



Histomorphologic Effect of *Citrus aurantiifolia* against Cadmium Chloride Induced Toxicity in the Liver and Kidney of Adult Wistar Rats

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Authors' contributions

This work was carried out in collaboration between both authors. Author EUE designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors EUE and FOA managed the analyses of the study. Author FOA managed the literature searches. Both authors read and approved the final manuscript.

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ABSTRACT

Aim: The study aimed to evaluate the protective effect of *Citrus aurantiifolia* against cadmium chloride toxicity induced in the liver and kidney of Albino Wistar rats histologically.

Methods: Twenty five adult female Albino rats weighing 150±11 to 200±50 g were used for the study. The rats were purchased from the Department of Pharmacology, College of Health Science, University Port Harcourt, Rivers State of Nigeria. They were randomly assigned into five groups (A B C D & E) with each group having five rats. Group A (Control) received only food and water for six weeks (6 weeks), Group B received food, cadmium water and treatment with lime juice for six weeks (6 weeks), Group C received food, water and lime juice for three weeks. After three weeks, they stopped lime and water and were induced with cadmium chloride for 3 weeks, Group D received food, cadmium chloride for 3 weeks and after 3 week they were given normal water and treated with lime for another 3 weeks, Group E received food and cadmium water for six weeks. At

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the end of the treatment, the liver and the kidney of each sacrificed rat were processed for paraffin sectioning and stained with Harris hematoxylin and eosin.

Results: Photomicrographs of Groups B and C which were induced with Cadmium Chloride and treated with citrus shows area of central necrosis and central vein congested with red blood cells and also the presence of inflammatory cells which are features of liver injury can be seen.

Conclusion: There was no significant protective effect of *Citrus aurantiifolia* against cadmium-induced liver injury in Albino Wistar rats. Also, there was no significant effect of cadmium-induced toxicity on the kidney of the Albino Wistar rat.

Keywords: Liver; *Citrus aurantiifolia*; kidney; cadmium chloride; albino rats; cancer; histomorphology.

1. INTRODUCTION

Cadmium (Cd) is a toxic metal widely distributed in the environment as a result of industrial and agricultural practices [1]. The source of Cd intake is mostly food, and most of the metal that is absorbed after oral exposure mainly accumulates in the liver and kidney, where it induces production of metallothionein (MT), a low molecular-weight protein that binds Cd with high affinity [2]. The following processes may be involved in the development of hepatotoxicity, activation of Kupffer cells that induce inflammation [3], Cd injury of hepatic endothelial cells obstructs the capillary lumen [4], thereby producing local hypoxia [5] and Cd induction of hepatic iron (Fe) causes depression, which may cause disturbances in Fe-dependent oxidative processes, e.g., adenosine triphosphate (ATP) synthesis [6]. It is thought that injured hepatocytes release a Cd-MT complex into the blood, which is then filtered in the kidneys through glomeruli and reabsorbed by proximal tubule epithelial cells, which are the target for extracellular Cd-MT. After degradation of the complex in these cells, Cd ions induce oxidative stress that causes, mitochondrial swelling, and loss of cristae and as well as inhibition of Na⁺-K⁺-ATPase. These events eventually lead to loss of ionic control and cellular injury [7].

Citrus aurantiifolia, which belongs to the Rutaceae family, is believed to reduce oxidative stress by inhibiting cellular lipid peroxidation and increasing cellular antioxidant systems [8,9]. Patil, et al. [10] have also reported that *Citrus aurantiifolia* (Lime) protect against the proliferation of cancer cells but there is limited data on its protective effects in other metal-induced toxicities. Since *Citrus aurantiifolia* juice contains potent antioxidants and suchtal-induced toxicities [11-14]. Significance of the study is to investigate effect of cadmium on the histology of the liver and kidney and the ability of *Citrus aurantiifolia* to ameliorate the toxicity of the

cadmium chloride and thus create awareness to general public about the health implication of cadmium chloride and thus encouraging individuals to take practical steps in preventing and controlling diseases associated with cadmium toxicities.

The study aimed to evaluate the protective effect of *Citrus aurantiifolia* against cadmium chloride toxicity induced in the liver and kidney of Albino Wistar rats histologically.

2. MATERIALS AND METHODS

2.1 Location of the Study

This study was carried out in the Department of Medical Laboratory Science, Faculty of Basic Medical Science, College of Health Science, Niger Delta University Wilberforce Island Bayelsa State of Nigeria.

2.2 Duration of Study

This study lasted for eight weeks.

2.3 Animal/Care

Twenty-five adult female Albino rats weighing 150±11 to 200±50 g were used for the study. The rats were purchased from the Department of Pharmacology, College of Health Science, University Port Harcourt, Rivers State of Nigeria. They were randomly assigned into five groups (A B C D & E) with each group having five rats. The rats were kept in their respective cages of an aluminium frame with metal nettings and plastic cages.

The rats were kept under laboratory condition of 25±5°C with 12 hour light/dark cycle for two weeks as an adaptation period. They were fed with pelletized grower feed manufactured by Grand Cereals Limited, Anambara State and water daily.

Table 1. Experimental design

Group A	Group B	Group C	Group D	Group E
Control, Received only food and water for six weeks (6 weeks)	Received food + cadmium water (CdCl ₂ .H ₂ O) + treatment with lime juice (1ml) for six weeks (6weeks)	Received food + water + Lime juice (1 ml daily) for three weeks. After three weeks, they stopped lime and water and were induced with 0.4 mg of cadmium chloride for 3 weeks	Received food + 0.4 mg of cadmium chloride for 3 weeks. After 3 week they stopped taking cadmium water. They give normal water and treated with lime (1 ml daily) for 3 weeks+	Received food and cadmium water for six weeks

The cages were washed and disinfected at intervals of two days while the cups and plates for the animal feeding were washed twice daily (morning and evening). Left overfeeds were thrown away and replaced with new ones likewise their drinking water.

The level of cadmium in their diet (water) was 0.4 g of cadmium dissolved in 1000 ml of distilled water. All treatments and procedures were performed following the protocol of the National Animal Care and Use Committee and Guidelines for the care and use of experimental animals [15].

2.4 Cadmium Preparation

0.4 g of Cadmium was dissolved in 1000 ml of distilled water and administered orally. All the rats were observed daily after dosing and then each rat was observed daily for thirty-two days.

2.5 Plant Collection and Extraction

A bulk of fresh lime used was purchased from swali market in yenagoa, Bayelsa state. The fresh limes were washed, peeled and squeezed into a clean glass cup and allowed to settle down. After settling, the juice was sieved and refrigerated to avoid the lime juice from getting bad.

Each rat was given 2 ml/kg body weight of fresh lime juice once daily, the administration was done orally with the aid of an oral gastric tube and the rats were observed daily for forty-two days.

2.6 Experimental Design

Rats were randomly divided into five groups. Each group had a total of five animals.

The oral route was chosen as a means of administration because the oral route is the main

mode of exposure of cadmium in humans and animals.

The cadmium chloride was dissolved in water and the rats were allowed to drink without altering the amount or volume of water consumed by each rat.

2 ml of Fresh lime juice was given based on the available literature data [16]. The treatment lasted for a total of six weeks.

2.7 Histological and Histochemical Studies

At the end of the experiment, the liver and the kidney of each sacrificed rat were dissected and trimmed of excess fat. They were fixed in 10% buffered formalin and processed for paraffin sectioning. It was dehydrated in different per cent of alcohol, cleared in xylene and embedded in paraffin blocks. Section of about 5 µm thickness was stained with Harris hematoxylin and eosin (H&E) for histological [1].

3. RESULTS AND DISCUSSION

Fig. 1 shows the morphology of the liver after administering Cadmium Chloride and Citrus juice. The slide labelled A is a photomicrograph of the control group showing normal structural morphology and Kupffer cells (K) while photomicrograph showing B and C represents Groups B and C which were induced with Cadmium Chloride and treated with citrus shows area of central necrosis (N) and central vein congested with red blood cells (R), presence of inflammatory cells (I), around the central vein as well as mild oedema (arrow) in the wall of the central vein. These features are markers of liver injury as seen in the above groups (B and C). But the severity of group D which was induced with 0.4 mg of cadmium chloride alone showed more severity which agrees with the work carried

out by Wlostowki, et al. [6] in which he stated that Cadmium accumulates in the liver and kidney where it induces the production of metallothionein (MT), a low molecular weight protein that binds cadmium with a high affinity. The protein forms a complex with cadmium and decreases its free concentration within the cell, thus decreasing the toxic potential of cadmium. When the binding capacity of metallothionein becomes saturated, the increased level of unbound cadmium ions initiates processes that can lead to liver and kidney injury. In the liver, cadmium exposure produces nonspecific inflammation of hepatocyte, swelling and mild necrosis [6]. From the workgroup B and C were induced with cadmium chloride and were treated with 1ml of fresh lime juice. With the above treatment, the liver of the animal still presented damage and showed no ameliorative effect from the treatment administered. Though according to Amal and fawzy [17] there was an ameliorative

effect on the liver of the rats treated with vitamin C and cadmium chloride at a dosage of 3 mg of cadmium per day and 2 g of vitamin C supplement per day. Though it was believed that the *Citrus aurantiifolia* would give an ameliorative effect on the liver of the rats treated with cadmium since *Citrus aurantiifolia* contains 29.1 mg of vitamin C, therefore, the presence of non-ameliorative effect could be dose dependent considering the volume of cadmium chloride consumed by the animals since there was no restriction on their consumption of cadmium per day and the volume of treatment may not have been enough to subdue the effect of cadmium to produce a positive result. The slide labelled D and E has a liver section "D" showing dilation of central blood vessel and inflammatory cell while liver section showing "E" show binucleated cell (arrow), Kupffer cell and hyperplasia (K) which also agrees with the research carried out by Wlostowski, et al. [6].

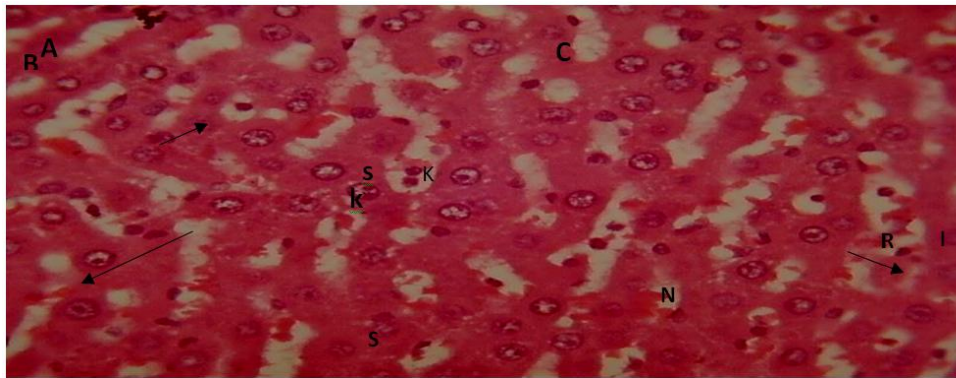


Fig. 1. Photomicrograph of control liver section showing normal structural morphology sinusoids(S), hepatocytes (arrow), Kupffer cell (K). Hematoxylin and eosin stain X400

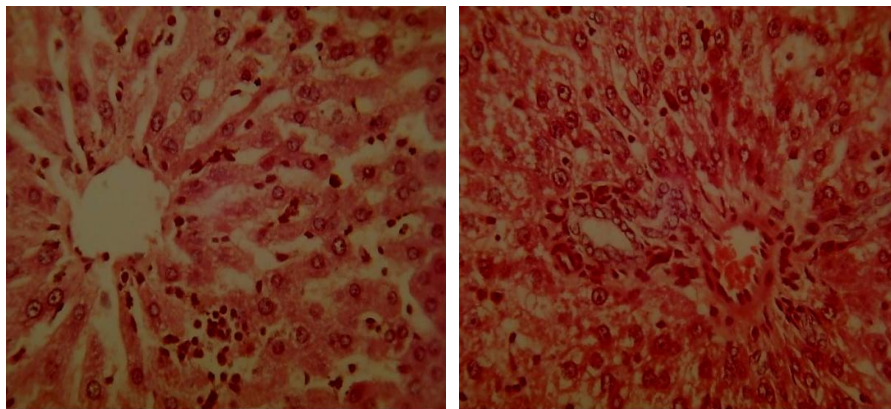


Fig. 2. Photomicrograph of the liver section showing a small area of necrosis (N), central vein filled with red blood cell (R), marked presence of inflammatory cell(I) around the central vein as well as mild oedema (arrow) in the wall of the central vein. hematoxylin and eosin stain x400

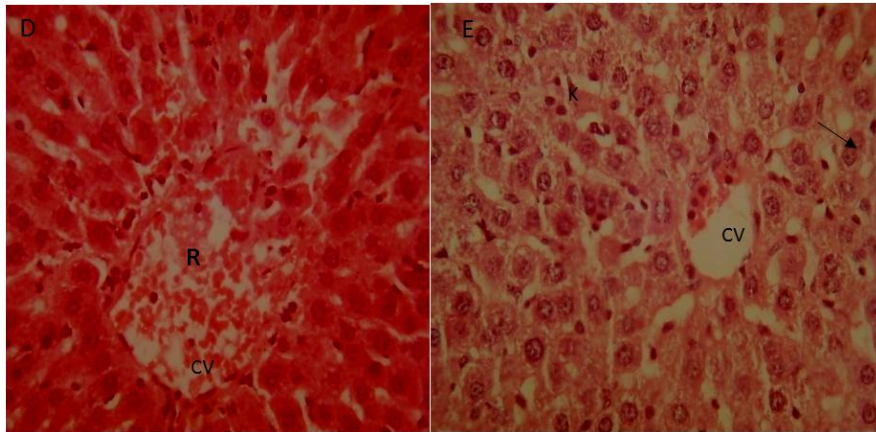


Fig. 3. Photomicrograph of liver section (D) showing congestion of central vein(CV) filled with red blood and inflammatory cells. Liver section (E) showing binucleated cell(arrow) , kupffer cell hyperplasia(K).hematoxylin and eosin stain x400

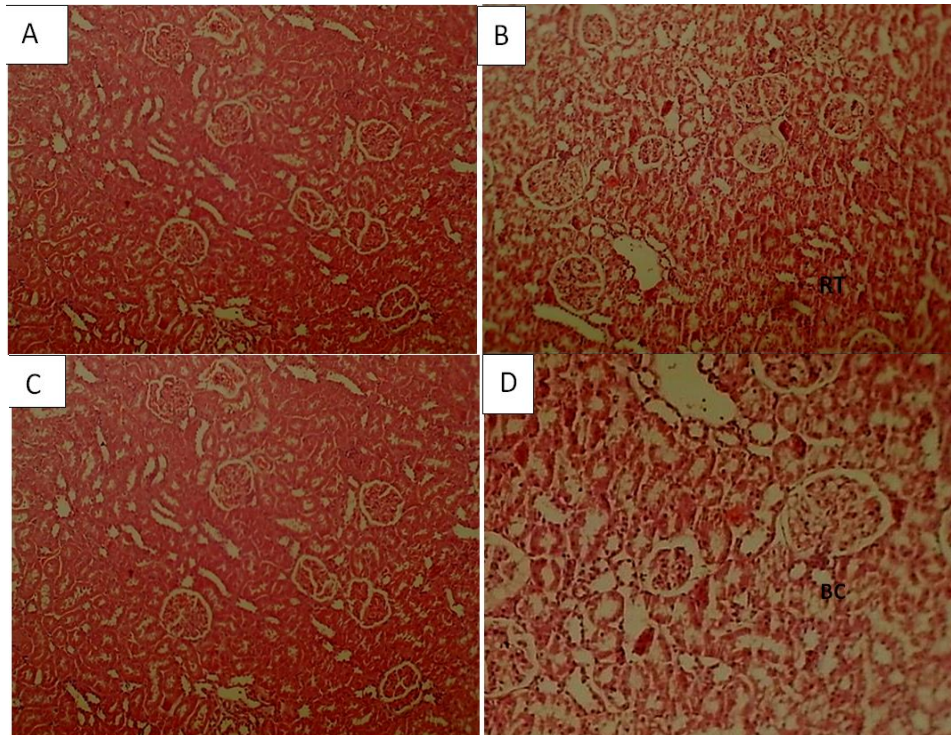


Fig. 4(A-D). Photomicrograph of kidney sections stained with hematoxylin and Eosin sections shows normal Bowman's capsule (BC), the renal tubules (RT) all are consistent with normal kidney histology

Fig. 4 contains slide A – D. A represents the control group, slide B, C and D were treated with lime and cadmium chloride. The section after being stained with eosin and hematoxylin show a normal Bowmans capsule (BC), renal tubules (RT). All are consistent with normal kidney

histology. There was no effect of cadmium-induced kidney toxicity on the kidney of Albino Wistar rat, which suggests that the duration of exposure to cadmium was not long enough to induce kidney damage on the rat and also, the dosage may also not have been enough to

induce the damage. Data from human studies suggest a latency period of approximately 10 years is required before the clinical manifestation of renal damage also depending on the intensity [18]. The period of 90 days and dosage of 3 mg Cadmium chloride is required to express the following effect, disruption of Bowman's capsule, degenerate cytoplasm of some cells of the renal tubules with necrotic nuclei. Cadmium also showed inflammatory cells, infiltration, dilation and congestion of blood vessels of the kidney [17].

4. CONCLUSION

In conclusion, there was no significant protective effect of *Citrus aurantiifolia* against cadmium-induced liver injury in Albino Wister rat. Also, there was no significant effect of cadmium-induced toxicity on the kidney of the Albino Wister rat.

CONSENT

It is not applicable.

ETHICAL APPROVAL

All authors hereby declare that "Principles of laboratory animal care (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the appropriate ethics committee.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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