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RESEARCH PAPER

HEPATOPROTECTIVE ROLE OF *MORINGA OLEIFERA* ETHANOLIC LEAF EXTRACT ON LIVER FUNCTIONS (BIOMARKERS) IN CADMIUM CHLORIDE INDUCED HEPATOTOXICITY IN ALBINO WISTAR RATS

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ABSTRACT

This study investigated the hepatoprotective role of ethanolic extract of *Moringa oliefera* (M.O) on liver function (Biomarkers) in cadmium chloride ($CdCl_2$)-induced hepatotoxicity on the liver of albino Wistar rats. Sixty-six adult male albino wistar rats weighing between 130g – 180g were used. LD_{50} was determined for both $CdCl_2$and MO using twenty-six (26) rats, while 40 rats were used for the experiment proper. The experimental rats were distributed into eight groups –A (control) and B, C, D, E, F, G and H, served as the treatment groups that received graded doses of $CdCl_2$or MOor both simultaneously or at separate periods. The animals were then euthanized for sample collection and analysis using standard methods. The results showed significant increase ($P<0.05$) in the serum levels of Alanine aminotransferase (ALT) (430.50 ± 149.20), Aspartate aminotransferase (AST) (421.10 ± 8.34) and Alkaline Phosphatase (ALP) (515.60 ± 21.78) of animals in group B, but some protective effect of MO in the treatment groups with significant decreases in ALT, AST and ALP levels. The study therefore concludes that ethanolic extract of *Moringa oleifera* showed appreciable hepatoprotective values on liver functions (biomarkers) in $CdCl_2$hepatotoxicity.

Key words: Liver Functions, Cadmium, *Moringa oleifera*, Hepatoprotective, Hepatotoxiciy

INTRODUCTION

Moringa oleifera is a tropical multipurpose plant that naturally grows in India, South Saharan of Africa and South America and has been used to combat malnutrition, especially among infants and nursing mothers (Anwar et al., 2007; Kumar, 2004).*Moringa oleifera* (*Moringaceae*) commonly known as Ben oil plant or drumstick plant in English language, '*Okweoyibo'* in Igbo, '*Gwara'* or '*Habiwal'* in Hausaand '*Adagbamaloye'* or '*Ewe Igbale'* in Yoruba grows rapidly in most regions of Nigeria (Anwar *et al*., 2007).The bark, sap, roots, leaves, seeds and flowers are used in traditional medicine(Sandoval and Jimeno, 2013).

Around the world, every part of the *Moringa oleifera* tree has been used effectively against varying ailments. Moringa tree contains many nutrients such as essential vitamins, essential minerals, phytochemicals, amino acids, antioxidants, anti-inflammatory nutrients, beta-carotene and it also contains Omega-3 and Omega-6 fatty acids (Anwar *et al*, 2007). The

antioxidants and nutrients present in Moringa leaf help to inhibit the activity of the free radicals in cells and tissue, especially on the liver giving a hepatoprotective effect on the liver cells (Ezejindu *et al,* 2014).
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 metabolism and detoxification of complex molecules. It neutralizes toxins, and manufactures bile which aids fat digestion and removes toxins through the bowels (Maton *et al*., 1993; Ajilore and Ayannuga, 2012). 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).

Cadmium chloride is an inorganic toxicant of great environmental and occupational concern, which was classified as a human carcinogen in 1993 (International Agency of Research on Cancer Monographs, 1993). Most of the available epidemiological information obtained from occupationally exposed populations in highly contaminated areas has established that excessive Cadmium exposure produces adverse health effects (Järup, 2000).Cadmium is known to cause multiple organ damage by promoting free radicals generation and forming complexes that interfere with mitochondrial and cell nucleus activities (McMurray and Tainer, 2003). People with higher levels of cadmium in their urine (evidence of chronic exposure to the heavy metal found in industrial emissions and tobacco smoke) appear to be nearly 3.5 times more likely to die of liver disease than those with lower levels of cadmium (Hopkins *et al.,* 2013).

Humans are generally exposed to cadmium by two main routes; inhalation and ingestion (Waisberg*et al*., 2003). Occupational exposure to cadmium occurs through working with cadmium containing pigments, plastic, glass, metal alloys and electrode material in nickel-cadmium batteries (Waisberg*et al*., 2003). Non occupational exposure occurs through food, water and cigarette smoking (Waisberg*et al*., 2003).Vinodini *et al*.(2014) suggested the *Moringa oleifera* canimprove the liver functions in cadmium chloride induced rats when pre-treated with *Moringa oleifera extract.Moringa Oleifera* has been reported to possess six times more potent antioxidant property than orange (Anwar *et al*., 2007). It has been used to scavenge reactive oxygen species in heavy metals induced organ damage in animal models (Siddhuraju and Becker, 2013).

The aim of this study is to evaluate the hepatoprotective effect of *Moringa oleifera* ethanolic leaf extract on liver function $CdCl_2$ -induced hepatotoxicity on the liver of Albino Wistar rats.

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MATERIALS AND METHODS

Location and Duration of Experiment: This study was conducted in the Department of Anatomy, Faculty of Basic Medical Sciences, College of Health Sciences, Nnamdi Azikiwe University, Nnewi Campus, Anambra State. The animals were acclimatized for two weeks and the actual experimental protocol lasted for 6 weeks.

Animals: Sixty-six (66) male albino Wistar Rats weighing between 130-180g (26 for LD_{50} determination and 40 for the experiment proper), were purchased from Nnamdi Azikiwe University Animal Farm, Nnewi, Nnewi North Local Government Area, Anambra state.

Plant: Fresh Moringa oleifera leaves were collected from a farm at Iyienu, Ogidi, Idemili North Local Government Area, Anambra state. Identification of this plant was carried out in the Department of Botany, Nanmdi Azikwe University (NAU). The leaves of fresh Moringa oleifera plant were shade dried under regulated temperature in a chamber for two (2) weeks, after which the leaves were pulverized into coarse form with high speed milling machine. The coarse form was then macerated in absolute ethanol. This was left to stand for 24 hours. After that, the extract was filtered using muslin cloth on a plug glass wool in glass column. The resulting ethanol extract was concentrated and evaporated to dryness using rotary evaporator at an optimum temperature which was between 40^0C and 45^0C to avoid denaturation of the active ingredients. The concentrated extract was stored in the refrigerator (10^oC) until use. This was done according to Ugwu*et al*., (2013).

Chemical: Cadmium Chloride was obtained from Link-Gate Chemical Store (a registered chemical shop) at Uga, Onitsha South Local Government Area, Anambra state. Other chemicals that were used for the liver function test such as dinitrophenylhydrazine reagent, NaOH, p-nitrophenol, diazotized sulfanilic and acid di-methylsulfoxide were also bought from the same store.

Median Lethal Dose: LD_{50} was carried out for both cadmium and Moringa oleifera ethanolic leave extract using modified Dietrich Lorke, (1983). LD_{50} of $CdCl_2$was 315.23mg/Kg, while LD_{50} of M.O was above 5000mg/Kg

Experimental Protocol: All experimental investigations were done in compliance with "humane animal" as stated in the "Guide to the care and use of Laboratory Animals Resources" (NRC, 2011). Acclimatization was done for two weeks.After acclimatization, animals were group as follows:

Group A (control group): Receiving feeds and distilled water only
Group B: $CdCl_2$ 50mg/Kg daily for 4 weeks
Group C: $CdCl_2$ 50mg/Kg + M.O 1000mg/kg for 4weeks.
Group D: $CdCl_2$ 50mg/Kg + M.O 2000mg/kg for 4weeks
Group E: M.O 1000mg/Kg for 2weeks then $CdCl_2$ 50mg/kg + moringa 1000mg/kg for 4weeks
Group F: M.O 2000mg/kg for 2weeks then $CdCl_2$ 50mg/kg + moringa 2000mg/kg for 4weeks
Group G: $CdCl_2$ 50mg/kg only for 4weeks then M.O 1000mg/kg for 2weeks
Group H: $CdCl_2$ 50mg/kg only for 4weeks then M.O 2000mg/kg for 2week

Animal Sacrifice and Collection of Blood Samples: At the end of the experiment (4weeks for Group A, B, C and D; 6weeks for E, F, G and H) of treatment (i.e. 24hours after last administration), animals were anesthetize with diethyl ether in a close jar, blood samples were collected directly from the apex of the heart (at the thoracic region) using 2ml syringes and put into plain serum bottle, and then serum were separated by centrifugation and was stored in a refrigerator of temperature -18^{o}c for biochemical analysis. Thereafter the animals were then euthanized with diethyl ether.

Biochemical Analysis: Blood samples collected and centrifuged were used for liver function assessment i.e. analyzing the activities of Alanine aminotransferase (ALT) and Aspartate aminotransferase (AST) as described by Reitman and Frankel, (1957), Alkaline phosphatase (ALP) as described by Kind et al,(1980) and total and direct biluribin as described by Dangerfield and Finlayson, (1953).

Statistical Analysis: Data were expressed as Mean ± Standard Deviation (SD) using tables. The differences between the groups were compared for statistical significance by using ANOVA and student 't' test. $p<0.05$ was taken as the significant level.

RESULT

Physical Observation: Daily oral administration of cadmium chloride (50mg/kg) to animals in Group B for 4weeks induced obvious symptom of toxicity in rats, as result of animals' sluggish attitude to food (rat diet) and water. Animals in Group G and H that were further treated with moringa for 2weekd after 4weeks treatment of moringa were initially showing sluggish attitude but gradually became responsive during the 2weeks treatment with moringa at different doses. However, animals in Group C, D, E and F (treated and pre-treated with moringa alongside cadmium chloride at different doses) showed no signs of sluggishness throughout the period of experiment.

Table 1: Showing mean and standard deviation (Mean ± S.D) of Liver function test for all groups

Groups	ALT	AST	ALP	DB	TB
Control (A)	15.00±0.00	10.00±0.00	120.00±0.00	0.50±0.00	0.20±0.00
$CdCl_2$ (B)	430.50±149.20*	421.10±8.34*	515.60±21.78*	0.58±0.01	0.30±0.00
$CdCl_2$ + M.O (C)	112.40±0.57	10.00±0.00	456.30±77.78*	0.23±0.05	0.39±0.13*
$CdCl_2$ + M.O (D)	15.00±0.00	10.00±0.00	299.25±2.47*	0.65±0.71	0.30±0.00
M.O then $CdCl_2$ (E)	180.15±0.21*	10.00±0.00	329.05±29.63*	0.34±0.11	0.30±0.00
M.O then $CdCl_2$ (F)	148.70±0.00*	10.00±0.00	239.20±74.67*	0.52±0.45	0.42±0.17*
$CdCl_2$ then M.O (G)	15.00±0.00	10.00±0.00	447.30±3.82*	0.20±0.00	0.30±0.00
$CdCl_2$ then M.O (H)	15.00±0.00	10.00±0.00	396.05±6.86*	0.20±0.00	0.30±0.00
	14.879	4855.019	20.763	0.733	1.574
	0.001	0.000	0.000	0.652	0.259

* Statistical significant difference ($p<0.05$)

Analysis of variance (ANOVA) showed significant difference in ALT ($p<0.05$), AST ($p<0.05$) and ALP ($p<0.05$) between the control groups and $CdCl_2$ group but showed no significant difference in DB ($p>0.05$) and TB ($p>0.05$). Post-hoc analysis was further used to determine the significant differences between the control group and all the test groups, and also between tests groups at different doses. This analysis showed that there was a significant difference in ALP ($p<0.05$) between control group and other treatment group. There was also a significance in ALP ($p<0.05$) between $CdCl_2$ group and other treatment group. However, there was no significant difference in ALT, AST, DB and TB level between control group and other treatment group (excluding $CdCl_2$ group) expect for group E and F in ALT level, and group C and F in TB level.

DISCUSSION

Biochemical markers such as Serum ALT, AST and ALP are used to detect chronic liver disease (Lemarie et al, 2004). The present study showed significant differences in the serum level of AST, ALP, ALT and TB ($P<0.05$) across the groups but showed no significant difference in Serum level of DB ($P>0.05$) as shown in Tables 1. There was a significant increase in the level of serum ALP, AST and ALT among animals in Group B (cadmium group) when compared to control. According to Naik (2010) cadmium hepatotoxicity has been revealed to be associated with up regulation or over production of reactive oxygen species which attack essential cell constituent and consequently causes release of intracellular content into circulation. In this study, the elevated serum levels of ALT, ALP and AST of animals in Group B may be due to hepato-cellular necrosis which causes increase in permeability of cell membrane resulting in the release or leakage of transaminases in the blood stream.

The increase in ALP represents general hepatic toxicity (Naik, 2010). Also, induction of ALP synthesis is the usual response of liver to any form of biliary obstruction (Mauro and Renze, 2008). This is in agreement with Mohammed and Azab, (2014) in their *study* on the effect of cadmium on liver and amelioration by aqueousextracts of fenugreek seeds, rosemary and cinnamon in guinea pigs (histological and biochemical study) revealing that the levels of serum ALT, AST and ALP were significantly elevated ($P < 0.05$) in cadmium chloride group when compared with the control group, and also in agreementwith Samir *et al*. (2012)who revealed that activities of ALT and AST were significantly elevated in rats treated with cadmium.

However, animals in Group C, D, E, F, G and H showed significant decrease in serum level of AST, ALP and ALT when compared to Group B (cadmium group). Also, animals in Group D, F and H treated with cadmium chloride and higher dose of moringa (2000mg/kg) at different approaches showed more significant decrease in serum level of ALT and ALP when compared with animals in Group C, E and G treated with cadmium chloride lower dose of moringa (1000mg/kg) at different approaches. This might be due to the hepatoprotective property of Moringa oleifera which may contribute to the stabilizing activity of the cell membrane preventing enzyme leakage.

Previous studies reported that hepatoprotective effect of moringa is due to the presence of quercetin and kaemferol (Selvakumar and Natarajan, 2008). This is in lineReetu *et al*., (2015) on the hepatoprotective activity of Moringa oleifera against Cadmium toxicity in rats in which result showed that albino rats exposed to cadmium chloride had a significant increase ($P\leq 0.01$) in AST, ALT and ALP level. Whereas, animals exposed treated with Moringa oliefera (500mg/kg) alongside Cadmium chloride (200mg/kg) showed a significant decrease ($P\leq 0.01$) in elevated ALP, AST and ALT when compared to the cadmium chloride treated group. This is also in line with Vinodini et al, (2014) who conducted a research on the evaluation of liver functions with Moringa oleifera leaf extract in Cadmium induced albino wistar rats revealing that pre-treatment with Moringa oleifera leaf extract in cadmium exposed rats showed a significant decrease in levels of AST and ALT as compared to the cadmium alone treated rats. It was suggested the Moringa oleifera can improve the liver functions in cadmium chloride induced rats as a result of its Hepatoprotective function.

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AUTHORS'S CONTRIBUTIONS:

All authors contributed in one way or the other in making this article a reality; in the financial aspect and the also in the statistical analysis