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Immature Coconut Water: A Renal Protective Agent in Wistar Rats

Research Article

Okafor CW[1], Ekezie J[1*], Ndubuka GI[2], Ezejiofor, T.I.N[3]

[1] Department of Biomedical Technology, School of Health Technology, Federal University of Technology, Owerri, Nigeria.
[2] Department of Anatomy, School of Health Technology, Federal University of Technology, Owerri, Nigeria.
[3] Department of Biotechnology, School of Biological Sciences, Federal University of Technology, Owerri, Nigeria.

Abstract

The biochemical characteristics of immature coconut water (ICW) have presented it as a therapeutic agent that can be used for preventive, management and curative purposes. Development of a more efficient and cost effective means of prevention and management of kidney disorders through antioxidant activities were the objectives for embarking on the current experiment. This experiment is aimed at investigating the protective effect of ICW against carbon tetrachloride (CCl_4) induced renal toxicity. Twenty(20) rats were fed on standard diet and divided into four groups. Rats in group 1 and group 2 were injected intraperitoneally (i.p) with olive oil. Group 1 received tap water only, while group 2 received ICW (100ml/kg body weight/day) only. Rats in Group 3 and Group 4 were injected i.p with CCl_4 (5ml/kg body weight). Group 3 received tap water, while group 4 received ICW (100ml/kg body weight/day) only. At the end of the experiment (1 week), blood was collected for biochemical analysis. The present findings revealed that, CCl_4 decreased theserum level of kidney markers and increased the serum levels of some electrolytes; but these effects were prevented by the prior administration ICW on rats. The present study concluded that ICW administration played a protective role against CCl_4- induced kidney damages in Wister rats. These protective effects were in the form of improving of kidney markers and electrolyte activities, in CCl_4-intoxicated rats. In the future, a dose dependent protective effect, and *in vitro* and *in vivo* regenerative effect of ICW on the kidney could be investigated.

Keywords : Protection; Coconut water; Serum; Kidney; Biochemistry.

Introduction

The abundance of polyunsaturated fatty acids and the presence of cytochrome P450 makes the kidney an organ particularly vulnerable to reactive oxygen species (ROS) attack [1-3]. The concentration of ROS reaches its peak <1-6 hours, depending on exposure and concentration or dose [4-8]. Carbon tetrachloride toxicity is dependent on the excessive production of the trichloromethyl radical (CCl_3) [9]. Early studies showed that free radicals, such as trichloromethyl (CCl_3) and oxygen-centered lipid radicals (LO or LOO, or both), are generated during CCl_4 metabolism by hepatic cellular cytochrome P450 [10].

A common manifestation and a mediator of cardiovascular, neurological, and numerous other complications of renal failure is oxidative stress [3]. It constitutes the mechanisms of production and progression of numerous renal diseases [11], as it mediates a wide range of renal impairments, from acute renal failure [12-16], rhabdomyolysis [12, 17] obstructive nephropathy [18, 19], hyperlipidemia [18, 20-22] and glomerular damage [23-25] to chronic renal failure and hemodialysis [26-29].

Despite the well-developed antioxidant systems to neutralize most detrimental effects of these oxidizing species in living organisms, a continuous production of oxidizing agent would eventually overwhelm it [30]. Therefore an antioxidizing action induced by antioxidizingagent would be vital in protecting against CCl_4-induced damage in the kidney [31].

The antioxiding action of Immature coconut water (ICW) has been reported [30-34], and it was observed to be more potent in fresh coconut water samples than in heated acid or alkali treated samples [35]. The protective effect of ICW against toxins has been studied, and its protective effect is evident from the

***Corresponding Author**:
Ekezie,Jervas,
Department of Anatomy, Federal University of Technology, P.M.B 1526 Owerri, Nigeria.
Tel: +234 806 5430037
E-mail ekeziejervas@gmail.com

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histopathological studies of the liver and kidney in ICW treated Wister rats, which did not show any distorted sinusoid and fatty infiltrations or necrosis, as observed in CCl_4-intoxicated rats [36].

In this present study, we investigated the protective effects of ICW against CCl_4-induced kidney toxicity in rats, by examining serum levels of kidney markers and some electrolytes.

Materials And Methods

Plant Materials

Young coconuts were (Cocosnucifera L.) collected from Eziobodo Community, in Owerri West, Imo State of Nigeria. It was authenticated and identified the department of Forestry & Wildlife, School of Agricultural Technology, Federal University of Technology, Owerri; as a dwarf (autogamous) Coconut (Cocosnucifera L. Arecaceae). The fresh immature coconut water (ICW) was obtained from the coconuts each time it is required for administration 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 of Health Guidelines for Care and Use of Laboratory Animals in Biomedical Research [37].

Chemical

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

Induction of renal totoxicity by CCl_4

Renal toxicity was induced by intraperitoneal injection of Carbon tetrachloride CCl_4, 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:

$$\text{Milligram Equivalent for renal toxicity induction} = \frac{(5\text{ml} \times \text{weight of rats (g)})}{1000\text{g}}$$

Determination of Dosage for the Administration of Immature Coconut Water (ICW) in Experimental Animals.

Immature coconut water (ICW) was administered through intragastric injection. The dosage was determined using 100ml/kg body weight, as a standard. Therefore the specific dosage for each Wister rat was calculated thus:

$$\text{Milligram Equivalent for ICW Administration} = \frac{(100\text{ml} \times \text{weight of rat (g)})}{1000\text{g}}$$

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 I: This group is made up of 5 male rats with weights ranging from 175g-200g. Rats were fed with rodent pellets and tap water (basal diet), and were injected intraperitoneally on the 7th day of the experiment with olive oil (0.5ml/kg body weight) only.

Group II: This group is made up of 5 male rats with weights ranging from 175g-200g. Rats were fed with rodent pellet, received ICW (100 ml/kg body weight/day) as their sole source of drinking water, and were injected intraperitoneally on the 7th day of the experiment with olive oil (0.5ml/kg body weight). This group served as positive control. The calculated dosage ICW was given in fragments of 3 times (i.e. 8am, 1pm, and 5pm) daily; via intragastric injection.

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 CCl_4 diluted with distilled water and Olive oil, at a ratio of 1:2:0.5 respectively. The dosage given was 5ml/kg body weight.

Group IV: This group is made up of 5 male rats with weights ranging from 175g-200g. Rats were fed basal diet and ICW (100 ml/kg body weight/day) as their sole source of drinking water [the calculated dosage of ICW was 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 CCl_4 diluted with distilled water and Olive oil, at a ratio of 1:2:0.5 respectively (the calculated dosage given was 5ml/kg body weight).

Collection of Blood Samples

At the end of the experiment, the animals were fasted overnight prior to the collection of samples. Blood was obtained from the control and experimental animals through Ocular puncture, using capillary tubes. 5 ml of blood samples were collected. The blood obtained was put into EDTA containers and plain blood sample containers. Serum was obtained from the blood and used for analysis.

Biochemical Techniques

Biochemical Reagents

Commercially obtained diagnostic kits were used to determine the serum levels of biochemical parameters, as follows: Randox Laboratories Limited were used for determination of serum urea and creatinine. Teco Diagnostics were used for determination of serum bicarbonate, chloride, potassium and sodium. Concentration of the biochemical constituents were calculated according to

the manufacturers' instructions, a standard protocol. The values obtained were used to analyze the biochemical condition of the experimental animals.

Statistical Data Analysis

The Biochemical Parameters were analyzed using SPSS. Analysis of variance (ANOVA) was first performed. Then a post-hoc test via Bonferroni for multiple comparisons was carried out. The Biochemical Parameters were considered statistically significant at $p<0.05$ and Confidence interval (CI) void of zero value.

Results and Discussion

The result of the biochemical activities of the wistar rat are shown in the tables that follow.

The decrease in the serum urea level was found to be significant ($p<0.0001$) compared to the normal control as shown in table 1.

There was a decrease in the serum creatinine level after CCl_4 intoxication which was significant ($p<0.0001$) compared to the normal control as shown in Table 2.

In this study also, bicarbonate (HCO^{3-}), chloride (Cl^-), potassium (K^+) and Sodium (Na^{2+}) levels in the negative control, were significantly elevated ($p<0.0001$) respectively, compared to the normal control group (Table 3-6).

In this study, carbon tetrachloride (CCl_4) treatment of Wister rats in the negative control resulted in decreased serum level of urea. The decrease in serum urea level was found to be significant ($p<0.05$) compared to the normal control as shown in table 1. This finding tends to support the reports obtained by previous experimental studies [38, 39]. Also, the reports on the effect of CCl_4 on serum creatinine level tends be contradicting among some investigators. While some investigators [39-42] found no significant effect of CCl_4 on serum creatinine level, our present study follows the report of other investigators [43] which observed a decrease in the serum creatinine level after CCl_4 intoxication which was significant ($p<0.05$) compared to the normal control as shown in table 2.

These alterations can be attributed to the disruption of the permeability of plasma, lysosomal, and mitochondrial membranes, as a result of the release of highly reactive oxides formed by the metabolism of CCl_4 [44, 45]. However, these trends were reverted in the experimental group as the serum urea and creatinine levels increase significantly. Elevation of these waste products in the kidney as depicted in table 1 & 2 are an indication of maintenance of renal function [46].

Maintenance of osmotic balance of the blood is done by reabsorption of ions, and it is one of the principal functions of the kidney [47]. In this study, bicarbonate (HCO^{3-}), chloride (Cl^-), potassium (K^+) and Sodium (Na^{2+}) levels in the negative control, were elevated significantly ($p<0.05$) compared to the normal control group (table 3-6). The significant increase in Na^{2+} concentration may indicate excess destruction of cells [47]. The serum increase of electrolytes is further evidence of renal impairment [48]. Although, a keen observation of the different levels of elevations show that they tend to vary among the various groups.

These renal protective effects were manifestations of antioxidant properties of the micronutrients contained in ICW. This is consistent with the report that micronutrients act directly to quench free radicals by donating electrons, or indirectly as a part of metalloenzymes [30].

Conclusion & Recommendation

It is considerable enough from the results of this study that ICW was able to suppress effectively, the effects of the radical oxidizing species produced from CCl_4 metabolism. This it achieved by exhibiting antioxidant activities.

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.

Table 1: Effects of CCl_4 and ICW on serum Urea activities of kidney in rat [Bonferroni (ANOVA post-hoc test) Multiple comparison Test output for Group Differences].

	Group	Mean Difference	Std. Error	P-value	95% Confidence Interval (CI)	
					Lower	Upper
Urea	Normal & Positive	52.86*	1.806	0	47.43	58.29
	Normal & Negative	-20.21*	1.806	0	-25.64	-14.78
	Normal & Experimental	-10.88*	1.806	0	-16.31	-5.45
	Positive & Negative	-73.07*	1.806	0	-78.5	-67.64
	Positive & Experimental	-63.74*	1.806	0	-69.17	-58.31
	Negative & Experimental	9.33*	1.806	0.001	3.9	14.77

* The mean difference is significant at the 0.05 level.

There was a decrease in the serum creatinine level after CCl_4 intoxication which was significant ($p<0.0001$) compared to the normal control as shown in table 2.

Table 2: Effects of CCl_4 and ICW on serum Creatinine activities of kidney in rat [Bonferroni (ANOVA post-hoc test) Multiple comparison Test output for Group Differences].

	Group	Mean Difference	Std. Error	P-value	95% Confidence Interval (CI)	
					Lower	Upper
Creatinine	Normal & Positive	0.77	0.339	0.23	-0.26	1.79
	Normal & Negative	-3.28*	0.339	0	-4.3	-2.26
	Normal & Experimental	-1.97*	0.339	0	-2.99	-0.95
	Positive & Negative	-4.05*	0.339	0	-5.07	-3.03
	Positive & Experimental	-2.74*	0.339	0	-3.76	-1.72
	Negative & Experimental	1.31*	0.339	0.008	0.29	2.33

* The mean difference is significant at the 0.05 level.

Table 3: Effects of CCl_4 and ICW on serum Chlorine (Cl^-) activities of kidney in rat [Bonferroni (ANOVA post-hoc test) Multiple comparison Test output for Group Differences].

	Group	Mean Difference	Std. Error	P-value	95% Confidence Interval (CI)	
					Lower	Upper
Chloride	Normal & Positive	-26.66*	0.688	0	-28.73	-24.59
	Normal & Negative	-42.66*	0.688	0	-44.73	-40.59
	Normal & Experimental	-52.44*	0.688	0	-54.51	-50.37
	Positive & Negative	-16.00*	0.688	0	-18.07	-13.93
	Positive & Experimental	-25.78*	0.688	0	-27.85	-23.7
	Negative & Experimental	-9.78*	0.688	0	-11.85	-7.71

* The mean difference is significant at the 0.05 level.

Table 4: Effects of CCl_4 and ICW on serum Potassium (K^+) activities of kidney in rat [Bonferroni (ANOVA post-hoc test) Multiple comparison Test output for Group Differences].

	Group	Mean Difference	Std. Error	P-value	95% Confidence Interval (CI)	
					Lower	Upper
Potassium	Normal & Positive	1.55*	0.038	0	1.44	1.67
	Normal & Negative	1.71*	0.038	0	1.6	1.83
	Normal & Experimental	-3.02*	0.038	0	-3.13	-2.9
	Positive & Negative	0.16*	0.038	0.004	0.047	0.28
	Positive & Experimental	-4.57*	0.038	0	-4.68	-4.45
	Negative & Experimental	-4.73*	0.038	0	-4.85	-4.62

* The mean difference is significant at the 0.05 level.

Table 5: Effects of CCl_4 and ICW on serum Sodium (Na^+) activities of kidney in rat [Bonferroni (ANOVA post-hoc test) Multiple comparison Test output for Group Differences].

	Group	Mean Difference	Std. Error	P-value	95% Confidence Interval (CI)	
					Lower	Upper
Sodium	Normal & Positive	0.59*	0.088	0	0.32	0.85
	Normal & Negative	16.91*	0.088	0	16.64	17.17
	Normal & Experimental	9.20*	0.088	0	8.93	9.47
	Positive & Negative	16.32*	0.088	0	16.06	16.59
	Positive & Experimental	8.61*	0.088	0	8.35	8.88
	Negative & Experimental	-7.71*	0.088	0	-7.97	-7.44

* The mean difference is significant at the 0.05 level.

Table 6: Effects of CCl_4 and ICW on serum Bicarbonate (CO_3^-) activities of kidney in rat [Bonferroni (ANOVA post-hoc test) Multiple comparison Test output for Group Differences].

	Group	Mean Difference	Std. Error	P-value	95% Confidence Interval (CI)	
					Lower	Upper
Bicarbonate	Normal & Positive	-10.03*	0.06	0	-10.21	-9.85
	Normal & Negative	-13.16*	0.06	0	-13.34	-12.98
	Normal & Experimental	-18.49*	0.06	0	-18.68	-18.31
	Positive & Negative	-3.14*	0.06	0	-3.32	-2.96
	Positive & Experimental	-8.47*	0.06	0	-8.65	-8.29
	Negative & Experimental	-5.33*	0.06	0	-5.51	-5.15

* The mean difference is significant at the 0.05 level.

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