


RESEARCH

Open Access



Protective effects of coconut water against the intraperitoneal infused carbon tetrachloride-induced toxicity—evaluations of biochemical, haematological and histopathological profiles in rats

Okezie Emmanuel^{1*} , Ifeanyichukwu Elekwa¹, Chidiebere Paul-Joseph¹, Victor C. Ude², Ozioma G. Egedezu¹, Solomon N. Ijioma³, Victor Obioma Amachaghi¹, Chikezie Uche-Ikonne⁴ and Eziuche A. Ugbogu¹

Abstract

Background: Coconut water is a relished beverage traditionally used as a remedy for childhood diarrhoea and gastroenteritis. It can be given to an individual who has hangover or suspected to have ingested a toxic substance. In this study, we evaluated the protective effect of coconut water against carbon tetrachloride (CCl₄)-induced toxicity in rats.

Results: Administration of coconut water decreased significantly albumin, aspartate transaminase (AST), alanine transaminase (ALT), urea, creatinine, bicarbonate (HCO₃⁻), total cholesterol, triglycerides, low-density lipoprotein, very low-density lipoprotein and malondialdehyde (MDA) levels compared to the non-pretreated group. Furthermore, high-density lipoprotein, glutathione (GSH), superoxide dismutase (SOD) and catalase (CAT) increased significantly ($p < 0.05$) in coconut water-pretreated groups compared to the negative control. There were no observed pathological changes in the coconut water-pretreated groups, but slight enlargement of the central veins and tubules was evident when compared to the negative control.

Conclusions: Therefore, intake of coconut water may be protective against toxicity induced by CCl₄ as its pretreatment elevated antioxidant parameters (GSH, SOD and CAT) and did not alter biochemical parameters in rats.

Keywords: Antioxidant, Carbon tetrachloride, Coconut water, Haematology, Toxicity

Background

Coconut water is a relished beverage gotten from coconuts (*Cocos nucifera* L) (Gordon and Jackson 2017). Previously, coconut water was mostly acquired as a fresh beverage in regions where it was accessible, by cutting open a green nut fresh off the tree; today, it is a \$300+ million business (Gordon and Jackson 2017). It is

a perennial plant that bears fruit 12–13 times a year for up to 60–70 years, providing between 30 and 75 fruits per year. The fruit, in the shape of a nut, grows on trees of different heights depending on the type of coconut and is an important source of money for many farmers and countries in the far East in particular, where it is utilized in a number of ways and exported to other countries across the world (Gordon and Jackson 2017). The fruit is a significant source of oilseeds, particularly in many developing nations such as the Philippines, Sri Lanka, India, Malaysia and portions of West Africa, Latin

*Correspondence: emmanuelokezie7@gmail.com

¹ Department of Biochemistry, Abia State University, PMB 2000, Uturu, Abia State, Nigeria

Full list of author information is available at the end of the article

America and the Caribbean (Gordon and Jackson 2017; Ghosh et al. 2014).

The chemical composition of coconut water has been widely studied (Yong et al. 2009; Awua et al. 2011; Prades et al. 2012). Compositionally, it is made of about 96% water and 6% solids. The major components of the solids in coconut water are soluble sugars, proteins and minerals with relatively high potassium content and low sodium content. Phytochemical compositions of coconut water have shown to contain phenolic compounds, flavonoids, tannins and phytates (Akpro et al. 2019). However, the content of coconut water is highly influenced by the type of cultivar of coconut and the stage of maturation of the nut (Gordon and Jackson 2017). The reports of Yong et al. (2009) revealed the presence of some water-soluble vitamins in coconut water such as thiamine, riboflavin, nicotinic acid, pantothenic acid, pyridoxine, biotin, folic acid and ascorbic acid. Salicylic acid, syringic acid, *m*-coumaric acid, *p*-hydroxybenzoic acid, *p*-coumaric acid, caffeic acid and catechin are among the phenolic constituents found in coconut water (Mahoyothee et al. 2016). Aside from amino acids and L-arginine, coconut water contains plant hormones such as auxin, 1, 3-diphenylurea and cytokinin. It also contains acid phosphatase, catalase, dehydrogenase, diastase, peroxidase, RNA polymerases and growth factors. More so, it contains some minerals including sodium, potassium, magnesium, iron, calcium, copper and phosphorous (Kaliyamoorthy et al. 2015).

Coconut water is unstable to air, and it is best consumed fresh in tropical climates. Fresh coconut water has traditionally been used for oral rehydration, treatment of childhood diarrhoea and gastroenteritis (Mandal et al. 2009). It provides several nutritional, physiological and medicinal benefits including antifungal, antioxidant, antibacterial, hypoglycaemic, anti-cancer, antitumor, anti-dermatophyte, antiviral, anti-parasitic and hepatoprotective properties (Loki and Rajamohan 2003; Mohamad et al. 2019; Elekwa et al. 2021). These biological activities have been attributed to their high concentration of bioactive chemicals. Researchers have also uncovered that virgin coconut oil has anti-inflammatory, analgesic, antipyretic, immune-stimulatory and anti-cancer (Intahpuak et al. 2010; Varma et al. 2019).

Pollutants and chemical xenobiotics emitted by industrial waste, such as CCl₄, can harm the liver and kidney by producing reactive oxygen species (ROS) (Dutta et al. 2018). ROS promotes oxidative stress, which has been linked to hepatorenal damages (Liu et al. 2011). ROS has also shown to induce cellular damage by lipid peroxidation and covalent binding, and an increase in ROS decreases oxidative stress defence enzymes. Its mode of action is evident in the liver cytochrome P₄₅₀ enzyme

system's reductive dehalogenation to create trichloromethyl free radical, which rapidly interacts with molecular oxygen to produce trichloromethylperoxyl radicals (Li et al. 2004). Excessive formation of CCl₄ free radicals can result in membrane lipid oxidation, which can progress to hepatorenal injury (Li et al. 2004). In this study, we investigated the protective effects of coconut water against CCl₄-induced toxicity in rats.

Methods

Collection and preparation of coconut water

Healthy tender coconuts were gathered from Ndi Ekpere Compound, Amayi Igbere in Bende Local Government Area of Abia State, Nigeria. The coconut was de-husked, and the epicarp was gently opened to avoid losing or contaminating the coconut water. Every day, new coconut water was collected and filtered in a sterile condition using a ready-to-use Whatman filter No 1.

Animal use and care

Healthy rats (male and female) (8 weeks old; weight 151.78 ± 19.45 g, n = 60) bred locally at the Department of Biochemistry, Faculty of Biological Sciences, Abia State University, Uturu, were used for the study. The rats were housed in a metal cage for at least a week before the commencement of the study to allow for acclimatization under natural atmospheric conditions. They were fed with standard rat chow (Vital Feed) and water *ad libitum*. The laboratory procedures and tests were carried out with the permission of the Abia State University Ethical Committee (ABSU/REC/BMR/0026) and in accordance with World Health Organization recommendations for 'good laboratory practice'.

Experimental procedure

Healthy Wistar rats (male, n = 30 and female, n = 30) were randomly divided into five groups. To prevent mating, the male and female rats were placed in separate cages. Groups 3–5 were treated with different doses of coconut water for 14 days. After the 14th day pretreatment with coconut water, groups 2–5 were given 1 mL/kg of CCl₄ (Merck, UK) diluted in olive oil at a 1:1 ratio by intraperitoneal (*i.p.*) route. The procedure is shown as:

Group 1: normal control- (distilled water; 1 mL/kg; *p.o.*)

Groups 2: negative control- CCl₄ only (1 mL/kg; *i.p.*)

Group 3: Coconut water (2 mL/kg; *p.o.*) + CCl₄ (1 mL/kg; *i.p.*)

Group 4: Coconut water (4 mL/kg; *p.o.*) + CCl₄ (1 mL/kg; *i.p.*)

Group 5: Coconut water (6 mL/kg; *p.o.*) + CCl₄ (1 mL/kg; *i.p.*)

The rats were sacrificed after 24 h of CCl_4 treatment. The rats were weighed and humanely sacrificed by cervical dislocation. The blood samples were collected through cardiac puncture and distributed into dry plain bottles for biochemical tests and ethylenediaminetetraacetic acid (EDTA) bottles for haematological investigations. The liver and kidney were harvested and used to assess their histopathology.

Haematological and biochemical assays

Red blood cell (RBC), white blood cell (WBC), platelets, RBC indices and WBC indices were measured in accordance with the procedure previously described by Bain et al. (2017). Randox diagnostic kits (Randox Laboratory Ltd., Co., Antrim, UK) were used to evaluate spectrophotometrically the biomarkers of kidney, liver and lipids. Urea, creatinine and electrolytes (Na^+ , K^+ , HCO_3^- and Cl^-) were the renal biomarkers studied. The hepatic biomarkers measured were alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP), bilirubin, total protein and albumin. Furthermore, lipids calculated included triglycerides (TAG), high-density lipoprotein (HDL), total cholesterol (TC) and low-density lipoprotein (LDL).

Antioxidant markers

Other researchers' methodologies were used to determine oxidative stress indicators. Techniques for measuring superoxide dismutase (SOD) were used as reported by Sun and Zigma (1978). Catalase was estimated as revealed by Aebi (1984). Malondialdehyde and

glutathione were evaluated using the methods described by Buege and Aust (1978) and Sedlak and Lindsay (1968), respectively.

Histopathological assessments

The liver and kidneys were fixed in 10% v/v formalin before being encased in paraffin wax and sectioned. Each specimen was cut at 5 μm and stained with haematoxylin and eosin dyes. Using X40 magnification light microscope, the stained portions were examined for histological changes and photomicrographs were taken.

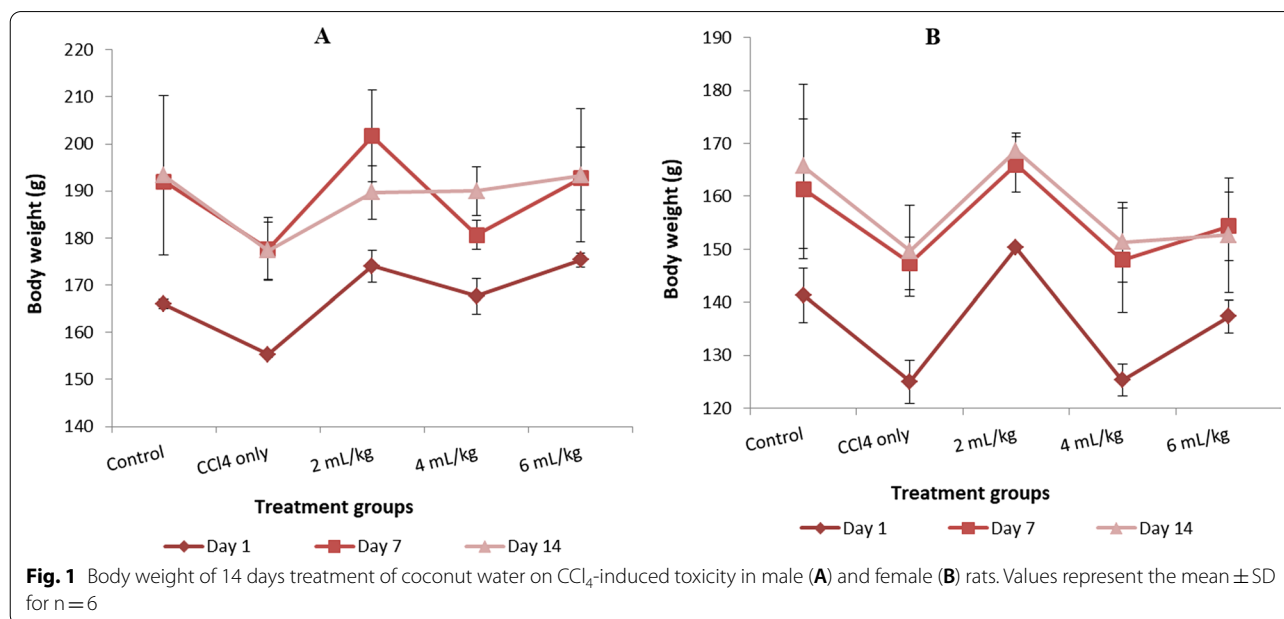
Statistical analysis

The generated data were represented as mean \pm standard deviation. To determine the difference between the groups, statistical analysis was performed with RTM statistical program (<https://cran.r-project.org/src/base/R3/>), version 3.0.3. A significant relationship was determined using the Tukey test post hoc at a 95% level of confidence.

Results

Body weight of 14 days treatment of coconut water on CCl_4 -induced toxicity

Figure 1 represents the protective effect of coconut water on body weight of CCl_4 -induced toxicity in both male and female rats. No significant changes ($p > 0.05$) in body weight were observed in both sexes in all the treatment groups.



Protective effect of coconut water on liver biomarkers of CCl₄-induced toxicity

Intake of coconut water decreased the values of albumin, AST and ALT compared to the negative control. However, total protein and bilirubin levels had a dose-dependent significant difference ($p < 0.05$) between the coconut water-pretreated groups (4 and 6 mL/kg) and the negative (CCl₄) control. ALP values did not show any significant difference in the male rats. In the female rats, there were significant differences ($p < 0.05$) in the values of albumin, AST, ALT, ALP and bilirubin among the coconut water-pretreated groups compared to the CCl₄ group (Table 1).

Protective effect of coconut water on kidney biomarkers of CCl₄-induced toxicity

Table 2 shows the data set obtained from the protective effect of coconut water on kidney function of CCl₄-induced toxicity in both sexes. There were significant differences observed in the values of urea, creatinine, Na⁺, K⁺ and HCO₃⁻ in the coconut water-pretreated groups compared to the CCl₄-treated group.

Protective effect of coconut water on haematological parameters of CCl₄-induced toxicity

In the CCl₄ group, the values of RBC, PCV, HB, platelet, MCV and neutrophils were decreased compared to the coconut water-treated groups. Furthermore, the values of WBC and lymphocytes were increased in the negative

control compared to coconut water-treated groups (Table 3).

Protective effect of coconut water on lipid profiles of CCl₄-induced toxicity

Table 4 shows the result obtained from the protective effect of coconut water on lipid profiles of carbon tetrachloride-induced toxicity in both male and female rats. Groups administered with coconut water had a significant decrease ($p < 0.05$) in the values of TC, triglycerides, LDL and VLDL. The values of the HDL decreased significantly in the CCl₄ group compared to the coconut water-treated groups.

Protective effect of coconut water on antioxidant enzymes of CCl₄-induced toxicity

The result presented in Table 5 is the protective effect of coconut water on antioxidant enzymes of CCl₄-induced toxicity in both male and female rats. Administration of coconut water caused a significant elevation in the values of GSH, SOD and CAT. The values of MDA decreased in the treatment group compared to its untreated counterparts in both male and female rats.

Protective effect of coconut water on histology section of liver and kidney

The liver and kidney histology sections of the male and female rats are presented in Figs. 2, 3, 4 and 5. The photomicrograph of the normal control showed a normal liver architectural design, but the treated rats showed a slight

Table 1 Protective effect of coconut water on liver biomarkers of carbon tetrachloride-induced toxicity in male (A) and female (B) rats

Parameters	Control	CCl ₄ only	Coconut water (mL/kg)		
			2 mL/kg	4 mL/kg	6 mL/kg
A					
Total protein (g/dl)	7.20 ± 0.07 ^a	5.65 ± 0.11 ^b	5.79 ± 0.09 ^b	6.03 ± 0.25 ^c	6.12 ± 0.15 ^a
Albumin (g/dl)	4.22 ± 0.04 ^a	3.37 ± 0.20 ^b	3.36 ± 0.43 ^b	3.40 ± 0.37 ^b	3.34 ± 0.56 ^b
AST (U/L)	18.67 ± 3.06 ^a	80.33 ± 2.52 ^b	74.67 ± 4.16 ^c	71.67 ± 0.58 ^c	70.67 ± 3.06 ^c
ALT (U/L)	10.33 ± 1.53 ^a	74.67 ± 4.16 ^b	64.67 ± 0.58 ^c	63.00 ± 1.00 ^c	63.00 ± 2.65 ^c
ALP (U/L)	58.70 ± 3.87 ^a	71.67 ± 0.58 ^b	72.83 ± 1.19 ^b	72.97 ± 0.50 ^b	71.97 ± 1.39 ^b
Bilirubin (g/dl)	0.50 ± 0.08 ^a	1.65 ± 0.10 ^b	1.41 ± 0.09 ^c	1.31 ± 0.09 ^c	1.27 ± 0.04 ^c
B					
Total protein (g/dl)	6.56 ± 0.40 ^a	5.16 ± 0.22 ^b	5.83 ± 0.04 ^c	5.46 ± 0.66 ^d	5.57 ± 0.33 ^c
Albumin (g/dl)	4.13 ± 0.29 ^b	2.91 ± 0.25 ^a	3.09 ± 0.06 ^c	2.97 ± 0.13 ^a	3.05 ± 0.07 ^c
AST (U/L)	22.33 ± 2.52 ^b	87.67 ± 1.53 ^a	80.00 ± 2.00 ^c	79.67 ± 4.73 ^c	70.00 ± 2.00 ^d
ALT (U/L)	12.00 ± 2.00 ^a	78.67 ± 3.51 ^b	65.67 ± 6.03 ^c	61.33 ± 1.15 ^c	60.00 ± 2.00 ^c
ALP (U/L)	58.57 ± 4.30 ^a	81.37 ± 3.81 ^b	71.33 ± 1.12 ^c	72.23 ± 1.72 ^c	72.10 ± 2.33 ^c
Bilirubin (g/dl)	0.45 ± 0.06 ^a	1.60 ± 0.25 ^b	1.06 ± 0.23 ^c	1.07 ± 0.29 ^c	0.98 ± 0.10 ^c

AST, aspartate aminotransferase; ALT, alanine transaminase; and ALP, alkaline phosphatase. Values are presented as mean ± SD, n = 6. Values in the row bearing the same letters of the alphabet are not statistically significant ($p > 0.05$) from one another. The data were analysed with one-way ANOVA and expressed as mean ± SD, then compared to controls, n = 6. Different letters (a, b or c) $p < 0.05$ both male and female rats show significant difference between the tested groups (2, 4 and 6 mL/kg) compared to the negative control

Table 2 Protective effect of coconut water on kidney biomarkers of carbon tetrachloride-induced toxicity in male (A) and female (B) rats

Parameters	Control	CCl ₄ only	Coconut water (mL/kg)		
			2 mL/kg	4 mL/kg	6 mL/kg
A					
Urea (mg/dl)	25.47 ± 1.36 ^a	27.13 ± 0.85 ^b	23.10 ± 0.62 ^a	24.77 ± 0.91 ^a	22.50 ± 1.48 ^c
Creatinine (mg/dl)	0.58 ± 0.05 ^a	0.68 ± 0.02 ^b	0.54 ± 0.03 ^a	0.51 ± 0.02 ^a	0.53 ± 0.06 ^a
Na ⁺ (mEq/L)	130.00 ± 1.48 ^a	120.57 ± 0.87 ^b	133.43 ± 1.56 ^a	134.67 ± 5.31 ^a	147.83 ± 0.99 ^c
Cl ⁻ (mEq/L)	89.45 ± 1.28 ^a	85.67 ± 0.75 ^a	87.66 ± 0.39 ^a	88.80 ± 0.70 ^a	96.54 ± 4.71 ^b
K ⁺ (mEq/L)	4.82 ± 0.08 ^b	4.12 ± 0.18 ^a	4.50 ± 0.22 ^c	4.61 ± 0.13 ^c	5.27 ± 0.40 ^a
HCO ₃ ⁻ (mEq/L)	23.53 ± 0.67 ^b	29.50 ± 1.01 ^a	25.83 ± 0.85 ^b	26.13 ± 0.64 ^c	25.23 ± 0.40 ^b
B					
Urea (mg/dl)	25.24 ± 0.83 ^a	23.77 ± 1.26 ^b	23.70 ± 0.28 ^b	24.78 ± 0.35 ^a	25.42 ± 0.30 ^a
Creatinine (mg/dl)	0.47 ± 0.03 ^a	0.66 ± 0.04 ^b	0.58 ± 0.03 ^c	0.61 ± 0.02 ^c	0.60 ± 0.05 ^c
Na ⁺ (mEq/L)	126.87 ± 1.68 ^a	118.10 ± 2.41 ^b	139.37 ± 0.75 ^c	144.27 ± 1.97 ^c	146.30 ± 1.05 ^c
Cl ⁻ (mEq/L)	94.90 ± 0.46 ^a	92.20 ± 1.71 ^a	97.30 ± 0.90 ^b	97.77 ± 1.21 ^b	101.43 ± 1.86 ^c
K ⁺ (mEq/L)	4.43 ± 0.32 ^a	4.10 ± 0.10 ^b	4.80 ± 0.10 ^c	5.27 ± 0.08 ^d	5.15 ± 0.15 ^d
HCO ₃ ⁻ (mEq/L)	24.50 ± 0.26 ^a	28.80 ± 0.70 ^b	25.63 ± 1.40 ^a	24.40 ± 1.31 ^a	23.70 ± 1.10 ^a

Na⁺, sodium ion; Cl⁻, chloride ion; K⁺, potassium ion; and HCO₃⁻, bicarbonate. Values in the row bearing the same letters of the alphabet are not statistically significant ($p > 0.05$) from one another. The data were analysed with one-way ANOVA and expressed as mean ± SD, then compared to controls, $n = 6$. Different letters (a, b or c) $p < 0.05$ both male and female rats show significant difference between the tested groups (2, 4 and 6 mL/kg) compared to the negative control

enlarged central vein compared to the negative control. More so, the hepatocytes appeared a bit distorted and many microcysts within the stroma were found (A-J). In the kidney histology (K-T), no pathological change was observed in the normal control unlike the CCl₄-treated groups. While the negative control had a slight dilated and shrunken glomerulus, a slight enlargement of the tubules was observed in the coconut water-treated group, although there were no pathological changes observed amongst the groups (Figs. 4, 5).

Discussion

In this study, we examined the protective effect of coconut water against CCl₄-induced toxicity in rats. The findings of the study revealed no significant changes in body weight in the experimental rats. This might be attributable to the brief period of CCl₄ delivery following coconut water pretreatment. In other reports, body weight measurement has been described as plausible evidence in toxicological research (Emmanuel et al. 2021).

Elevations of liver biomarkers; enzymes (AST, ALT and ALP), bilirubin, albumin and total proteins are strong indicators for liver insults (Orieke et al. 2019; Ugbogu et al. 2019; Emmanuel et al. 2021). When the liver is under severe attack, these enzymes are released into the blood and thus give insight on the hepatic status of the liver (Ugbogu et al. 2019). Dutta et al. (2018) reported that CCl₄ is hepatotoxic by producing ROS. Increased ROS has been associated with decreased antioxidant

levels (Emmanuel et al. 2021). In the present study, the negative control had elevated levels of liver enzymes and administration of coconut water decreased the values of albumin, AST and ALT. Our findings correlate with the results of other researchers (Anurag and Rajamohan 2003). Anurag and Rajamohan (2003) showed that coconut water counteracted an increased level of liver enzymes caused by isoproterenol exposure. The reported hepatoprotective activities of coconut water might be linked to its antioxidant activities (Tsai et al. 2009).

A functional renal system may be related to the kidney's filtration capacity, commonly known as the glomerular filtration rate (GFR). A reduction in GFR may produce an increase in urea and creatinine in the serum, indicating renal disease (Jose 2014). Additionally, in kidney test, a high serum urea level indicates renal failure, which usually progresses to severe kidney damage in long-term instances (Gowda et al. 2010). In our study, urea, creatinine and HCO₃⁻ significantly decreased in the coconut water-treated groups when compared to the negative control. Abnormal elevations of creatinine, urea and electrolytes levels are implicated in kidney insults (Ugbogu et al. 2019; Emmanuel et al. 2021).

Haematological parameters are those that relate to blood and blood-forming organs. The quantity and shape of RBC, WBC and platelets are important in disease diagnosis and monitoring (Owoeye et al. 2011). In this work, groups pretreated with coconut water prior to CCl₄ induction had a significant haematoprotective effect

Table 3 Protective effect of coconut water on haematological parameters of carbon tetrachloride-induced toxicity in male (A) and female (B) rats

Parameters	Control	CCl ₄ only	Coconut water (mL/kg)		
			2 mL/kg	4 mL/kg	6 mL/kg
A					
RBC ($\times 10^{12}/L$)	5.13 \pm 0.15 ^a	4.47 \pm 0.15 ^a	5.00 \pm 0.10 ^a	5.30 \pm 0.10 ^a	6.07 \pm 0.15 ^a
PCV (%)	46.67 \pm 1.53 ^a	40.00 \pm 1.00 ^b	47.67 \pm 3.21 ^a	50.00 \pm 1.00 ^c	52.67 \pm 1.53 ^c
HB (g/dL)	14.43 \pm 0.38 ^a	13.27 \pm 0.25 ^b	14.60 \pm 0.53 ^a	14.97 \pm 0.25 ^a	15.07 \pm 0.15 ^a
WBC ($\times 10^9/L$)	7.23 \pm 0.35 ^a	10.13 \pm 0.35 ^b	6.93 \pm 0.31 ^a	8.43 \pm 0.38 ^a	7.50 \pm 0.30 ^a
Platelet ($\times 10^9/L$)	29.00 \pm 2.65 ^a	17.67 \pm 2.52 ^b	31.67 \pm 1.53 ^a	29.33 \pm 4.16 ^a	26.33 \pm 4.04 ^a
MCV (fl)	90.93 \pm 1.14 ^a	89.57 \pm 0.99 ^a	95.30 \pm 5.15 ^a	94.30 \pm 0.10 ^a	87.37 \pm 3.16 ^a
MCH (pg)	28.10 \pm 0.46 ^a	29.70 \pm 0.46 ^a	29.20 \pm 0.72 ^a	28.23 \pm 0.12 ^a	24.87 \pm 0.90 ^b
MCHC (g/L)	309.33 \pm 2.64 ^a	331.70 \pm 2.12 ^b	306.73 \pm 9.98 ^a	299.33 \pm 1.15 ^a	286.57 \pm 6.85 ^a
Neutrophil (%)	60.67 \pm 2.31 ^a	41.33 \pm 3.06 ^b	48.33 \pm 1.53 ^c	50.00 \pm 1.00 ^c	52.00 \pm 1.00 ^c
Lymphocyte (%)	32.00 \pm 2.00 ^a	51.33 \pm 3.21 ^b	45.33 \pm 2.52 ^c	42.67 \pm 1.53 ^c	41.00 \pm 1.00 ^c
Monocyte (%)	4.67 \pm 0.58 ^a	3.67 \pm 0.58 ^a	3.33 \pm 0.58 ^a	4.00 \pm 0.00 ^a	3.33 \pm 0.58 ^a
Eosinophil (%)	2.67 \pm 0.58 ^a	3.33 \pm 0.58 ^a	2.67 \pm 0.58 ^a	2.67 \pm 1.15 ^a	2.67 \pm 0.58 ^a
Basophil (%)	0.00 \pm 0.00	0.00 \pm 0.00	0.00 \pm 0.00	0.00 \pm 0.00	1.00 \pm 0.00
B					
RBC ($\times 10^{12}/L$)	6.80 \pm 0.50 ^a	5.90 \pm 0.40 ^a	6.10 \pm 0.60 ^a	6.90 \pm 0.06 ^a	7.00 \pm 0.90 ^a
PCV (%)	48.30 \pm 2.50 ^a	43.60 \pm 2.00 ^b	48.30 \pm 3.50 ^a	52.00 \pm 3.60 ^c	57.70 \pm 3.00 ^c
HB (g/dL)	15.80 \pm 0.81 ^a	14.80 \pm 0.20 ^b	15.70 \pm 0.50 ^a	16.00 \pm 0.60 ^a	16.10 \pm 0.90 ^a
WBC ($\times 10^9/L$)	9.90 \pm 0.82 ^a	11.67 \pm 0.78 ^b	8.90 \pm 0.42 ^a	10.00 \pm 0.67 ^a	9.10 \pm 0.20 ^a
Platelet ($\times 10^9/L$)	51.30 \pm 2.50 ^a	44.60 \pm 3.70 ^b	37.80 \pm 3.60 ^c	45.00 \pm 2.40 ^b	37.30 \pm 1.58 ^c
MCV (fl)	90.30 \pm 3.70 ^a	88.70 \pm 3.10 ^a	93.20 \pm 2.80 ^a	86.80 \pm 2.40 ^a	93.40 \pm 4.70 ^a
MCH (pg)	29.10 \pm 1.60 ^a	29.30 \pm 0.81 ^a	29.37 \pm 0.80 ^a	26.20 \pm 1.30 ^b	26.70 \pm 1.25 ^b
MCHC (g/L)	313.40 \pm 3.50 ^a	325.50 \pm 9.90 ^b	307.70 \pm 12.40 ^a	295.50 \pm 8.80 ^c	271.20 \pm 7.10 ^d
Neutrophil (%)	65.60 \pm 3.00 ^a	36.60 \pm 2.00 ^b	44.30 \pm 3.20 ^c	45.60 \pm 1.50 ^c	47.00 \pm 3.60 ^c
Lymphocyte (%)	27.60 \pm 1.50 ^a	56.60 \pm 2.15 ^b	49.30 \pm 2.80 ^c	48.30 \pm 2.80 ^c	45.60 \pm 2.90 ^d
Monocyte (%)	4.60 \pm 0.58 ^a	4.30 \pm 0.58 ^a	4.30 \pm 0.58 ^a	4.30 \pm 0.58 ^a	4.00 \pm 0.60 ^a
Eosinophil (%)	4.33 \pm 0.58 ^a	3.33 \pm 0.58 ^b	3.67 \pm 0.58 ^b	3.67 \pm 0.58 ^b	3.33 \pm 1.15 ^b
Basophil (%)	0.00 \pm 0.00	0.00 \pm 0.00	0.00 \pm 0.00	0.00 \pm 0.00	0.00 \pm 0.00

RBC, Red blood cells; PCV, packed cell volume; HB, haemoglobin; MCV, mean corpuscular volume; MCH, mean corpuscular haemoglobin; MCHC, mean corpuscular haemoglobin concentration; and WBC, white blood cell. Values in the row bearing the same letters of the alphabet are not statistically significant ($p > 0.05$) from one another. The data were analysed with one-way ANOVA and expressed as mean \pm SD, then compared to controls, $n = 6$. Different letters (a, b or c) $p < 0.05$ both male and female rats show significant difference between the tested groups (2, 4 and 6 mL/kg) compared to the negative control

in both male and female rats compared to the negative control. In the negative control, the levels of RBC, PCV and HB were decreased, and this may be associated with the toxic free radicals generated from CCl₄ which consequently affected haematopoiesis in the experimental animals as revealed in other studies (Rahmouni et al. 2011). Therefore, haematological parameters are very sensitive and are crucial for predicting the toxicological and pathological effect of potentially toxic substances in both human and animals (Kong et al. 2016). Previous study on the effects of CCl₄ on haematological parameters showed that acute CCl₄ toxicity led to transient decrease in the HB concentration and reticulocyte count as well as PCV and RBC counts by extension which is in consonant with

this present study (Saba et al. 2010; Emmanuel et al. 2021).

The rats administered with coconut water prior CCl₄ induction had decreased levels TC, triglycerides, LDL and VLDL, while HDL levels increased compared to the untreated group. This demonstrates that CCl₄ could disrupt lipid metabolism. Atherosclerosis and coronary heart disease have been linked to higher levels of cholesterol, triglycerides, LDL and VLDL (Sandhya and Rajamohan 2008; Emmanuel et al. 2021). Our findings confirmed the previously reported evidence that coconut water lowered the increased levels of TC, triglycerides, LDL and VLDL in rats induced by a high cholesterol fat feed (Sandhya and Rajamohan 2006).

Table 4 Protective effect of coconut water on lipid profiles of carbon tetrachloride-induced toxicity in male (A) and female (B) rats

Parameters	Control	CCl ₄ only	Coconut water (mL/kg)		
			2 mL/kg	4 mL/kg	6 mL/kg
A					
TC (mg/dL)	133.57 ± 4.04 ^a	152.57 ± 2.65 ^b	144.20 ± 2.13 ^c	142.83 ± 1.96 ^c	135.47 ± 2.12 ^a
HDL-C (mg/dL)	76.83 ± 1.21 ^a	65.43 ± 1.70 ^b	68.10 ± 3.35 ^b	72.47 ± 2.60 ^c	73.80 ± 3.35 ^c
Triglycerides (mg/dL)	155.00 ± 6.85 ^a	179.47 ± 3.05 ^b	170.50 ± 1.08 ^c	172.43 ± 2.90 ^c	167.97 ± 2.62 ^c
LDL-C (mg/dL)	25.73 ± 3.41 ^a	45.24 ± 35.89 ^b	42.19 ± 5.13 ^b	36.55 ± 3.58 ^c	28.07 ± 5.36 ^a
VLDL-C (mg/dL)	31.00 ± 1.37 ^a	35.89 ± 0.61 ^a	34.10 ± 0.22 ^a	33.82 ± 0.70 ^a	33.59 ± 0.52 ^a
B					
TC (mg/dL)	142.20 ± 4.40 ^a	149.80 ± 5.70 ^b	144.20 ± 2.80 ^a	144.20 ± 2.20 ^a	142.30 ± 3.40 ^a
HDL-C (mg/dL)	77.20 ± 2.10 ^a	67.80 ± 1.80 ^b	70.90 ± 2.20 ^c	72.90 ± 2.00 ^c	73.70 ± 2.90 ^c
Triglycerides (mg/dL)	171.70 ± 4.50 ^a	186.80 ± 4.10 ^b	178.00 ± 2.50 ^c	178.20 ± 2.20 ^c	178.70 ± 2.30 ^c
LDL-C (mg/dL)	30.70 ± 3.20 ^a	45.30 ± 3.80 ^b	37.70 ± 2.80 ^c	35.50 ± 1.60 ^c	32.90 ± 1.90 ^a
VLDL-C (mg/dL)	34.30 ± 1.80 ^a	37.30 ± 1.84 ^b	35.60 ± 1.50 ^c	35.60 ± 1.40 ^c	35.70 ± 1.90 ^c

TC, total cholesterol; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; and VLDL-C, very low-density lipoprotein cholesterol. Values in the row bearing the same letters of the alphabet are not statistically significant ($p > 0.05$) from one another. The data were analysed with one-way ANOVA and expressed as mean ± SD, then compared to controls, $n = 6$. Different letters (a, b or c) $p < 0.05$ both male and female rats show significant difference between the tested groups (2, 4 and 6 mL/kg) compared to the negative control

Table 5 Protective effect of coconut water on antioxidant enzymes of carbon tetrachloride-induced toxicity in male (A) and female (B) rats

Parameters	Normal control	CCl ₄ only	Coconut water (mL/kg)		
			2 mL/kg	4 mL/kg	6 mL/kg
A					
GSH (U/L)	49.53 ± 1.81 ^a	46.98 ± 2.67 ^b	48.13 ± 3.06 ^a	49.35 ± 1.16 ^a	49.67 ± 2.35 ^a
SOD (U/L)	21.97 ± 2.48 ^a	17.94 ± 1.52 ^b	18.52 ± 1.03 ^b	21.82 ± 0.65 ^a	22.44 ± 0.81 ^a
CAT (U/L)	12.52 ± 2.12 ^a	10.35 ± 0.75 ^b	10.89 ± 0.79 ^b	12.63 ± 0.77 ^a	11.62 ± 0.85 ^a
MDA (U/L)	0.51 ± 0.06 ^a	0.99 ± 0.10 ^b	0.98 ± 0.13 ^b	0.81 ± 0.06 ^b	0.81 ± 0.05 ^b
B					
GSH (U/L)	48.55 ± 4.03 ^a	45.25 ± 2.71 ^b	49.49 ± 3.47 ^a	51.36 ± 0.88 ^a	52.49 ± 1.71 ^a
SOD (U/L)	22.85 ± 1.60 ^a	19.81 ± 1.55 ^b	22.37 ± 0.53 ^a	22.57 ± 1.31 ^a	24.26 ± 0.81 ^c
CAT (U/L)	12.31 ± 1.11 ^a	10.56 ± 0.60 ^b	11.05 ± 1.31 ^a	12.17 ± 0.19 ^a	11.89 ± 1.00 ^a
MDA (U/L)	0.66 ± 0.05 ^a	1.09 ± 0.11 ^b	0.88 ± 0.04 ^c	0.87 ± 0.06 ^c	0.77 ± 0.03 ^c

GSH, glutathione; SOD, superoxide dismutase; CAT, catalase; and MDA, malondialdehyde. Values in the row bearing the same letters of the alphabet are not statistically significant ($p > 0.05$) from one another. The data were analysed with one-way ANOVA and expressed as mean ± SD, then compared to controls, $n = 6$. Different letters (a, b or c) $p < 0.05$ both male and female rats show significant difference between the tested groups (2, 4 and 6 mL/kg) compared to the negative control

CCl₄ has been found to be toxic by causing the production of free radicals capable of interacting with sulphur-containing proteins, which can impair antioxidant activity (Tsai et al. 2009). This study revealed that CCl₄ has a negative effect on antioxidants, as seen by a decrease in SOD, GSH and CAT and an increase in MDA. Our findings are consistent with the findings of Rahmouni et al. (2011) that discovered that CCl₄ may reduce erythrocyte GSH, SOD and CAT activities while increasing erythrocyte MDA levels.

In the histology of the liver and kidney, pathological changes were evident; however, there were slight enlargement of the central veins and tubules, respectively, compared to the negative control. Coconut water administration revealed a dose-dependent protection compared to the untreated group. Injuries in the liver included deformed hepatocytes, dilated sinusoids, microvascular steatosis and an enlarged central vein, while congested blood vessels and dilated tubules were detected in the kidney of the negative control. These findings support the

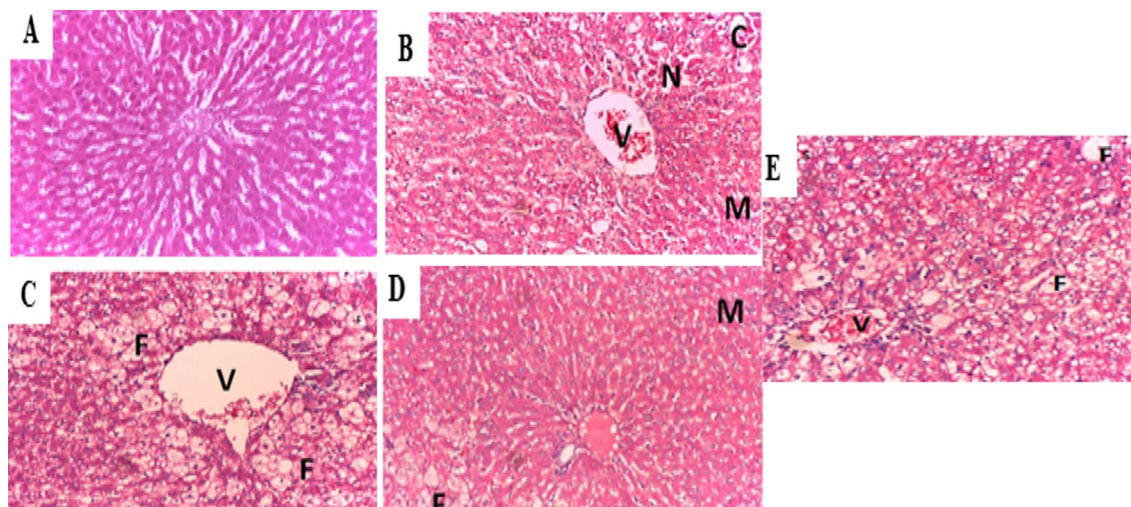


Fig. 2 Protective effect of coconut water on histology section of liver against CCl₄-induced toxicity in male rats (A–E). **A** Control, **B** CCl₄ only, **C** 2 mL/kg, **D** 4 mL/kg, **E** 6 mL/kg. V: central vein, S: sinusoids, NS: unmarkable sinusoids, M: multicystic space. The liver sections were stained with haematoxylin and eosin staining (H&E) solution and observed under an Olympus light microscope fixed with camera, at a magnification (40×)

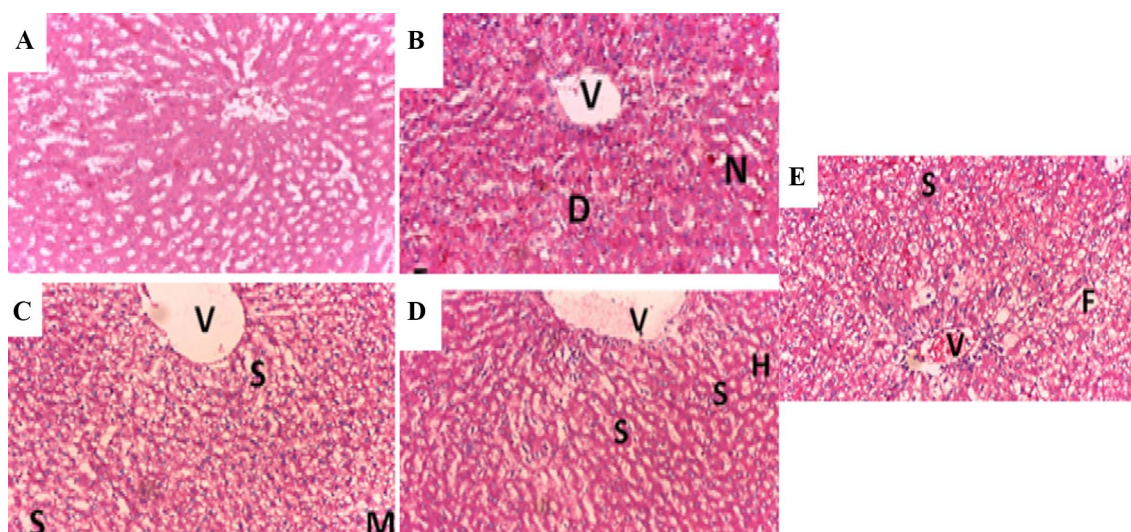


Fig. 3 Protective effect of coconut water on histology section of liver against CCl₄-induced toxicity in female rats (A–E). **A** Control, **B** CCl₄ only, **C** 2 mL/kg, **D** 4 mL/kg, **E** 6 mL/kg. V: central vein, S: sinusoids, NS: unmarkable sinusoids, M: multicystic space, D: dilated cystic space, H: distorted hepatocytes. The liver sections were stained with haematoxylin and eosin staining (H&E) solution and observed under an Olympus light microscope fixed with camera, at a magnification (40×)

assertion that CCl₄ has haematotoxic, hepatotoxic and nephrotoxic potentials (Rahmouni et al. 2011; Elshater et al. 2013; Emmanuel et al. 2021).

Conclusions

Our findings show that coconut water protects rats from CCl₄-induced toxicity. Its pretreatment for 14 days following CCl₄ toxicity elevated GSH, SOD,

CAT levels and decreased MDA levels. These data show that coconut water may protect against lipid peroxidation by scavenging the harmful radicals produced by CCl₄ and thereby normalizing the quantity and distribution of lipids in rats’ systemic circulation. The observed increase in these antioxidants may be related to coconut water’s hepato/reno-protective potentials and reduced cellular oxidative damage.

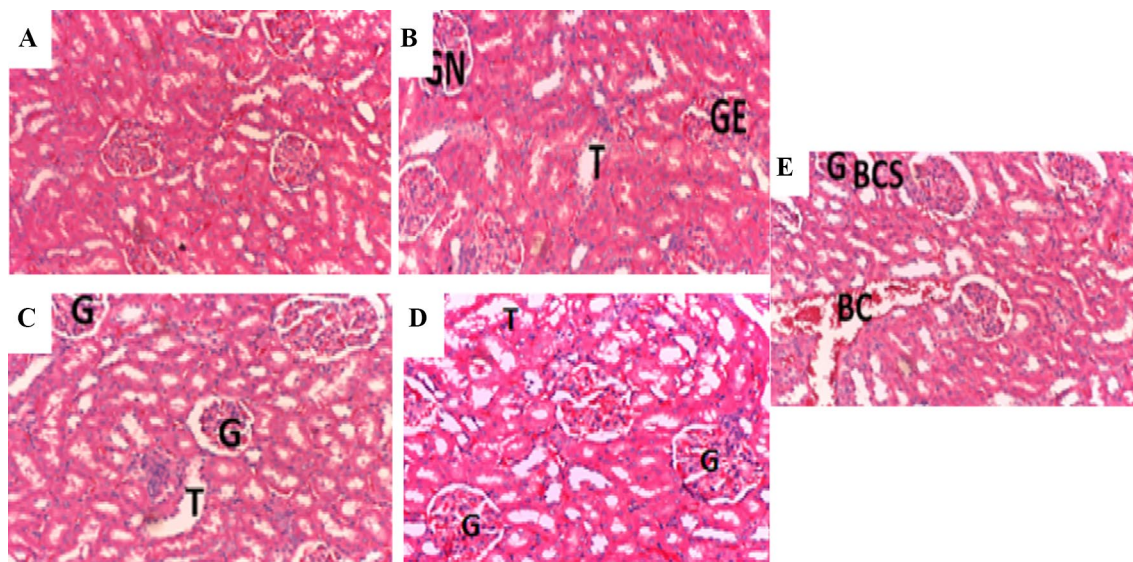


Fig. 4 Protective effect of coconut water on histology section of kidney against CCl₄-induced toxicity in male rats (A–E). **A** Control, **B** CCl₄ only, **C** 2 mL/kg, **D** 4 mL/kg, **E** 6 mL/kg. G: glomeruli, T: tubules, D: glomerular degeneration, GS: shrunken glomerula, BCS: Bowman’s space, B: Bowman’s capsule, D: dilated tubules, GN: network of the glomerulus. The kidney sections were stained with haematoxylin and eosin staining (H&E) solution and observed under an Olympus light microscope fixed with camera, at a magnification (40×)

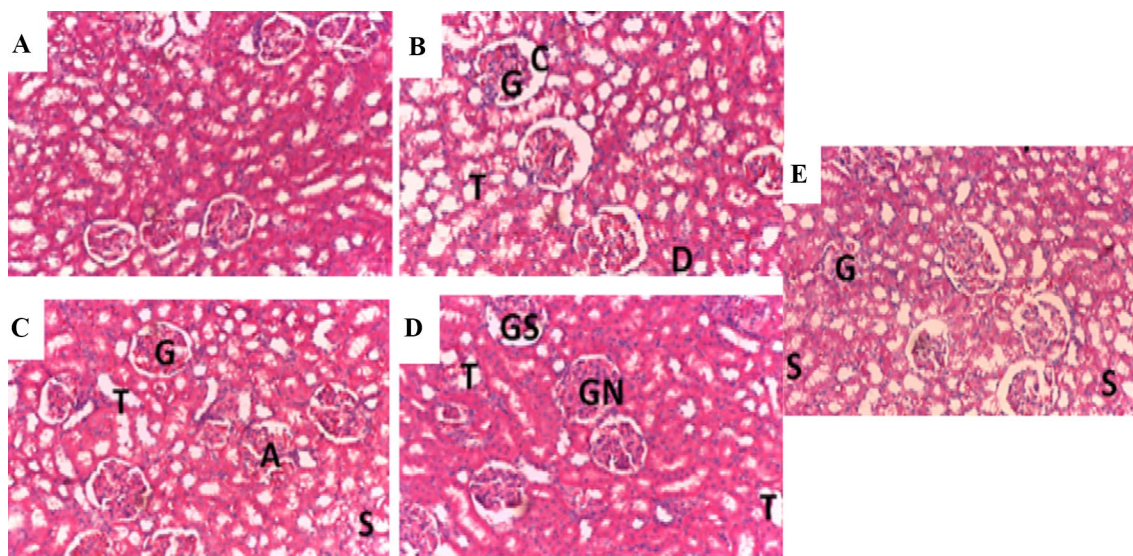


Fig. 5 Protective effect of coconut water on histology section of kidney against CCl₄-induced toxicity in female rats (A–E). **A** Control, **B** CCl₄ only, **C** 2 mL/kg, **D** 4 mL/kg, **E** 6 mL/kg. G: glomeruli, T: tubules, D: glomerular degeneration, GS: shrunken glomerula, BCS: Bowman’s space, B: Bowman’s capsule, D: dilated tubules, GN: network of the glomerulus. The kidney sections were stained with haematoxylin and eosin staining (H&E) solution and observed under an Olympus light microscope fixed with camera, at a magnification (40×)

Notwithstanding the health benefits of coconut water, it can be noted that its contents may be influenced and varied by the type of cultivar, geographical location and the stage of maturation of the nut.

Abbreviations

ALT: Alanine transaminase; AST: Aspartate transaminase; ALP: Alanine phosphatase; CAT: Catalase; CCl₄: Carbon tetrachloride; Cl⁻: Chloride ion; CVD: Cardiovascular disease; EDTA: Ethylenediaminetetraacetic acid; HB: Haemoglobin; HCO₃⁻: Bicarbonate; HDL: High-density lipoprotein; GSH: Glutathione; K⁺: Potassium ion; LDL: Low-density lipoprotein; MCV: Mean corpuscular volume; MCH: Mean corpuscular haemoglobin; MCHC: Mean corpuscular

haemoglobin concentration; MDA: Malondialdehyde; Na⁺: Sodium ion; PCV: Packed cell volume; RBC: Red blood cell; ROS: Reactive oxygen species; SOD: Superoxide dismutase; TC: Total cholesterol; VLDL: Very low-density lipoprotein; WBC: White blood cell.

Acknowledgements

Not applicable.

Author contributions

OE, IE and EAU conceptualized the work, sourced the literature and wrote the original draft. CP,VCU, OGE, SNI, VOA and CU wrote the original draft and read the manuscript. All authors have read and approved the manuscript.

Funding

This study was sponsored by Tertiary Education Trust Fund Institutional Base Research Grant from Abia State University, Uturu, Nigeria.

Availability of data and materials

All the data used for this manuscript are available on request from the corresponding author.

Declarations

Ethics approval and consent to participate

The laboratory procedures and tests were carried out with the permission of the Abia State University Ethical Committee (ABSU/REC/BMR/0026) and in accordance with World Health Organization recommendations for 'good laboratory practice'.

Consent for publication

Not applicable.

Competing interests

The authors declare none whatsoever.

Author details

¹Department of Biochemistry, Abia State University, PMB 2000, Uturu, Abia State, Nigeria. ²Department of Medical Biochemistry, College of Medicine, Enugu State University of Science and Technology, PMB 01660, Enugu, Nigeria. ³Department of Zoology and Environmental Biology, Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria. ⁴Department of Public Health, Abia State University, PMB 2000, Uturu, Abia State, Nigeria.

Received: 23 December 2021 Accepted: 29 June 2022

Published online: 15 July 2022

References

- Aebi H (1984) Catalase in vitro. In: Method in enzymology, vol 105. Academic Press, New York, pp 121–126 [https://doi.org/10.1016/S0076-6879\(84\)05016-3](https://doi.org/10.1016/S0076-6879(84)05016-3)
- Akpro LA, Gbogouri GA, Konan BR, Issali AE, Konan KJL, Brou KD, Nemlin GJ (2019) Phytochemical compounds, antioxidant activity and non-enzymatic browning of sugars extracted from the water of immature coconut (*Cocos nucifera* L.). *Sci Afr* 6:e00123. <https://doi.org/10.1016/j.sciafr.2019.e00123>
- Anurag P, Rajamohan T (2003) Cardioprotective effect of tender coconut water in experimental myocardial infarction. *Plant Foods Human Nutri* 58(3):1–12
- Awua AK, Doe ED, Agyare R (2011) Exploring the influence of sterilization and storage on some physicochemical properties of coconut (*Cocos nucifera* L.) water. *BMC Res Notes* 4(1):451
- Bain BJ, Bates I, Laffan MA (2017) Dacie and Lewis practical haematology. Elsevier Health Sciences, New York
- Buege JA, Aust SD (1978) Microsomal lipid peroxidation. *Methods Enzymol* 52:302–310
- Dutta S, Chakraborty AK, Dey P, Kar P, Guha P, Sen S, Kumar A, Sen A, Chaudhuri TK (2018) Amelioration of CCl₄ induced liver injury in Swiss albino mice by antioxidant rich leaf extract of *Croton bonplandianus* Baill. *PLoS ONE* 13(4):e0196411
- Elekwali I, Ude VC, Emmanuel O, Amachaghi VO, Ugobogu EA (2021) *In vivo* studies on the ameliorative effect of coconut water against carbon tetrachloride induced toxicity in rats. *Biomarkers*. <https://doi.org/10.1080/1354750X.2021.1946848>
- Elshter AEA, Salman MMA, Mohamed SA (2013) The hepato-ameliorating effect of *Solanum nigrum* against CCl₄ induced liver toxicity in albino rats. *Egypt Acad J Biol Sci* 5(1):59–66
- Emmanuel O, Okezie UM, Iweala EJ, Ugobogu EA (2021) Pretreatment of red palm oil extracted from palm fruit (*Elaeis guineensis*) attenuates carbon tetrachloride induced toxicity in Wistar rats. *Phytomed plus* 1:100079. <https://doi.org/10.1016/j.phyplu.2021.100079>
- Ghosh PK, Bhattacharjee P, Mitra S, Poddar-Sarkar M (2014) Physicochemical and phytochemical analyses of copra and oil of *Cocos nucifera* L. (West Coast Tall Variety). *Inter J Food Sci*. <https://doi.org/10.1155/2014/310852>
- Gordon A, Jackson J (2017) Case study: application of appropriate technologies to improve the quality and safety of coconut water. *Food Safety Qual Syst Dev Countries*. <https://doi.org/10.1016/B978-0-12-801226-0.00007-4>
- Gowda S, Desai PB, Kulkarni SS, Hull VV, Math AAK, Vernekar SN (2010) Markers of renal function tests. *North Am J Med Sci* 2:170–173
- Intahphuak S, Khonsung P, Panthong A (2010) Anti-inflammatory, analgesic, and antipyretic activities of virgin coconut oil. *Pharmaceut Biol* 48(2):151–157
- Jose HS (2014) Overview of urea and creatinine. *Lab Med* 45(1):e19–e20. <https://doi.org/10.1309/Im920sbznzpjrgut>
- Kaliyamoorthy J, Rajasekaran S, Nagarajan M, Vijayarengan P (2015) Bioactive enzyme activity and medicinal properties of tender coconut (*Cocos nucifera* L.). *Int J Modern Biochem* 4(1):10–14
- Kong BH, Tan NH, Fung SY, Pailoor J (2016) Sub-acute toxicity study of tiger milk mushroom *Lignosus tigris* Chon S. Tan Cultivar E sclerotium in Sprague Dawley rats. *Front Pharmacol* 7:246
- Li JX, Pang YZ, Tang CS, Li ZQ (2004) Protective effect of taurine on hypochlorous acid toxicity to nuclear nucleoside triphosphatase in isolated nuclei from rat liver. *World J Gastroenterol* 10:694–698
- Liu Q, Kong B, Li G, Liu N, Xia X (2011) Hepatoprotective and antioxidant effects of porcine plasma protein hydrolysates on carbon tetrachloride-induced liver damage in rats. *Food Chem Toxicol* 49(6):1316–1321
- Loki AL, Rajamohan T (2003) Hepatoprotective and antioxidant effect of tender coconut water on CCl₄ induced liver injury in rats. *Indian J Biochem Biophys* 40:354–357
- Mahayothee B, Koomyart I, Khuwijitjaru P, Siriwongwilaichat P, Nagle M, Müller J (2016) Phenolic compounds, antioxidant activity, and medium chain fatty acids profiles of coconut water and meat at different maturity stages. *Int J Food Propert* 19(9):2041–2051
- Mandal SM, Dey S, Mandal M, Sarkar S, Maria-Neto S, Franco OL (2009) Identification and structural insights of three novel antimicrobial peptides isolated from green coconut water. *Peptides* 30:633–637
- Mohamad NE, Yeap SK, Abu N, Lim KL, Zambari NR, Nordin N, Sharifuddin SA, Alitheen LK, NB, (2019) *In vitro* and *in vivo* antitumor effects of coconut water vinegar on 4T1 breast cancer cells. *Food Nutr Res* 10:63
- Orieke D, Ohaeri OC, Ijeh II, Ijioma SN, Achi NK (2019) Acute and sub-acute evaluations of methanolic leaf extract of *Corchorus olitorius* in experimental animal models. *Asian J Res Anim Vet Sci* 2(4):1–12
- Owoeye O, Onwuka SK, Farombi EO (2011) *Vernonia amygdalina* leaf extract and alpha-tocopherol alleviated gamma radiation-induced hematological and biochemical changes in rats. *Int J Biol Chem Sci* 5(5):1978–1992
- Prades A, Dornier M, Diop N, Pain JP (2012) Coconut water uses, composition and properties: a review. *Fruits* 67(2):87–107
- Rahmouni F, Hamdaoui L, Badraoui R, Rebai T (2011) Protective effects of *Teucrium polium* aqueous extract and ascorbic acid on hematological and some biochemical parameters against carbon tetrachloride (CCl₄) induced toxicity in rats. *Biomed Pharmacother* 91:43–48
- Saba AB, Oyagbemi AA, Azeez OI (2010) Amelioration of carbon tetrachloride-induced hepatotoxicity and haemotoxicity by aqueous leaf extract of *Cnidioscolus aconitifolius* in rats. *Nig J Physiol Sci* 25:139–147
- Sandhya VG, Rajamohan T (2006) Beneficial effects of coconut water feeding on lipid metabolism in cholesterol-fed rats. *J Med Food* 9(3):400–407
- Sandhya VG, Rajamohan T (2008) Comparative evaluation of the hypolipidemic effects of coconut water and lovastatin in rats fed fat-cholesterol enriched diet. *Food Chem Toxicol* 46(12):3586–3592

- Sedlak J, Lindsay RH (1968) Estimation of total, protein-bound, and non-protein sulfhydryl groups in tissue with Ellman's reagent. *Anal Biochem* 25:1192–1205
- Sun M, Zigma S (1978) An improved spectrophotometer assay of superoxide dismutase based on epinephrine autoxidation. *Anal Biochem* 90:81–89
- Tsai CF, Hsu YW, Chen WK, Chang WH, Yen CC, Ho YC, Lu FJ (2009) Hepatoprotective effect of electrolyzed reduced water against carbon tetrachloride-induced liver damage in mice. *Food Chem Toxicol* 47(8):2031–2036
- Ugbogu EA, Akubugwo EI, Ude VC, Emmanuel O, Okomba NO, Ibe C, Onyero O (2019) Safety evaluation of an aqueous extract of *Termitomyces robustus* (Agaricomycetes) in Wistar rats. *Inter J Med Mushrooms* 21(2):193–203
- Varma SR, Sivaprakasam TO, Arumugam I, Dilip N, Raghuraman M, Pavan KB, Rafiq M, Paramesh R (2019) *In vitro* anti-inflammatory and skin protective properties of virgin coconut oil. *J Trad Complement Med* 9(1):5–14
- Yong JW, Ge L, Ng YF, Tan SN (2009) The chemical composition and biological properties of coconut (*Cocos nucifera* L.) water. *Molecules* 14:5144–5164

Publisher's Note

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Submit your manuscript to a SpringerOpen[®] journal and benefit from:

- ▶ Convenient online submission
- ▶ Rigorous peer review
- ▶ Open access: articles freely available online
- ▶ High visibility within the field
- ▶ Retaining the copyright to your article

Submit your next manuscript at ▶ [springeropen.com](https://www.springeropen.com)
