ORIGINAL ARTICLE ISSN: 2455-5274

Histocytoarchitectural Changes of Wistar Rat's Kidney Exposed to Young Coconut Water Before and After Induction with Carbon Tetrachloride

Ekezie Jervas[1], Gideon IhebuzoNdubuka[2], Okafor Chimaobi Wilson[2], Iwuji Samuel Chidi[2]

[1]Department of Anatomy, Federal University of Technology, Owerri, Nigeria.

[2]Department of Biomedical Technology, Federal University of Technology, Owerri, Nigeria.

Date of Submission: ***24-10-2017***

Date of Acceptance: ***28-01-2017***

Date of Publishing: ***04-07-2017***

ABSTRACT

Background: Young coconut water (YCW) has been used by individuals to boast immunity and that of the experimental animals. In this study an attempt was made to investigate the protective effect of YCW against the Carbon tetrachloride (CCl4) induced renal toxicity in rats. **Methods:** A total of 20 male adult wistar rats which were not previously subjected to any experiment were divided into four groups. Each group has five rats. Group 1 (Normal control) received basal diet, olive oil and water. Group 2 (Positive control) received basal diet, olive oil and YCW (100ml/kg). Group 3 (Negative control) received basal diet, water and CCl4 diluted with water and olive oil. Group 4 (Experimental group) received basal diet and YCW (100ml/kg) and then intoxicated with CCl4 diluted with water and olive oil. After day 7, the rats were sacrificed and their kidneys were collected and processed histologically following standard protocols. **Results:** Group 1 (Normal control) displayed normal histocyto-archetature of the kidney. Group 2 (Positive control) revealed hyperplasia with mild inflammatory response. Group 3 (Negative control) showed hyper-cellularity, mild cystic spaces, necrosis, and loose glomerular membrane indicative of high inflammatory response. Group 4 (Experimental group) revealed moderate cellular activities in line with moderate inflammatory response. **Conclusion:** The administration of YCW on the rat before intoxication with CCl4 suppresses the deleterious effect of CCl4 on the experimental group.

Keywords: *Kidney, coconut water, carbon tetrachloride.*

INTRODUCTION

The toxicity of Carbon tetrachloride (CCl4) has been reported to be dependent on the excessive production of the trichloromethyl radical (CCl3), which reacts with oxygen to form the more toxic trichloromethylperoxyl radical (CCl3O2).[1] It can cause the formation of reactive oxidizing species (ROS) in many vital tissues of which a higher concentration was found to be distributed in the kidney than the liver after a systemic administration of CCl4 in rats.[2,3] Toxic free radicals lead to marked lipid peroxidation that result in excessive damage to cell membranes and in the development of a number of pathological changes in renal impairment.[4,5] These toxic renal effects occur via the destruction of renal mitochondrial function including the calcium flux across mitochondrial membranes.[6]

Although living organisms have well developed antioxidant systems to neutralize most detrimental effects of these oxidizing species,[7] they can also be exhausted by continuous production of the oxidizing species.In this sense, an antioxidizing action induced by antioxidizing agent would play an important role in protecting against CCl4-induced damage.

Name & Address of Corresponding Author
Dr. Ekezie Jervas
Department of Anatomy,
Federal University of Technology,
P.M.B 1526 Owerri Nigeria
E mail: ekeziejervas@gmail.com

The antioxiding action of young coconut water (YCW) has been reported,[7-10] and it was observed to be highest in fresh coconut water samples and decreased significantly on heating, acid or alkali treatments or dialysis.[11] The protective effect of YCW against toxins has been studied, and its hepatoprotective effect is evidenced from the histopathological studies of the liver in YCW treated Wister rats, which did not show any fatty infiltration of necrosis, as observed in CCL4-intoxicated rats.[12]

In this present study, we investigated the protective effects of young coconut juice against CCl4-induced renal toxicity in rats by examining the renal cyto-architecture.

MATERIALS ANDMETHODS

Plant Materials

Youngcoconuts(Cocosnucifera L.) were collected from Eziobodo Community, in Owerri-West Local Government Area., Imo State, Nigeria. It was authenticated and identified by the department of Forestry & Wildlife, School of Agricultural Technology, Federal University of Technology, Owerri, as a dwarf (autogamous) Coconut (Cocosnucifera L. Arecaceae). The fresh young coconut water (YCW) was obtained from the coconuts each time it is required for administered on the Wister rats.

Animal

A total of 20 adult male Wistar rats with body weights ranging from 175g to 200g obtained from Animal house of the Department of Forestry & Wildlife, School of Agricultural Technology, Federal University of Technology, Owerri, Nigeria were used in the study. The animals were allowed acclimatization in the laboratory conditions for two weeks before the commencement of the study. During which, the experimental animals were housed in cages, kept on a 12 h/12 h light/dark cycle and had free access to standard rodent pellet diet and water ad libitum. The experimental procedures adopted in this study were in strict compliance with the United States National Institutes ofHealth Guidelines for Care and Use of Laboratory Animals in Biomedical Research (1985, no. 85-23).

Chemical

Carbon tetrachloride (Riedel-de Haen AG Seelze-Hannover), Olive oil and other chemicals and solvents were of highest grade commercially available.

Induction of renaltoxicity by CCl4

Renal toxicity was induced by the intraperitoneal injection of Carbon tetrachloride CCl4, diluted with distilled water and vector (Olive oil) in the ratio of 1:2:0.5 respectfully. Dosage was determined using 5ml/kg body weight, as a standard. Therefore, the specific dosage for each Wister rat was calculated thus:

Milligram Equivalent for renaltoxicity induction =

$$\frac{5\text{ml} \times \text{weightofrats (g)}}{1000\text{g}}$$

Experimental Group and Protocol

The rats were divided randomly into 4 groups comprising of 5 rats in each group. They were all fed with the same diet throughout the experimental period. The experimental design is described as follows:

Group	Protocol
Group I	This group is made up of 5 male rats with weights ranging from 175g-200g. Rats were fed only with basal diet and tap water.
Group II	This group is made up of 5 male rats with weights ranging from 175g-200g. Rats were fed normal basal diet, injected i.p with Olive oil and received YCW(100 ml/kg body weight/day) as their sole source of drinking water [the calculated dosageof YCWwas given in fragments of 3 times (i.e 8am, 1pm, and 5pm) daily; via intragastric injection. This group served as positive control.
Group III	This group is made up of 5 male rats with weights ranging from 175g-200g. Rats were fed basal diet and tap water, and then they were intoxicated via intraperitoneal injection on the 7th day of the experiment with CCl4 diluted with distilled water and Olive oil, at a ratio of 1:2:0.5 respectively. The dosage given was 5ml/kg body weight. This group served as the negative control
Group IV	This group is made up of 5 male rats with weights ranging from 175g-200g. Rats fed basal diet and young coconut water(100 ml/kg body weight/day) as their sole source of drinking water [the calculated dosage of YCWwas given in fragments of 3 times (i.e 8am, 1pm, and 5pm) daily; via intragastric injection], and then they were intoxicated via intraperitoneal injection on the 7th day of the experiment with CCl4 diluted with distilled water and Olive oil, at a ratio of 1:2:0.5. The dosage given

	was 5ml/kg body weight. This group served as the experimental group(the calculated dosage given was 5ml/kg body weight, on the first day of the experiment).

Tissue collection, processing and examination

At the end of the experiment, the overnight fasted animals (the control and experimental animals) were sacrificed and the kidney samples were collected for histological analysis. The kidney tissues were cut in smallpieces and immersed in neutral buffered formalin 10% and processed for histological studies, using standard methods.[14] The extent of CCl4-induced necrosis was evaluated by assessing the morphological changes in the kidney sections stained with hematoxylin and eosin (H and E). Photomicrographs were taken using digital microscope eyepiece SCOPETEK DCM 500, 5.0 mega pixels.

RESULTS

Then results obtained are shown in Plate 1-4 below:

Microscopic Examination of the Kidney

[Figure 1] (Normal control group): Showing a section typical of the kidney. The cortex is seen to be housing the tuft of the glomerulus with slightly loose basement membrane. The interstitiumis stained red. The collecting tubules appear normal in architecture. No histopathological lesion was seen.

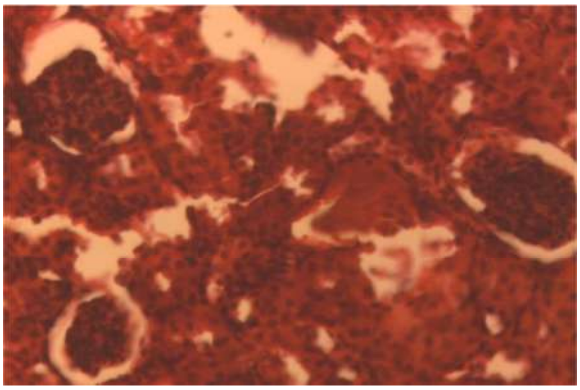

Figure 1: Transverse section of kidneyGroup I (Normal control) H & E Stain. X 400.

[Figure 2] (Positive control group): Showing a section of the kidney glomerular loss, podicytes remaining and hyperplasia. Features are in line with that of an inflammatory response.

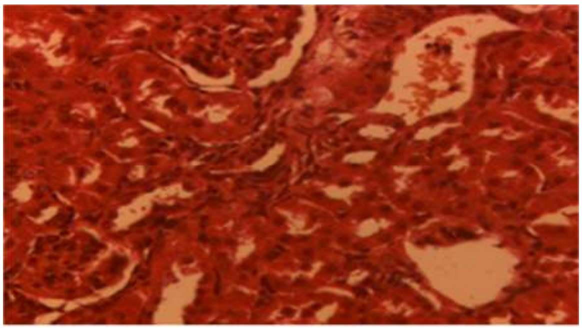

Figure 2: Transverse section of kidney in Group II (Positive control group) H & E Stain. x400.

[Figure 3] (Negative control group): Showing a section of the kidney with hypercellularity, mild cystic spaces and mild necrosis, loosed glomerular membrane. Features are in line with that of an inflammatory response.

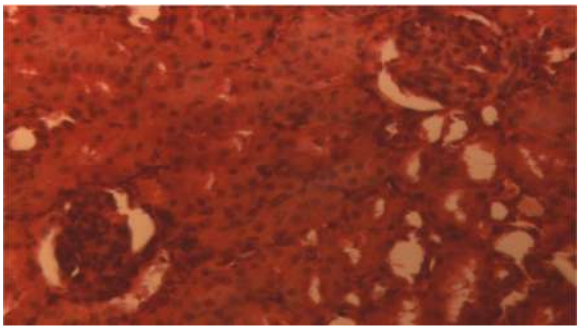

Figure 3: Transverse section of kidneyGroup III (Negative control group)H & E Stain. x400.

[Figure 4] (Experimental group): Showing a section of the kidney with moderate cellular activity in line with that of a moderate inflammatory response.Cyto-architecture was protected.

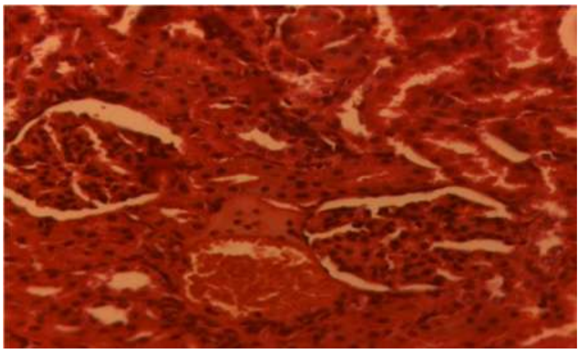

Figure 4: Transverse section of kidneyGroup III (Experimental group)H & E Stain. x400.

DISCUSSION

It has been found that the metabolism of CCl4 involves the production of free radicals. These free radicals initiate the peroxidation of membrane poly unsaturated fatty acids, cell necrosis, GSH depletion, membrane damage and loss of antioxidant enzyme activity.[15-17] There have been several reports which clearly demonstrated that in addition to hepatic toxicity and disorders in the lungs, testis and blood, caused by the free radical generated by CCl4, it also induces kidney disorders.[18-21] Therefore, the effort towards the eradication and prevention of kidney disorders and hepatic damage by eliminating free radicals and prevent lipid peroxidation is necessary.

In this study, the cyto-architecture of the kidney was examined. The analysis of thenormal control group [Figure 1] showed a kidney cyto-architecture with normal features, with slightly loose basement membrane of the glomerulus, but no histopathological lesion was seen. However, these normal features were enhanced in the [Figure 2] (positive control group), suggesting a renal protective activity of the coconut water through itsvasorelexant and antihypertension action,[22] and electrolytic effect.[23]

In the negative control group [Figure 3] the cyto-architectural features of the kidney were distorted with hypercellularity, mild cystic spaces and mild necrosis. This indicates an inflammatory response as result of oxidative stress injury in the kidney.[24,25] However, the cyto-architecture of plate 4 (experimental group) showed reduced distortion, with slight inflammatory response. Thus, improved cyto-architectural features in plate 2 and plate 4, compared to plate 1 and 3 respectively, supports documented histological reports.[26-28] This attenuation of the CCl4 induced kidney disorder by young coconut water showed its free radical scavenging activity,[12,29,30] and as a renal protective agent.

CONCLUSION

This present study showed that YCW is safe for consumption as it significantly improved the cyto-architecture of the kidney. Also,the toxic effect of CCl4 on the kidney wasfound to be greatly reduced.

Recommendation:
There is need for an in vitro and an in vivo investigation into the curative and regenerative effect of young coconut water on renal cells.

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How to cite this article: Jervas E, Ndubuka GI, Wilson OC, Chidi IS. Histocytoarchitectural changes of Wistar Rat's Kidney exposed to Young Coconut Water before and after induction with Carbon tetrachloride. Acad. Anat. Int. 2017;3(1):44-48.

Source of Support: Nil, **Conflict of Interest:** None declared.