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Cadmium-induced hepatorenal-toxicity in rats: Possible ameliorative effect of *Talinum triangulare*

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ABSTRACT: This study investigated the effect of graded doses of aqueous extract of *T. triangulare* on cadmium-induced renal- and hepatotoxiity in rats. Male wistar rats (n = 30), divided into 5 groups; control (n = 6) and test groups (n = 24) were used for the study. The test was divided into groups that received only cadmium (cadmium only) and 3 groups that received cadmium plus graded doses of herbal extract (400, 600 and 1000 mg Kg^{-1} body wt) respectively. Cadmium chloride, 3 $mgkg^{-1}$ body wt.sc was administered only on day 1 of the 14 day study period. Cadmium significantly ($p<0.05$) reduced liver ALT, AST and ALP activities relative to the control. This was accompanied by a significant ($p<0.05$) elevation in plasma activities of these enzymes when compared with the control. It also significantly ($p<0.05$) increased organ/body wt ratio, plasma creatinine and urea levels relative to the control. Though treatment with graded doses of the herbal extract provided varying degrees of mitigation against these cadmium-induced alterations, the highest dose of 1000 $mgKg^{-1}$ body wt was the most effective dose.

Keywords: Cadmium; *T. triangulare;* Hepato-renal toxicity; Environmental pollution; Bioaccumulation.

Introduction

Cadmium is a well known environmental and industrial toxicant, causing significant metabolic alterations and injuries to biological systems at different level (1). It is particularly hazardous because it bioaccumulates in living cells and is neither biodegraded nor biotransformed as a mode of detoxication. Some cadmium has been found in all natural materials that have been analyzed (2). Cadmium has been linked to osteomalacia (3), gonadotoxicity, hepatotoxicity and renal toxicity (3, 4). The most severe form of cadmium toxicity in humans is tai-itai disease (5).

The kidneys and liver are among the major target organs of cadmium accumulation and intoxication (1). With acute exposure, cadmium primarily accumulates in the liver (6). This toxicity is mediated through the formation of free radicals in amounts that overwhelm the natural antioxidant defence system of man and animals (7).

It is well known that the toxic effects of xenobiotics can be modified by other substances (8). Many natural agents which are known to possess antioxidant properties have been used in the management of cadmium toxicity (9,10). Hepatoprotective activities of hydroxyl and polyhydroxyl-organic compounds found in vegetables, fruits and some herbs have been reported (11). Investigation into the phytochemical constituents of *T. triangulare*, (12), revealed the presence of Omega 3 fatty acids and high levels of nutritionally important antioxidant vitamins (vitamins C, E and betacarotene) and minerals (magnesium) as well as soluble fibres (pectin). The antinutrients present in the plant include saponins, phytate, tannic acid and oxalate (13). Phytate has been reported to have metal-chelating and antioxidant effects (14,15). It has been shown that phytate is well absorbed in rodents based on its distribution to various organs as early as 1hr after administration (16).

Synthetic metal chelators are currently used in the management of cadmium toxicity. However, one of the major criticisms against their continued use is that they lead to higher degree of kidney damage than will be caused by cadmium alone (17). Medicinal plants have the advantage of having little or no side effects (18).

Some of them have been used by trado-medical practitioners for hundreds of years in many countries of the world. The aim of this study is to investigate the effect of *T. triangulare*, which contains natural metal chelating principles, on cadmium-induced hepatic and renal toxicity in rat. Hepatic and renal toxicity were assessed by determining the plasma and liver activities of Alanine and Aspartate transferases (ALT and AST) and alkaline phosphatise (ALP) activities, plasma creatinine, and urea levels in rat.

Materials and Methods

The experiment was conducted on 30 male albino rats (Wistar strain) with an average weight of 187.67 ± 4 g. They were housed in stainless steel cages with wire mesh floors and allowed an acclimatization period of 2 weeks prior to the beginning of the study. Throughout the 14 days treatment period, animals were allowed free access to commercial rat chow (BFFM Ltd, Ewu, Nigeria) and water. They were randomly divided into 5 groups of 6 rats each. Group 1(control) was both cadmium (Cd) and aqueous extract of *T. triangulare* (ET) free. Rats in group 2 received Cd only while those in groups 3, 4 and 5 received Cd and graded doses of ET (400, 600 and 1000 mg Kg^{-1}) respectively. The herbal extract was administered daily by gavage while Cd (3 mg Kg-1) and /or its vehicle (normal saline) were administered subcutaneously, on day 1 of study. The rats were weighed once every week and accordingly, the dose of Cd and ET were adjusted on a weekly basis. These treatments were carried out in accordance with the principles of laboratory animal care (NIH publication no. 85-93, revised 1985).

Preparation of *Talinum triangulare P*lant Extract

T. triangulare (1000g) leaves, collected from a village near Benin City, were sun-dried and pulverized with mortar and pestle. It was soaked overnight in 3L of distilled water. Thereafter, it was filtered with fine muslin cloth and concentrated to a constant weight at $40^{O}C$ in a rotavapor apparatus. The residue was collected and stored in a refrigerator. The concentrate was then reconstituted into a stock solution of 400 mg / ml in distilled water. The appropriate volume of this solution (calculated on the basis of animal weight) was administered daily by gavage.

Preparation of Plasma Samples

At the end of the two weeks study period, animals were sacrificed under chloroform anesthesia. Fresh blood was collected in heparinized tubes and centrifuged at 3,500 rpm for 5 minutes. The resultant plasma was neatly collected into a clean plain sample bottle.

Preparation of Tissue Homogenate

Weighed portions of the tissues were homogenized in ice cold normal saline. The homogenates were centrifuged at 3,500 rpm for 15mins and the clear supernatant obtained was immediately used for the analysis of antioxidant enzymes.

Biochemical analysis

Glutamic-oxaloacetic transaminase (GOT) also known as Aspartate transaminase (AST) activity was determined by monitoring the concentration of oxaloacetate hydrazone formed with 2,4-dinitrophenylhydrazine. AST activity (IU/L) in each sample was extrapolated from a standard calibration curve provided by the commercial test kit manufacturer, Randox Laboratories, U.K. Similarly, Alanine transaminase (ALT) also known as Glutamic-pyruvic transaminase (GPT) was measured by monitoring the concentration of pyruvate hydrazone formed with 2,4–dinitrophenylhydrazine. Again, ALT activity (IU/L) in each sample was extrapolated from a standard calibration curve provided by the commercial test kit manufacturer, Randox Laboratories, U.K.

The activity of alkaline phosphatase (ALP) was determined according to the method described by GSCC *(DGKC),* (1972) and as modified by Amino and Giese (19). The substrate 4 – nitrophenylphosphate (colourless) in the presence of alkaline phosphatase is oxidized to 4 –nitrophenyl (yellow) at an alkaline pH as indicated. The ALP activity in IU/L was calculated as 3300 multiplied by the change in absorbance/minute.

In the determination of creatinine, the method employed was the colorimetric method with an initial deproteinization of the sample (20). Concentration of creatinine in plasma was calculated as A_{sample} / $A_{standard}$ X concentration of standard (mg/dL), where concentration of standard =2mg/dL

Plasma Urea was determined according to the method of (21). Urea in plasma is hydrolyzed to ammonia in the presence of urease. The ammonia is then measured photometrically by Berthelot's reaction. The concentration of urea in each sample was calculated as A_{sample} / $A_{standard}$ X concentration of standard (mg/dL), where concentration of standard =80mg/dL

Statistical analysis

The results were expressed as means ± SD. Statistical analysis was by one way Analysis of Variance (ANOVA). The differences between the means were tested by Turkey Kramer Multiple Range tests. Values of $P<0.05$ were considered statistically significant.

Results

The effects of *T. triangular*e on cadmium-induced changes in alanine aminotransferase (ALT), Aspartate aminotranferase (AST) and alkaline phosphatase (ALP) activities in plasma and liver are presented in Table 1.

Table 1: The Effect of *T. triangulare* on Alanine amino transaminase (ALT), Aspartate transaminase (AST) and Alkaline phosphatase (ALP) in liver and blood of cadmium-exposed rat.

TREATMENT GROUPS	PARAMETERS					
	Liver			Plasma		
	ALT (U/g tissue)	AST (U/g tissue)	ALP (U/g tissue)	ALT (U/L)	AST (U/L)	ALP (U/L)
Control	0.507 ± 0.015	3.640 ±0.037	0.074 ±0.014	63.680 ±2.110	51.000 ±1.004	87.703 ±5.910
Cd Only	0.467 ± 0.004*	2.771 ±0.416*	0.055 ±0.011*	72.401 ±0.690*	66.002 ±1.716*	119.015 ±3.102*
CdET400	0.483 ±0.008*	3.750 ±0.409[a]	0.167 ±0.028 [a]*	70.920 ±1.282*	51.330 ±1.151 [a]	119.083 ±7.295*
CdET600	0.510 ±0.012 [a]	3.093 ±0.194	0.201 ±0.012 [a]*	67.691 ±2.771 [a]	33.331 ±2.520 [a] *	107.417 ±2.556*
CdET1000	0.487 ±0.007	3.260 ±0.095	0.107 ±0.004 [a]	63.322 ±0.160 [a]	39.667 ±1.528 [a] *	78.875 ±10..557[a]

Values are means ±SD (n=6)

*Values are significantly ($p<0.05$) different from control

[a] Values are significantly ($p<0.05$) different from the group that was treated with cadmium only group

Table 2: Effect of *T. triangulare* on organ/body wt ratio, plasma creatinine, urea and total protein levels in cadmium-exposed rats

TREATMENT GROUPS	PARAMETERS					
	Liver weight (g)	Liver/body wt ratio	Kidney weight (g)	Kidney/body wt ratio (x 10^{-2})	Plasma	
					Creatinine µmol/L	Urea µmol/L
Control	7.001 ±0.420	0.037 ±0.002	1.301 ±0.080	0.600 ±0.010	0.082 ±0.005	9.693 ±3.343
Cd Only	8.625 ±0.620*	0.047 ±0.004*	1.247 ±0.031	0.687 ±0.049*	0.099 ±0.004*	15.539 ±0.670 [a]*
CdET400	8.951 ±0.305*	0.048 ± 0.001*	1.170 ±0.020	0.680 ±0.020*	0.097 ±0.002*	9.906 ±0.853 [a]
CdET600	7.350 ±0.331 [a]	0.043 ±0.003*	1.174 ±0.042	0.723 ±0.015 *	0.101 ±0.001*	8.842 ±1.200 [a]
CdET1000	7.572 ±0.435 [a]	0.042 ±0.004	1.132 ±0.101*	0.717 ±0.025*	0.090 ±0.008	8.093 ±1.485 [a]

Values are means ±SD (n=6)

*Values are significantly ($p<0.05$) different from control

[a] Values are significantly ($p<0.05$) different from the group that was treated with cadmium only group

Results show that cadmium significantly ($p<0.05$) reduced ALT, AST and ALP activities in the liver relative to the control. This was accompanied by a corresponding increase ($p<0.05$) in the plasma levels of these enzymes, relative to the control. In the liver, treatment with the herbal extract at a dose of 600 mg Kg^{-1} body wt resulted in significantly ($p<0.05$) higher levels of ALT and ALP when compared with the cadmium only group. The graded doses of *T. triangulare* offered significant ($p<0.05$) protection against cadmium-induced reduction in liver ALP activity relative to the cadmium-only group. However, only the doses of 600 and 1000 mg herbal extract Kg^{-1} body wt had a similar effect on liver ALT activity relative to the control. Cadmium-induced increase in plasma ALT and AST was significantly ($p<0.05$) reduced by the herbal extract at doses of 600 and 1000 mg Kg^{-1} body wt when compared to the cadmium only group. Only the dose of 1000 mg herbal extract Kg^{-1} body wt significantly ($p < 0.05$) decreased the cadmium-induced elevation in plasma ALP activity whereas the 600 and 1000 mg Kg^{-1} body wt reduced ALT activity to levels that were not statistically different from control.

The organ/body wt ratio of cadmium-exposed rats was presented in Table 2. A significant ($p<0.05$) increase in organ / body weight ratio was observed in both the kidney and liver of all the cadmium-exposed rats when compared with the control. In this study, cadmium toxicity of the kidney was assessed by the estimation of creatinine and urea levels in plasma. Cadmium treatment resulted in a significant ($p<0.05$) increase in urea and creatinine when compared with the control. Although treatment with the graded doses of *T. triangulare* effectively reduced urea to levels that were not statistically different from control, only the highest dose of the extract had a similar effect on the plasma creatinine level (Table 2).

Discussion

The present study examined the effect of graded doses of aqueous extract of *T. triangulare* on the liver and kidney of cadmium-exposed rats. Cadmium reduced the activities of ALT, AST and ALP in the liver. This effect on these marker enzymes have been reported in our earlier publication (9) and by others (22). Transaminases play an important role in amino acid metabolism and are found in the cells of almost all

bodytissues. Alanine aminotransferase (ALT) is the most specific marker of liver cell damage while ALP is considered an enzyme of hepatocyte plasma membrane (23). Thus an increase in the plasma activities of these enzymes, as observed in this study, is related to liver damage.

Cadmium has been reported to accumulate primarily in the liver and kidney because these organs are rich in metallothionein (24). The presence of more cadmium in the liver than in the kidney, muscle and testes after a single intraperitoneal administration has been demonstrated (25). They reported redistribution from the liver to other tissues, particularly the kidney. Two mechanisms have been proposed to account for cadmium toxicity of the liver. One involves a direct action of free cadmium (not bound to metallothionein) ions (26) and the other, the formation of reactive oxygen species which may overwhelm the body's antioxidant defence system (7) and damage the hepatocyte membranes. Upon destruction and consequent increased permeability of the hepatocyte membrane, these enzymes are released into the blood and their levels are elevated. The highest dose of *T. triangulare* extract used in this study demonstrates protective action against cadmium-induced hepatocyte damage, since they normalized the activities of liver and plasma enzymes, ALT, AST and ALP. This positive effect might not be unrelated to the high flavonoid and phytate content of *T. triangulare* extract (13, 27)

The kidneys are the primary means by which urea and creatinine are eliminated from the body. These are almost entirely cleared by glomerular filtration. Cadmium toxicity of the kidney has been associated with the impairment of renal tubular and glomerular functions (28). This may account for the elevated levels of urea and creatinine in the plasma of cadmium-exposed rats, observed in the present study. The damaging effect of cadmium on the kidney has also been reported by others (3, 22). The highest dose of *T. triangulare* extract (1000 $mgKg^{-1}$body wt) exhibited significant mitigation against cadmium-induced elevation of both urea and creatinine levels in plasma. Though the other doses of the extract protected against increase in plasma urea level, they were not effective in protecting against the elevation of plasma creatinine.

This study has shown that though treatment with *T. triangulare* offered varying degrees of protection against cadmium-induced decrease in liver ALT, AST and ALP activities, the 1000 mg Kg^{-1} body wt was the most effective.

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