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Amelioration of Cadmium Toxicity in the Liver and Kidney of Wistar Rats by Combined C*itrus sinensis* and *Manihot esculenta* Leaf Extract

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

This study investigated the ameliorative role of combined C*itrus sinensis* and *Manihot esculenta* leaf extract in Cadmium toxicity in the kidney and liver of Wistar Rats. Extracts of cassava and orange leaf were obtained using standard protocols. Twelve adult female rats (165±2g) divided into 3 groups

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were treated as follows: Group 1 (Control group): given only normal rat feed and drinking water; Group 2 (Cd group (received): given a Cd (30mg/kg body weight) orally on first day and normal rat feed and water for the remaining days; Group 3 (Cd + combined leaf extract group): given Cd as in Group 2 above and combined extract at a dosage of 200mg/kg body weight orally everyday for14 days. They also received normal rat feed and water. A marked reduction in alanine amino transferases (ALT), aspartate aminotransferase (AST), Lactate dehydrogenase (LDH) and alkaline phosphatase (ALP) activities in the liver upon exposure to Cd with a corresponding upsurge in the activities of these enzymes in the serum was observed. In addition, Cd exposure led to significant rise in the levels of urea, creatinine and electrolytes in the serum. However there was significant amelioration in these parameters upon treatment of Cd-ingested rats with the combined leaf extract. This study showed that Cd induces hepato and nephro-toxicities in experimental animals but *Manihot esculenta* and *Citrus sinensis* leaves can be employed together in the amelioration of hepatic and neprotic injuries occasioned by Cd exposure.

Keywords: Citrus sinensis; Manihot esculenta; cadmium-toxcity; amelioration; liver; kidney.

1. INTRODUCTION

The fact that the environment in which humans live is polluted by chemicals and heavy metals, due largely to increased anthropogenic activities, has been echoed and reechoed by researchers [1-3]. One of such heavy metal that is of great concern to the scientific community is Cadmium (Cd), which has deleterious toxicological effects in plants and animals because it can bio-accumulate in tissues [4-7]. It blocks the activity of enzymes and biological processes that require zinc, calcium and magnesium; it instigates oxidative stress, depletes tissue antioxidants and induces peroxidation of membrane lipids [8-10]. Furthermore, mutations, cancer, and apoptosis have been attributed to Cd toxicity [11-15].

Humans are usually exposed to Cd via ingestion and inhalation [1,16]. It has been proven that the kidney and liver are the two principal organs in which Cd toxic effects are manifested resulting in disturbance of normal function of hepatic enzymes, damage of tubules, liver diseases, renal failure and osteoporosis [17-19].

The toxic effects of Cd as well as those of other heavy metals can be ameliorated by plant extracts and natural substances with adequate antioxidant properties that can subdue reactive species instigated by Cd [20,21]. Plants contain abundant bioactive molecules such as anthocyanins, phenolics, flavonoids, tocopherols, vitamins, minerals, carotenoids, lignins and tannins which offer protection against damage by reactive species such as hydroxyl radical and hydrogen peroxide [22,23]. Over the years, progresses in discovery of novel pharmaceuticals have relied heavily on phytochemicals in plants [24,25]. These bio-molecules are present in significant quantities in the leaves of *Manihot esculenta* (cassava) and *Citrus sinensis* (sweet orange) [26-27].

Cassava root is an important agro-industrial component derived from cassava. However, cassava leaves, which are left over in substantial amounts have limited utility backed by scientific data, though they have been used traditionally in relieving headache, sores, fever, and other diseases [27,28]. *C. sinensis* leaf is an invaluable source of vitamins and other antioxidants, is eaten worldwide, and is used traditionally for the management of cramps, anxiety, obesity, cough, constipation, diarrhea and hypertension, but if left over may pollute the environment and become breeding ground for insects [29].

Therefore, to provide sound backing to the judicious utilization and optimize the potentials of cassava and orange leaves, scientific data as regards their bioactivity, antioxidant prowess and health benefits are needed [30,31]. Also, although polyherbal preparations have been proven to give better results than single plant extracts [32], studies on their use in ameliorating Cd toxicity are scarce. To this end, this study evaluated the ameliorative influence of combined C*itrus sinensis* and *Manihot esculenta* leaves extract in Cd-mediated toxicity in the kidney and liver of Wistar rats.

2. MATERIALS AND METHODS

2.1 Experimental Area

The experiment was carried out at the Biochemistry Laboratory of Edwin Clark University located in Kiagbodo town, Delta State, Nigeria.

2.2 Chemical and Reagents

The chemicals and regents used were of high standard. Assay kits manufactured by Randox Laboratories Ltd, U.K. were used for the biochemical assays.

2.3 Collection and Handling of Plant Leaves

C*itrus sinensis* and *Manihot esculenta* leaves were collected from a local farm in Kiagbodo, Delta State, Nigeria and were identified botanically (ECU/04/2023). After sorting the leaves to eliminate dead matter and debris, they were dipped in clean water for further cleaning. Thereafter, they were removed from the stalk and dried under shade for 2 weeks. The dried leaves were thereafter grounded into fine powder using an electric blender. The grounded leaves were kept in sealed container until further use.

2.4 Extraction Process

Simple maceration technique was utilized in the extraction of bio-molecules from the plant leaves. Exactly 200g of the grounded plants was immersed in 500ml of ethanol for duration of 2 days with temperature maintained at 25^0C. The mixture was stirred from time to time. Thereafter, it was filtered with whatmann filter paper. Ethanol in the filtrate obtained was removed with the aid of a rotary evaporator to yield a viscous extract used for the study.

2.5 Experimental Animals

Twelve female Wistar rats with average weight of 165g were used in the study. They were procured from the animal center of the Faculty of Basic Medical Sciences in Delta State University, Abraka, Delta State, Nigeria and transported to the animal research center of Edwin Clark University, Kiagbodo Delta State, where they were allowed to acclimatize for one week before the experiment began. The handling of the rats followed acceptable international animal care procedures and protocols

2.6 Experimental Design

With four rats in a group, the rats were randomized into 3 groups and treated as stated below for two weeks:

Group 1 (Control group): given only normal rat feed and drinking water.

Group 2 (Cd group (received): given a Cd (30mg/kg body weight) orally on the first day of the experiment and normal rat feed and water for the remaining days.

Group 3 (Cd + combined leaf extract group): given Cd as in Group 2 above and combined extract at a dosage of 200mg/kg body weight orally everyday for 14 days. They were also given normal rat feed and water.

2.7 Sample Collection

After two weeks of administration the animals in each group were sacrificed with an anesthesia (di-ethyl). Blood and tissue samples were collected and processed for biochemical assays using standard laboratory protocols.

2.8 Biochemical Tests

Serum kidney and liver function indicators such as serum total protein (TP), aspartate aminotransferase (AST), alkaline phosphatase (ALP), lactate dehydrogenase (LDH), alanine aminotransferase (ALT), creatinine, urea, and electrolytes (sodium and potassium) were assayed using standard methods as shown in Table 1.

2.9 Statistical Analysis

The results that were recorded from this study were analyzed by analysis of variance (ANOVA) and LSD (least significant difference) test to reveal the significant difference between the mean values of the measured parameters in the respective groups. A significant change was considered acceptable at $p<0.05$. Results of the biochemical estimations are reported as mean ± SD of triplicate readings.

Table 1. Kidney and liver function indicators assay methods

Assay	Authority	Principle/Remark
Total Protein	[33]	In a medium with high pH, cupric ions, interact with protein peptide bonds to yield a coloured complex equivalent to the protein in the sample.
LDH	[34]	LDH catalyses the formation of lactate from pyruvate along with the production of NAD from NADH

Assay	Authority	Principle/Remark
ALT	[35]	ALT is assayed by monitoring the amount of pyruvate hydrazone formed with 2, 4-dinitrophenyl hydrazine at a wave length of 546nm (530-550 nm).
AST	[35]	AST is measured by monitoring the concentration of oxalocetate hydrazone formed with 2, 4-dinitrophenylydrazine at a wavelength of 546 nm.
ALP	[36]	ALP catalyses the release of P-nitrophenol when P-nitrophenylphosphates reacts with water.
Urea	[37]	Urease catalyses the breakdown of Urea to ammonia
Creatinine	[38]	Creatinine in solution with high pH reacts with picric acid to form a coloured complex, which is equivalent to the quantity of creatinine in the sample
Sodium and Potassium	Randox Assay Kits	

3. RESULTS

3.1 Ameliorative Influence of Combined C*itrus sinensis* and *Manihot esculenta* Leaves Extract in Cadmium-mediated Toxicity in the Liver of Wistar Rats

The ameliorative influence of combined C*itrus sinensis* and *Manihot esculenta* leaves extract on the activities of aminotransferases in the serum and liver of Cd treated rats is presented in Table 2. A marked reduction in the activities of both transaminases (ALT and AST) in the liver upon exposure to Cd (Group 2) was seen as compared to the un-exposed rats (Group 1). Conversely, an upsurge in the actions of both transaminases was witnessed in the serum of rats Cd-ingested rats (Group 2) in relation with the control (Group 1). The result indicates that exposure to Cd caused a lowering in the actions of the enzymes in the liver, but instigated an enhancement of their activities in the serum.

The activities of both transaminases measured in the serum of Cd-exposed rats treated with the combined leaf extract (Group 3) was markedly lower than that of rats given only Cd (Group 2). Conversely, the activities of the enzymes in the liver of rats given the extract (Group 3) were strikingly higher than Cd-exposed rats that did not receive the extract (Group 2). The inference from this result is that the management of rats given Cd with the extract causes a lowering of the activities of the transaminases in the serum but increased their activities in the liver.

The ameliorative role of combined C*itrus sinensis* and *Manihot esculenta* leaves extract on the activities of alkaline phosphatase (ALP) and Lactate dehydrogenase (LDH) in the serum and liver of Cd treated rats is presented in Table 3.

The activities of ALP and LDH in the serum and liver of control rats were 242.33, 279.56, 222.56 and 278.67 respectively. These were appreciably elevated to 405.00 and 274 in the serum respectively while a reduction in their activities to 184.88 and 110.56 was seen in the liver respectively. When rats Cd-ingested were administered the extract (Group 3), there was a marked reduction in the actions of both enzymes in the serum, with a corresponding amplification in the liver, compared to Cd-exposed rats that didn't take the extract (Group 2). The result proposes that Cd toxicity brought about a noticeable amplification in the activities of LDH and ALT in the serum with a significant decline in the liver. However, administration of the extract to Cd-ingested rats (Group 3) reversed the Cd-induced modulation in the actions of the enzymes.

The ameliorative role of Combined C*itrus sinensis* and *Manihot esculenta* leaves extract on the levels of total protein in the serum and liver of Cd-treated rats is presented in Table 4.

The highest protein level of 29.00 was recorded in the serum of rats maintained on Cd alone (Group 2). This value was significantly higher that total protein levels in the serum of rats in the control group and Cd-exposed rats that received the extract (Group 3) an indication that Cd toxicity caused a rise in total protein levels in the serum. A similar trend was seen in the liver total protein values, but with better amelioration by the extract.

Table 2. Ameliorative influence of combined C*itrus sinensis* and *Manihot esculenta* leaves extract on the activities of aminotransferases in the liver and serum of Cd treated rats

Groups	ALT (U/L)		AST (U/L)	
	SERUM	LIVER	SERUM	LIVER
1 (Control)	45.15 ± 1.20[a]	84.23 ± 2.23[a]	30.66 ± 1.82[a]	92.40 ± 2.41[a]
2 (Cd)	81.04 ± 2.01[b]	39.54 ± 1.76[b]	62.01 ± 2.24[b]	42.20 ± 2.12[b]
3 (Cd + Extract)	50.66 ± 1.11[a]	51.32 ± 1.88[c]	42.04 ± 1.22[c]	90.43 ± 3.02[a]

Data is shown as mean ± standard deviation (SD). Figures shown in the same column having different alphabetical superscripts are appreciably different from each other at p<0.05

Table 3. Ameliorative influence of combined C*itrus sinensis* and *Manihot esculenta* leaves extract on the activities of alkaline phosphatase (ALP) and Lactate dehydrogenase (LDH) in the liver and serum of Cd treated rats

Groups	ALP (U/L)		LDH (U/L)	
	SERUM	LIVER	SERUM	LIVER
1 (Control)	242.33 ± 5.89[a]	279.56 ± 4.45[a]	222.56 ± 5.34[a]	278.67 ± 4.83[a]
2 (Cd)	405.00 ± 5.84[b]	184.88 ± 4.67[b]	274.65 ± 5.45[b]	110.56 ± 3.34[b]
3 (Cd+Extract)	295.00 ± 4.23[c]	282.22 ± 5.04[a]	245.58 ± 4.08[c]	290.54 ± 4.86[a]

Data is shown as mean ± standard deviation. Figures shown in the same column having different alphabetical superscripts are significantly different from each other at p<0.05

3.2 Ameliorative Influence of Combined *Citrus sinensis* and *Manihot esculenta* Leaves Extract in Cadmium-mediated Toxicity in the Kidney of Wistar Rats

The ameliorative influence of combined C*itrus sinensis* and *Manihot esculenta* leaves extract on the levels of urea and creatinine in the serum of Cd treated rats is presented in Table 5.

The Serum urea level in the control rats was 85.3±6.5. This was significantly elevated (p<0.05) to 125±5.0 in rats exposed to Cd (Group 2) and thus Cd administration brought about a noteworthy increase in serum urea. When Cd-exposed rats were treated with the leaf extract (Group 3), there was a noteworthy reduction (p<0.05) in the level of serum urea (96.67) compared to rats exposed to Cd alone (Group 2), indicating that the extract considerably lowered serum urea levels.

Similarly, the serum creatinine level in the control rats was 0.76±0.12. This was significantly elevated (p<0.05) to 1.91±0.04 in rats exposed to Cd (Group 2) an indication that Cd administration instigated a noteworthy rise in serum creatinine levels. However, upon treatment of Cd-exposed rats with the combined leaf extract (Group 3), a marked decrease (p<0.05) in the level of serum creatinine was noticed (1.3±0.10) compared to rats exposed to Cd alone (Group 2). The inference is that feeding of the extract drastically lowered serum creatinine level in Cd-exposed rats.

The ameliorative influence of combined C*itrus sinensis* and *Manihot esculenta* leaves extract on the activities levels of serum electrolytes (sodium and potassium) of Cd treated rats is presented in Table 6. The level of the electrolytes (Na and K) in the serum of rats administered Cd alone (Group 2) was much higher (p<0.05) compared to that recorded for the control pointing to the fact that exposure to Cd may have triggered increase in the electrolytes levels. When Cd-exposed rats were managed with the combined leaf extract (Group 3), there was a pronounced decrease (p<0.05) in electrolytes level in the serum sodium relative to rats which received Cd but were not given the extract (Group 2). This shows that the extract markedly lowered serum electrolytes levels.

Table 4. Ameliorative influence of combined C*itrus sinensis* and *Manihot esculenta* leaves extract on the levels of total protein in the serum and liver of Cd treated rats

Groups	TP(g/100 ml)	
	SERUM	LIVER
1 (Control)	16.33 ± 1.12[a]	5.23 ± 0.56[a]
2 (Cd)	29.00 ± 2.10[b]	7.87 ± 0.68[b]
3 (Cd+ Extract)	21.01 ± 1.23[c]	5.29 ± 0.45[a]

Data is shown as mean ± standard deviation (SD). Figures shown in the same column having different alphabetical superscripts are significantly different from each other at p<0.05

Table 5. Ameliorative influence of combined C*itrus sinensis* and *Manihot esculenta* leaves extract on the levels of urea and creatinine in the serum of Cd treated rats.

GROUPS	Urea (mg/dl)	Creatinine (mg/dl)
Group 1 (Control)	85.3±6.5[a]	0.76±0.12[a]
Group 2 (Cd)	125±5.0[b]	1.91±0.04[b]
Group 3 (Cd+ Extract)	96.67±2.1[c]	1.3±0.10[c]

Data is shown as mean ± standard deviation. Figures shown in the same column having different alphabetical superscripts are appreciably different from each other at p<0.05

Table 6. Ameliorative influence of combined C*itrus sinensis* and *Manihot esculenta* leaves extract on the levels electrolytes (sodium and potassium) in the serum of Cd treated rats

GROUPS	Na^+	K
Group 1 (Control)	24.33±3.5[a]	1.3±0.10[a]
Group 2 (Cd)	72.00±5.3[b]	4.1±0.26[b]
Group 3 (Cd+ Extract)	48.33±3.51[c]	2.3±0.31[c]

Data is shown as mean ± standard deviation. Figures shown in the same column having different alphabetical superscripts are appreciably different from each other at p<0.05

4. DISCUSSION

In this study Cd toxicity in the liver was witnessed in noteworthy reduction in the activities of ALT, AST, LDH and ALP in the liver accompanied with an increase in their activities in the plasma. Cd also caused marked increase in levels of total protein in the liver. These results agree with the report of several similar findings on Cd toxicity [4,10,39-41]. ALT and AST are key enzymes needed in the metabolism of amino acids in the liver, whose upsurge in the plasma has been tied to damage of hepatic membrane – an indication of hepatic injury [4,42]. According to Renuka et al. [43], necrosis of liver cells leads to an increase in their membrane permeability and subsequent leakage of their contents into the plasma. It has been shown previously that because the liver, as well as, the kidney are rich in metallothionein, they are primes sites of Cd bio-accumulation and hence its toxic effects [4,44-46].

The kidney participates actively in maintaining homeostasis and in the process of detoxifying and subsequent removal of toxicants and other waste products. This makes it a prime target of heavy metal poisoning. Cd is international recognized as a nephro-toxicant [47]. Thus it was not surprising that in this study, animals exposed to Cd had significantly higher levels of serum urea, creatinine and electrolytes (Na^+ and K), which are indices of kidney damage and it is in agreement with the report of other researchers [48]. Increase in serum levels of creatinine and urea have been linked to a number of reasons, chief of which is their ineffective removal from the circulatory system due to impairment of renal tubular and glomerular functions [45,49]. As noted by Schnellman and Kelly [50], Cd-induced peroxidation of membrane lipids decreases membrane fluidity, which can shift Na-K ATPase from their normal positions with electrolyte imbalance as the resulting consequence. This also confirms the assertion by Patra et al. [51] that alterations in transport pathways is one way through which Cd toxicity is manifested.

The combined extract employed in this study displayed abundant ameliorative action against cadmium-induced hepato-nephrotic damage, since it brought the actions of ALT, AST, ALP and LDH as well as the amounts of total protein, urea, creatinine, sodium and potassium to normal. This positive effect might not be unconnected to the antioxidant activity of the extract as demonstrated in a previous study [10,52]. Both plants have been shown to contain significant quantities of flavonoids, carotenoids and other useful phytochemicals that can shield cells from the harmful effects of free radicals and chelate heavy metals [31,52-54].

5. CONCLUSION

In conclusion, the result of the study depicted that cadmium exposure causes hepato and nephro-toxicities in experimental animals but the combined extract of *Manihot esculenta* and *Citrus sinensis* leaves effectively ameliorated the Cd-induced damage. Thus the leaves of *Manihot esculenta* and *Citrus sinensis* can be deployed together in the amelioration of hepatic and neprotic injuries occasioned by Cd exposure. More studies are however required to spotlight the specific mechanisms of this amelioration as well as the specific biomolecules responsible it.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts.

ETHICAL APPROVAL

Animal Ethic committee approval has been collected and preserved by the author(s).

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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