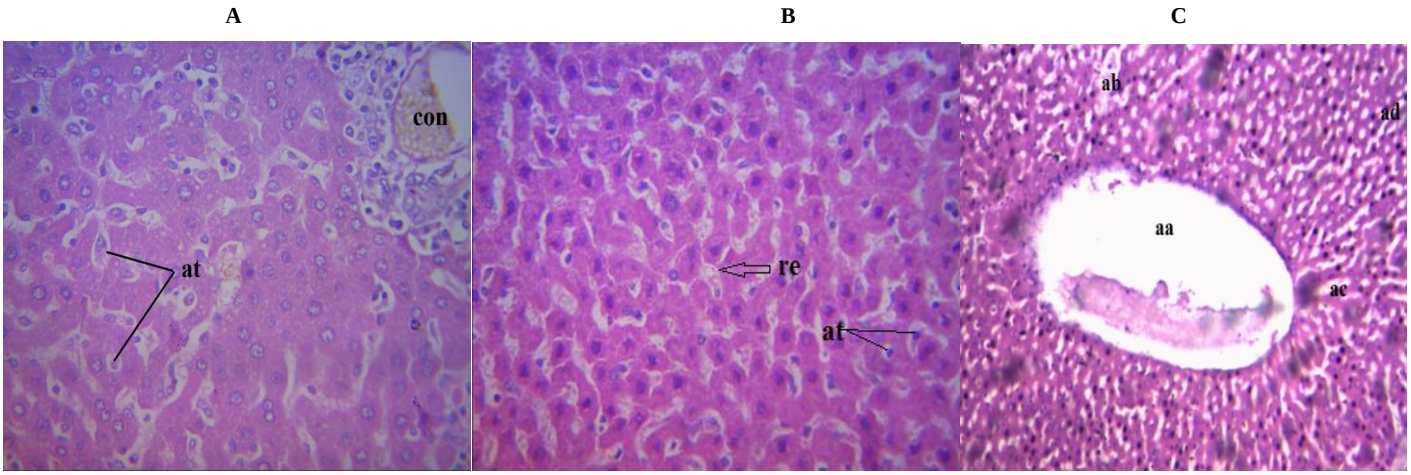

Figure 2: Photomicrograph of liver sections of Wistar rats after 30 days

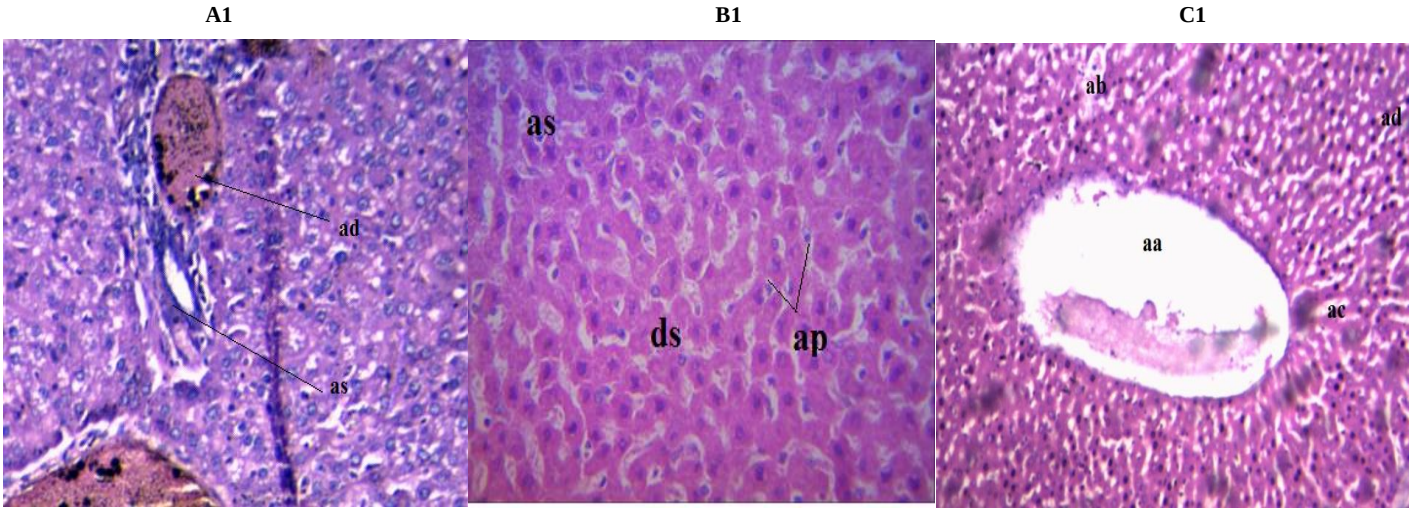

Figure 3: Photomicrograph of liver sections of Wistar rats after 60 days

Histopathology

Figure 2 shows the photomicrograph of liver sections of the negative control group (A), D3O (B) and normal control group (C) after the 30 days of challenge and treatment. In the negative control group (Cd only), the hepatic lobules are seen to be slightly blurred having marked distinction with infiltration and congestion (con). There is noticeable hemorrhage and some fatty acid change (at). In the D3O-treated group (B), slight blurred trabeculae are observed. The hepatic lobules are blurred with marked distinction (re), the cytoplasm of some of the cells show empty vacuoles (at), there is infiltration of erythrocytes and mononuclear cells. In the normal control group (C), presence of lobules, pentagonal in shape is observed with central veins (aa). Peripheral hepatic tetrads in the connective tissue are seen. These hepatocytes (ad) are organized in trabeculaes which are radiantly running from the central venous supply there is a distinction from the sinusoids (ab) which holds the Kupffer cells (ac).

Figure 3 shows photomicrograph of liver sections of the different groups after the 60 days of treatment. In the Cd-only challenged group

(A1), the hepatic lobules are slightly blurred with marked distinction with infiltration and congestion (ad). There is noticeable hemorrhage and some fatty acid change (as). In the D3O-treated group (B1), slight blurred trabeculaes are seen. The hepatic lobules are blurred with marked distinctive fatty changes (ds). The cytoplasm of some of the cells show empty vacuoles and there is infiltration of erythrocytes and mononuclear cells in the sinuses (as). Also, the Kupffer cells are distinguishable in the sinusoid walls (ap). The normal control group (C1) showed presence of lobules that are pentagonal in shape. There are central veins (aa), and peripheral hepatic tetrads in the connective tissue. The hepatocytes (ad) are organized in trabeculaes which are radiantly running from the central venous supply there is a distinction from the sinusoids (ab) which holds the Kupffer cells (ac). The histological studies of the liver sections showed a progressive and high level of cell necrosis and increased number of Kupffer cells with presence of mononuclear cells in the $CdCl_2$ only-challenged group, indicative of toxicity (Figure 2A and 3A1). Histopathology of the Wistar rats treated with D-3-O-methyl-chiroinositol showed absence of cell necrosis and decrease in Kupffer cells (Figure 2B and 3B1). Sinuses overfilled with blood was minor as compared to $CdCl_2$ only-challenged group. The $CdCl_2$ only-challenged group showed necrotic and increased density of nucleated chromatins and there were significant sinuses overfilled with blood showing severe toxicity and damage. From the results of the study, it was evident that D3O administration led to a partial repair of the hepatocytes damage induced by chronic cadmium exposure in the Wistar rats when compared to normal liver sections (Figure 2C and 3C1).

Conclusion

It could be concluded that administration of cadmium chloride at (2.5 mg/kg bw) for 2 months caused significant ($p < 0.05$) elevation in liver function enzyme levels, whereas the daily administration of D-3-O-methyl-chiroinositol after $CdCl_2$ challenge restored levels of liver function to normalcy. $CdCl_2$-administration exhibited severe alterations in the histology of the liver tissues. The administration of D-3-O-methyl-chiroinositol ameliorated liver tissues possibly through facilitating recovery of the hemorrhagic and inflammatory development and through its antioxidant potentials.

Conflict of interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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