

# Protective Effect of Immature Coconut Water on Hepatocytes against Carbontetrachloride-induced Liver Damage in Wister Rats

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**Abstract:** *The biochemical characteristics of immature coconut water (ICW) have presented it as a therapeutic agent that can be used extensively in the field of biochemistry and medicine for both industrial, and preventive, management and curative purposes. Development of a more efficient and cost effective means of prevention and management of liver 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<sub>4</sub>) induced hepatotoxicity. 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<sub>4</sub> (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 and liver samples were collected for biochemical and histopathological analysis. The present findings revealed that, CCl<sub>4</sub> elevated serum enzyme activities of liver and some biochemical parameters, but these effects were prevented by the prior administration ICW on rats. Histopathologically, a significant cyto-architectural distortion, hepatocyte degeneration, necrotic cells, fatty liver, and inflammatory cell migration were observed in the liver of CCl<sub>4</sub> treated group. The present study concluded that ICW administration played a protective role against CCl<sub>4</sub>-induced liver damages in Wister rats. These protective effects were in the form of improving liver enzyme activities, blood biochemical parameters and histological features of liver in CCl<sub>4</sub>-intoxicated rats. In the future, a dose dependent protective effect, and in vitro and in vivo regenerative effect of ICW on hepatocytes, could be investigated.*

**Keywords:** Protection, Hepatocyte, Immature coconut water (ICW), Histopathology, Serum

## 1. Introduction

The liver is the largest gland and the second largest organ in the body. It weighs approximately 1500 g and accounts for approximately 2.5% of adult human body weight [1]. It has a complex architecture, and performs a myriad of functions in the body [2, 3]. Drugs and toxins or viral infection can cause extensive damage to hepatocyte, reducing function and regeneration, and thereby leading to liver failure [4, 5]. Liver diseases remain one of the more serious health problems [6, 7], as it accounts for it 7.9% of the total medical admissions [8] and will become the 14<sup>th</sup> most common cause of death in 2030 [9].

Toxicity of CCl<sub>4</sub> is a well characterized murine model for the study of oxidative damage in vivo [10], as it has been widely used for experimental induction of liver damage [11-13]. CCl<sub>4</sub> metabolism by the hepatic cytochrome P450 generates the trichloromethyl free radical, which readily interacts with molecular oxygen to form trichloromethylperoxyl radicals that are hepatotoxic [14, 15]. Hepatotoxicity of CCl<sub>4</sub> can lead to cell injury and liver damage, in a similar way as what happens in the cases of acute hepatitis [16, 17]. Thus, the effort towards the prevention of hepatic damage by eliminating free radicals and prevent lipid peroxidation, is justified. The protective effects of various natural and synthetic products against hepatotoxicity have been observed to have been reported [18-20], with varying degrees of protection.

Immature coconut water (ICW) is the liquid found inside green coconut before the coconut matures and turns acidic [21]. It contains the various vitamin B complexes[22], vitamin C (15 mg/100mL), and a free amino acid L-arginine (30 mg/dL), which significantly reduce lipid peroxidation [23]. It is also highly rich in Inorganic ions such as K (290 mg %), Na (42 mg %), Mg (10 mg %), P (9.2 mg %) etc. These Micronutrients act directly to quench free radicals by donating electrons, or indirectly as a part of metallo enzymes [24].

In this present study, we investigated the protective effects of young coconut juice against CCl<sub>4</sub>-induced liver toxicity in rats by examining serum levels of liver enzymes.

## 2. Materials and Methods

### Plant Materials

Young coconuts were (*Cocosnucifera* L.) collected from Eziobodo Community, in Owerri-West Local Government Area of 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 YCW 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 of 175-200g 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 experimental animals were housed in air-conditioned rooms at 23-25°C, kept on a 12 h/12 h light/dark cycle and had free access to standard rodent pellet diet and water ad libitum. The animals were acclimatized in the laboratory conditions for a week before the commencement of the study. 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 (1985, no. 85-23) (25).

### 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 toxicity by CCl<sub>4</sub>

Liver toxicity was induced by the intraperitoneal injection of Carbon tetrachloride CCl<sub>4</sub>, 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 ICW in Experimental Animals.

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:

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

## 3. Experimental Group and Protocol

The rats were divided randomly into 4 groups comprising 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 7<sup>th</sup> day of the experiment with olive oil (0.5ml/kg body weight) only.

**Group II:** This group is made up 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 7<sup>th</sup> day of the experiment with olive oil (0.5ml/kg body weight). This group served as positive control. The

calculated dosage of 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 5 male rats with weights ranging from 175g-200g. Rats fed basal diet and tap water, and then they were intoxicated via intraperitoneal injection on the 7<sup>th</sup> day of the experiment with CCl<sub>4</sub> 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 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 7<sup>th</sup> day of the experiment with CCl<sub>4</sub> 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).

## 4. 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 Chemicals

Commercially obtained diagnostic kits were used to determine the serum levels of biochemical parameters, as follows: Randox Laboratories Limited was used for determination of serum Aspartate aminotransferase (ASP), Alanine aminotransferase (ALT), and Teco Diagnostics were used for determination of serum Acid phosphatase (ACP). Concentration of the biochemical constituents was calculated according to the manufacturers' instructions. The values obtained were used to analyze the biochemical condition of the experimental animals.

### Statistical Data Analysis

The Results of the Biochemical Parameters were analysed using SPSS. The statistical analysis of variance (ANOVA) test and further test via Bonferroni (ANOVA post-hoc test) for multiple comparisons were performed. Where  $p < 0.05$  and Confidence interval (CI) void of zero value were considered statistically significant.

## 5. Results

### Biochemical Data Analysis of Blood Serum

The biochemical activities of AST, ALT and ACP were estimated in serum samples as the liver function biomarkers. These results are given in Table 1. The CCl<sub>4</sub> treatment markedly affected the liver specific enzymes. It was found that a significant ( $p < 0.05$ ) increase in serum AST, ALT and ALP activities of CCl<sub>4</sub> treated rats. This result suggests that these hepatic biomarkers were elevated in the serum due

to release of the enzymes from damaged liver. However as significant decrease ( $p < 0.05$ ) was observed in the respective serum activities of rats given Camel milk + CCl<sub>4</sub> compared with CCl<sub>4</sub> treated group. In the other hand, the

activities of ACP showed insignificant changes ( $p > 0.05$ ) in all treated groups.

**Table 1:** Effects of CCl<sub>4</sub> and ICW on serum Aspartate transferase (ASP) activities of liver 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
AST	Normal & Positive	1.09*	0.105	0.000	0.78	1.41
	Normal & Negative	-0.70*	0.105	0.000	-1.02	-0.39
	Normal & Experimental	-0.34*	0.105	0.031	-0.65	-0.02
	Positive & Negative	-1.80*	0.105	0.000	-2.11	-1.48
	Positive & Experimental	-1.43*	0.105	0.000	-1.75	-1.12
	Negative & Experimental	0.37*	0.105	0.018	0.05	0.68

**Table 2:** Effects of CCl<sub>4</sub> and ICW on serum Alanine transferase (ALT) activities of liver in ratBonferroni (ANOVA post-hoc test)Multiple comparison Test output for Group Differences

	Group	Mean Difference	Std. Error	P-value	95% Confidence Interval (CI)	
					Lower	Upper
ALT	Normal & Positive	1.71*	0.330	0.001	0.72	2.70
	Normal & Negative	-8.61*	0.330	0.000	-9.60	-7.62
	Normal & Experimental	-4.67*	0.330	0.000	-5.66	-3.68
	Positive & Negative	-10.32*	0.330	0.000	-11.31	-9.33
	Positive & Experimental	-6.38*	0.330	0.000	-7.37	-5.39
	Negative & Experimental	3.94*	0.330	0.000	2.94	4.93

**Table 3:** Effects of CCl<sub>4</sub> and ICW on serum Acid phosphatase (ACP) activities of liver in ratBonferroni (ANOVA post-hoc test)Multiple comparison Test output for Group Differences

	Group	Mean Difference	Std. Error	P-value	95% Confidence Interval (CI)	
					Lower	Upper
ACP	Normal & Positive	2.63*	0.193	0.000	2.05	3.21
	Normal & Negative	-1.49*	0.193	0.000	-2.07	-0.91
	Normal & Experimental	0.72*	0.193	0.011	0.14	1.29
	Positive & Negative	-4.12*	0.193	0.000	-4.70	-3.54
	Positive & Experimental	-1.92*	0.193	0.000	-2.50	-1.34
	Negative & Experimental	2.20*	0.193	0.000	1.62	2.78

## 6. Discussion

In this study, carbon tetrachloride (CCl<sub>4</sub>) treatment of Wister rats in the negative control resulted in elevated serum levels of hepatic enzymes such as; AST, ALT and ACP. These elevations were significant ( $p < 0.05$ ) compared to the normal control as shown in table1-3. The increased serum levels of the hepatic enzymes may be due to the cell membrane and mitochondria damages as a result of CCl<sub>4</sub> induction, thereby resulting to their release into circulation as reported in other studies (26-28). Also, this study confirms the cyto-architectural distortion reported in other studies on hepatotoxicity, where cell injury and liver damage occurred after CCl<sub>4</sub> (29-35).

Treatment of Wister rats with ICW prior to CCl<sub>4</sub> intoxication in the experimental group, was found to suppress significantly ( $p < 0.05$ ) the increase of serum AST, ALT and ACP activities induced by CCl<sub>4</sub> treatment in rats. This finding implies that ICW challenge to protect liver tissue from CCl<sub>4</sub> injury. Suppression of the elevation of liver enzymes after CCl<sub>4</sub> intoxication may be due to the prevention of the intracellular enzyme leakage from membranes (36). The attenuation of CCl<sub>4</sub> induced liver damage by ICW showed its free radical scavenging and

membrane stabilizing activities [23, 37, 38]. However, this can be as a result of its high vitamins and micronutrients content [22, 24,39]. The efficacy of ICW as a hepatoprotective agent has been observed in this study; where it has shown the capacity to reduce the harmful effect, while retaining the hepatic physiology that would have been distorted by a hepatotoxin [40].

## 7. Conclusion

The result of this study showed that ICW caused a protective effect against CCl<sub>4</sub>-induced liver damage and as well improved the biochemical parameters. Therefore, ICW may be used as a therapeutic agent to protect against toxic effects of CCl<sub>4</sub> and other chemical agents that may harm the liver.

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