Ilam University of Medical Sciences

Plant Biotechnology Persa

Online ISSN: 2676-7414

Homepage: https://pbp.medilam.ac.ir

Extracts from *Dacryodes edulis* Restored Renal Functions Altered by CCl4 in Rats

Patricia N. Akunne[1*], Chidube A. Alagbaoso[2], Noghayin E. J. Orhue[3]

[1]Department of Applied Chemical and Natural Sciences, Micheal and Cecilia Ibru University, Agbara, Ughelli, Delta State, Nigeria. Email: akunepat@gmail.com
[2]Department of Biomedical Engineering, University of Texas Southwestern Medical Center, Dallas, Texas, USA. Email: chidube.alagbaoso@utsouthwestern.edu
[3]Department of Biochemistry, Faculty of Life Sciences, University of Benin, Benin City, Edo State, Nigeria. Email: noghayin.orhue@unbien.edu
Corresponding Author, Department of Applied Chemical and Natural Sciences, Micheal and Cecilia Ibru University, Agbara, Ughelli, Delta State, Nigeria. Email: akunepat@gmail.com

Article Info

Article type:
Original Article

Article History:
Received: 21 August 2023
Received in revised form: 23 December 2023
Accepted: 23 December 2023
Published online: 31 December 2023

Keywords:
Environmental toxic chemicals, Carbon tetrachloride, *Dacryodes edulis*, Kidney damage, Kidney function

Abstract

Objective: The human body has evolved defense mechanisms for handling some toxic chemicals. However, the defense mechanisms are often overwhelmed resulting in tissue damage and disease. Recent studies have reported the therapeutic potential of *Dacryodes edulis* (*D. edulis*), but there is little or no report of its protective effects on the kidneys upon exposure to toxicants. In this study, the protective effect of *D. edulis* on the kidneys upon exposure to carbon tetrachloride (CCl_4), a toxic environmental chemical, was investigated.

Methods: An animal model of kidney damage from CCl_4 was established with Wistar albino rats. The rats were orally treated with aqueous and methanol extracts of *D. edulis* at concentrations ranging from 100-400 mg/kg b. weight for 21 days followed by oral administration of CCl_4 in olive oil for an additional one day. Plasma concentrations of electrolytes, activity of antioxidant enzymes, hematology, and kidney histology were studied to determine the effect of CCl_4 on the kidneys.

Results: The activity of catalase, superoxide dismutase, glutathione peroxidase, and malondialdehyde concentration in the kidneys increased significantly, indicating that CCl_4 caused reactive oxygen species (ROS) production. A reduced concentration of reduced glutathione (GSH) was also observed in the kidneys of rats that received only CCl_4. Aqueous and methanol extracts of *D. edulis* restored the activity of antioxidant enzymes and GSH concentration to their normal levels, which suggested that *D. edulis* possessed free radical scavenging properties. Plasma concentrations of Na^+, K^+, and Cl^-as well as urea and creatinine concentrations that were high upon CCl_4 exposure were significantly lowered on treatment with 400 mg/kg b. weight of both aqueous and methanol extracts of *D. edulis*.

Conclusion: The altered plasma electrolytes, urea, and creatinine concentrations could be attributed to the damaging effects of ROS on the kidneys. Furthermore, both hematological and histological analyses indicated that D. edulis extracts protected the kidneys from the toxic effects of CCl_4. These data suggested that *D. edulis* possessed protective bioactive components against kidney damage caused by CCl_4.

[DOI: 10.61186/pbp.5.2.100]

 Publisher: Ilam University of Medical Sciences

How to cite: N. Akunne P, A. Alagbaoso CH, E. J. Orhue N. Extracts from *Dacryodes edulis* Restored Renal Functions Altered by CCl4 in Rats. Plant Biotechnology Persa 2023; 5(2): 100-121.

Introduction

Humans are constantly exposed to toxic environmental chemicals, and large numbers of them cause serious damage to body tissues and organs. Body organs such as the kidneys are highly susceptible to damage caused by these chemicals because some of the chemicals undergo some biochemical transformations by enzymes found in the kidneys. An important example of a toxic environmental chemical, which has been used to model damages caused by toxicants is carbon tetrachloride (CCl4) [1]. Carbon tetrachloride causes severe damage to both the liver and the kidneys because the enzymes located in these organs transform it into its toxic form. Kidney damage results in a decrease in renal function, leading to a rise in nitrogenous waste products in the blood. Nitrogenous wastes such as urea, creatinine, uric acid, and other toxic products are normally filtered out of the blood and excreted through the urine, but when the kidneys are damaged, the waste products are trapped in the blood unable to be excreted. Furthermore, the kidneys play important roles in regulating the osmolarity and pH of the blood, as well as maintaining homeostasis of electrolyte concentration. The kidneys also help blood formation in the bone marrow through the formation and secretion of the hormone, erythropoietin [2].

Transformation of CCl_4 in the liver and kidneys by cytochrome P450 enzymes results in the formation of its trichloromethyl and proxychloromethyl free radicals, which cause serious damage to cells through oxidative stress and lipid peroxidation [3]. Peroxidation of membrane lipids results in cellular leakage, leading to an increase in the activity of cellular components, especially enzymes, in the blood [4]. Damage to plasma membranes by reactive oxygen species (ROS) usually results in cell death.

Medicinal herbs have shown great potential in suppressing oxidative damage caused by toxic chemicals. *Dacryodes edulis (D. edulis)* is a member of the *Burseracaea* family, found in the humid tropical zone of non-flooded forests [5], and is grown in some African countries, such as Nigeria, Ghana, and Cameroon. The fruits of *D. edulis* have been reported to possess bioactive compounds with beneficial health effects and are used by traditional herbalists for treating fever, oral disease, and ear infections. Extracts from the plant contain secondary metabolites that have been shown to have antimicrobial and antioxidant activities [6]. In Nigeria the vital oil of *D. edulis* resins has significant antibacterial and antifungal properties, inhibits lipid peroxidation, and prevents oxidative damage in humans during aging, cancer, atherosclerosis, and diabetes because of the antioxidant attributed (mono and sesquiterpenes) (Koudou *et al.,* 2004). Ethanol extract of D. edulis seeds is a source of hepatoprotective drug production (Orhue, 2015) by inhibiting lipid peroxidation and triglyceride accumulated in the liver of the rats administered due to its high content of flavonoids and alkaloids and reducing HDL levels in rats (Leudeu *et al.*, 2006). An array of chemical constitutes have been isolated from the plant such as terpenes, flavonoids, tannins, alkaloids, and saponins and the stem exudates contain tannin, saponin, and alkaloids with implications in the treatment of a variety of skin diseases and inflammation (Okwu and Nnamdi, 2008). Traditional herbal medicines have in the recent past attracted the interest of scientific communities as alternative therapies. Previous findings by Chimaobi et al [7] indicated that extracts from the leaves of *D. edulis* possessed the ability to scavenge free radicals such as DPPH, superoxide, and hydroxyl, as well as high reducing power because of the flavonoids and phenolic compounds it contained. Therefore, to further support the medicinal attributes of *D. edulis*, we investigated the protective activity of aqueous and methanol extracts from the seeds and stem bark of *D. edulis* against CCl_4-induced kidney damage in rats.

[DOI: 10.61186/pbp.5.2.100]

Materials and Methods

Preparation of Samples

The seeds and stem bark of *D. edulis* were obtained from Ologbo village in Edo State, Nigeria. Seeds from ripe fruits of *D. edulis* were used for this study. The seeds and stem bark were chopped into pieces, air-dried, and pulverized using a milling machine. The powdery samples were extracted with 4 L of water or methanol by soaking 500 g of the sample for 48 and 72 h respectively with regular stirring, followed by filtration through a cheesecloth and Whatman filter paper No 2 and lyophilization.

Animals

The animals used for this study were adult male Wistar rats weighing 145-170 g. They were acclimatized to laboratory conditions for seven days with free access to food and water (*ad libitum*). The rats were housed in clean cages placed in well-ventilated rooms with standard housing conditions (under humid tropical conditions) throughout the experiment. All animals received care according to the criteria outlined in the Guide for the Care and Use of Laboratory Animals prepared by the National Academy of Science and published by the National Institute of Health [8]. The animal study was approved by the Ethics Committee on Animal Care of the Faculty of Life Sciences, University of Benin.

Experimental Design

Animals were divided into eight (8) groups of 7 rats each. Carbon tetrachloride (CCl_4) was diluted with olive oil in a ratio of 1:1 (v/v) and administered orally at a dose of 3 mL/kg bw by gavage. Group A served as control and animals in this group received only feed and water. Group B served as the negative control which orally received only CCl_4, while groups C, D, and E orally received 100, 200, and 400 mg/kg bw of aqueous extracts of *D. edulis* stem bark or seeds respectively for 22 days. Groups F, G, and H orally received 100, 200, or 400 mg/kg bw of methanol extract of *D. edulis* stem bark or seeds respectively for 22 days. The weight of the animals was recorded daily, and the animals were monitored closely throughout the study. At the end of the administration period, the animals were sacrificed, the kidneys were excised, and blood was collected for histopathological examination, and hematological as well as renal function determination respectively.

Creatinine Determination

The method of Bohme [9] outlined in a diagnostic kit (RANDOX: CR2337, United Kingdom) was followed to determine creatinine concentration. Briefly, a working reagent was prepared by mixing equal volumes of picric acid and sodium hydroxide. The working reagent (1000 µL) was put in two test tubes labeled A and B, and 100 µL of the standard solutions was added to the test tubes. The tubes were vortexed and allowed to stand for 30 sec. Absorbance, A1, and A2 were read at 492 nm at 2 min apart. Creatinine concentration was calculated as:

$$\frac{Absorbance\ of\ sample}{Absorbance\ of\ standard} \times Concentration\ of\ standard$$

Urea Determination

The method of Weatheburn [10] outlined in a diagnostic kit (RANDOX: UR3825, United Kingdom) was followed to determine urea concentration. The procedure outlined in the kit was strictly followed. In the kit, reagent R1 was prepared by transferring R1a into R1b and mixed gently, and reagent R2 was diluted with 660 mL of distilled water while reagent R3 was diluted with 750 mL of distilled water. The sample or standard (10 µL) was added to three sets of test tubes labeled A, B, and C. Test tubes A and B contained the standard while test tube C contained the sample, and 100 µL of R1 was then

[DOI: 10.61186/pbp.5.2.100]

added in the respective tubes, vortexed, and left to stand at 37°C for 10 min. Reagent R1 (Phosphate buffer, (pH = 7.0), Sodium salicylate, Sodium nitroprusside, EDTA) and Reagent R3 (Sodium hydroxide, Sodium hypochlorite) (2.5 mL each) were also added in the tubes, mixed, and incubated at 37°C for 15 min. Absorbance was measured at 546 nm. Urea concentration was calculated as follows:

$$\frac{Absorbance\ of\ sample}{Absorbance\ of\ standard} \times Concentration\ of\ standard$$

Chloride Determination

The method of Tietz et al [11] was followed as outlined in a diagnostic kit (TECO: C501-480, USA). Chloride reagent (1000 µL) was added to sample or standard tubes containing 10 µL of the respective solution, vortexed, and incubated for 5 min. Absorbance was measured at 505 nm, and chloride concentration was calculated as follows:

$$\frac{Absorbance\ of\ sample}{Absorbance\ of\ standard} \times Concentration\ of\ standard$$

Potassium Determination

The method of Terri and Sesin [12] was followed as outlined in a diagnostic kit (TECO: P605-50, USA). The sample or standard (10 µL) was added to test tubes, and 1000 µL of sodium reagent was added, vortexed, and incubated for 3 min. Absorbance was measured at 500 nm, and chloride concentration was calculated as follows:

$$\frac{Absorbance\ of\ sample}{Absorbance\ of\ standard} \times Concentration\ of\ standard$$

Sodium Determination

The method of Maruna [13] was followed as outlined in a diagnostic kit (TECO: S600-50, USA). The sample or standard (50 µL) was added to different tubes, followed by 1000 µL of filtrate reagent, and shaken vigorously for 3 min. The tubes were centrifuged at 1500 ×g for 10 min and the supernatant was carefully collected. The acid reagent (1000 µL), supernatant (50 µL), and color reagent (50 µL) were added into a test tube and mixed. Absorbance measured at 550 nm was used to calculate sodium concentration as follows:

$$\frac{Absorbance\ of\ sample}{Absorbance\ of\ standard} \times Concentration\ of\ standard$$

Superoxide dismutase

Determination of SOD concentrations was carried out by the methods of Misra and Fridovich (1972). Three tubes were set, 200 µl of distilled water, samples, and 2.5 ml of carbonated buffer (0.05M), were pipetted into two different test tubes namely A and B respectively thereafter, 300 µl of fresh epinephrine (0.3 mM) added to B and C, and absorbance was taken at 30, 60, 90 and 120 seconds at 480 nm. The activity of SOD was taken using % inhibition = Δ absorbance sample – Reference standard / Δ absorbance sample × 100

SOD activity = % inhibition/ weight of tissue in the volume of solution used in assay × 50

Catalase

Procedure

Catalase was determined by the Goth (1991) method. 550 µL of phosphate buffer (PH 7.4) was pipetted across the three tubes A, B, and C, 500 µL distilled water and samples were put in A and B respectively followed by 500 µL of 0.2M H_2O_2 and were allowed to stay for 3 minutes on bench. Then, 1000 µL of H_2SO4 (6M) was added to all tubes

[DOI: 10.61186/pbp.5.2.100]

followed by 7mL of $KMNO_4$. The absorbance was taken at 480 nm immediately.

Activity of catalase was calculated using; = Δ absorbance sample × VT /M ×W ×L

VT = Total volume of reaction samples

M= Molar extinction co-efficient of H_2O_2 (40 cm^{-1})

W = Weight (g) of tissue in the volume of sample used for assay

L= Light path 1 cm

Malondialdehyde (MDA) Procedure

Determination of MDA concentrations was carried out by Buege and Aust (1983). Two sets of tubes were set A and B, 1 ml of distilled water and samples were pipetted into various tubes and TCA-TBA-HCI solution was also pipetted and carefully mixed. The test tubes were covered with foil paper, heated for 15 minutes in a boiling water bath of 1000C, and cooled. Through centrifuging, the flocculent was precipitated out at 1000 rpm for 10 minutes. The absorbance sample was read at 532 nm against blank. MDA concentration was determined using A × Vt /M× V× W.

A = Absorbance sample, VT = Total volume of the reaction mixture (µL), M = Molar extinction coefficient 1.56×10^5 cm^{-1}, v = Volume of the sample used in the assay (µL), and W= Weight (g) of tissue in the volume of sample used for the assay.

Reduced Glutathione (GSH) Procedure

GSH concentrations were determined by Ellman's (1959) methods. Two test tubes were set, 1000 µL of 5% of trichloroacetic acid (TCA) was pipette into tubes A and B and 500 µL of plasma was pipette into A and centrifuged. 500 µL of Ellman's reagent were then added to both tubes after centrifuging containing supernatant, followed by 3 ml of phosphate buffer. At an absorbance of 412 nm samples were read.

Reduced glutathione concentration = (Absorbance $_{sample}$ / Absorbance $_{standard}$) × concentration of standard.

Glutathione Peroxidase (GPx) Procedure

GPx concentrations were carried out using Nyman's (1959) methods. 1.5 ml distilled water, 2.5 ml of H_2O_2, pyrogallol, and phosphate buffer (PH 7.4), were pipetted into the test tube followed by 200 µL of plasma. Samples were mixed and allowed to stand for 30 minutes at room temperature. The deep brown color was formed and determined at an absorbance of 430 nm. Glutathione peroxidase activity was estimated using.

= A × Vt × Df / M × V × W

A = Absorbance sample, VT = Total volume of the reaction mixture (µL), Df = Dilution factor, M = Molar extinction coefficient 12 cm^{-1}, v = Volume of the sample used in the assay (µL), and W= Weight (g) of tissue in the volume of sample used for the assay.

Hematology assay

A modern hematology automated analyzer was used. A small sample of blood is aspirated into a chamber and diluted with an isotonic saline solution. Two dilutions were prepared by the analyzer: lysed and not lysed. White blood cell (WBC) and hemoglobin values were measured (first dilution) by displaying the values on the screen on the instrument. Meanwhile, the analyzer processes the second dilution where red blood cells (RBC), hematocrit (HCT), mean corpuscular volume (MCV), and platelet count (PLT) were measured (Chapman, 2000; Zandecki *et al.*, 2007). These small portions of the diluted fluid in each bath flowed past a small aperture.

[DOI: 10.61186/pbp.5.2.100]

Histopathological Examination

A representative kidney tissue from each group was excised from the rats and preserved with 10% Neutral buffered formalin for histopathological analysis. A tissue section measuring 3-5 mm in thickness was cut out and placed in a tissue cassette. Cut-out tissue was processed with Leica TP2010 automatic tissue processor for 18 h and subjected to fixation (with 10% Neutral buffered formalin), dehydration (with increasing concentration of isopropyl alcohol), clearing or de-alcoholization (with xylene), and finally impregnation or infiltration (with molten paraffin wax). Tissues were then embedded in paraffin wax using a thermoscientific automated tissue embedding machine and sectioned to get ultra-thin sections at five (5) microns. They were then floated out from the thermoscientific digital floating bath on the frosted end of pre-labeled slides and dried on the thermoscientific digital slimline hot plate. They were further dried in a Thermo Scientific hot air oven overnight and subjected to hematoxylin and eosin staining. Stained slides were mounted in DPX and allowed to dry before viewing under the microscope with X_{10} and X_{40} magnification and photomicrographs were taken.

Data Analysis

All results are expressed as mean standard error-free mean (SEM). The levels of homogeneity among the groups were assessed using One-way Analysis of Variance (ANOVA) followed by Duncan's Post Hoc test. All analyses were done using SPSS Software Version 16.0 and P values < 0.05 were considered statistically significant.

Results

From the results obtained, 200 and 400mg/kg aqueous and methanol seed and stembark of *D. edulis* were able to prevent the rats from the alterations that could have resulted from the exposure to CCl_4. thereby showing a significant difference when compared to those with CCl_4 only without treatment.

Effect of extracts of D. edulis Stem bark on plasma urea, creatinine, potassium (K), sodium (Na), and chloride (Cl) of rats treated with CCl_4.

A significant increase was observed in the plasma urea, creatinine, potassium (K^+), sodium (Na^+), and chloride (Cl^-) concentrations of animals administered with only CCl_4 as compared to those of the control group (Table 1). Alterations induced by CCl_4 were silenced by methanol and aqueous extracts of D. edulis stem bark, especially at 200 and 400 mg/kg

Table 1: *Effect of extracts of D. edulis stem bark on renal function parameters of CCl_4-treated rats.*

Groups	Urea (mg/dl)	Creatinine (mg/dl)	K (mg/dl)	Na (mg/dl)	Cl (mg/dl)
Control	38.98±6.04^{a}	0.55±0.002^{a}	5.14±0.03^{a}	56.43±5.02^{a}	128.05±2.02^{b}
CCl_4 only	69.81±10.01^{c}	1.25±0.002^{c}	18.42±0.04^{c}	126.64±4.03^{d}	155.12±2.50^{d}
AQSB 100mg/kg	73.66±8.09^{d}	1.22±0.002^{c}	18.22±0.03^{c}	157.07±2.03^{f}	155.01±2.002^{d}

[DOI: 10.61186/pbp.5.2.100]

AQSB 200mg/kg	69.81±8.01^{c}	1.16±0.003^{b}	14.68±0.04^{b}	134.89±6.05^{e}	145.66±6.01^{c}
AQSB 400mg/kg	43.74±3.01^{b}	1.05±0.003^{ab}	10.11±0.03^{b}	119.28±4.03^{c}	122.84±5.02^{b}
MTSB 100mg/kg	78.46±6.01^{d}	1.20±0.002^{c}	12.87±0.03^{b}	151.08±6.04^{f}	158.26±6.004^{d}
MTSB 200mg/kg	46.03±8.02^{b}	1.06±0.002^{ab}	9.96±0.02^{a}	112.56±3.03^{c}	98.44±4.02^{a}
MTSB 400mg/kg	40.49±2.00^{b}	0.99±0.003^{a}	7.76±0.09^{a}	98.52±4.22^{b}	97.89±4.03^{a}

Results are expressed as mean ± SEM (n = 7). AQSB stands for Aqueous extract of D. edulis stembark, while MTSB stands for Methanol extract of D. edulis stem bark. Values with different superscripts along the same column are significantly different at $p < 0.05$.

Effect of extracts of D. edulis seeds on plasma urea, creatinine, potassium (K), sodium (Na), and chloride (Cl) of rats treated with CCl_4

The results obtained showed a significant increase in plasma urea, creatinine, potassium (K^+), sodium (Na^+), and chloride (Cl^-) concentrations of animals administered with only CCl_4 as compared to those of rats in the control group (Table 2). Methanol and aqueous extracts from *D. edulis* seeds administered orally had a suppressive effect on the alterations induced by CCl_4 at a dose of 200 and 400 mg/kg.

Table 2: *Effect of extracts of D. edulis seed on kidney function parameters of CCl_4-treated rats*

Groups	Urea (mg/dl)	Creatinine (mg/dl)	K (mg/dl)	Na (mg/dl)	Cl (mg/dl)
Control	41.91±6.01^{b}	0.49±0.012^{a}	4.04±1.79^{a}	57.41±6.01^{a}	121.67±6.01^{a}
CCl_4 only	60.34±4.18^{e}	1.20±0.01^{c}	16.16±0.03^{c}	122.02±8.02^{d}	158.26±7.03^{d}
AQSD 100mg/kg	70.21±6.01^{f}	1.18±0.02^{c}	16.90±0.19^{c}	143.41±8.01^{f}	157.32±3.23^{d}
AQSD 200mg/kg	50.84±7.01^{d}	1.10±0.01^{b}	11.76±0.25^{b}	137.88±9.01^{e}	141.87±4.11^{c}
AQSD 400mg/kg	47.87±5.01^{bc}	1.08±0.001^{b}	10.83±0.14^{b}	111.86±6.02^{c}	132.66±6.01^{b}
MTSD 100mg/kg	62.26±2.02^{e}	1.17±0.003^{c}	17.85±0.11^{c}	123.53±6.04^{d}	156.29±5.02^{d}
MTSD 200mg/kg	40.22±3.01^{b}	1.09±0.001^{b}	10.32±0.93^{b}	110.52±6.01^{c}	136.53±8.02^{b}
MTSD 400mg/kg	38.17±2.02^{a}	1.05±0.02^{b}	9.91±0.10^{b}	87.38±4.02^{b}	128.49±3.02^{a}

[DOI: 10.61186/pbp.5.2.100]

Results are expressed as mean ± SEM (n = 7). AQSD stands for Aqueous extract of *D. edulis* seed, while MTSD stands for Methanol extract of *D. edulis* seed. Values with different superscripts along the same column are significantly different at $p < 0.05$.

Hepatoprotective Phase Effect of Extracts of D. edulis Stembark on Oxidative Stress Biomarkers in the Kidney of CCl_4-induced Rats

Administration of carbon tetrachloride resulted in a significant ($p<0.05$) increase in the activities of CAT, SOD, GPx, as well as MDA but significantly decreased the concentration of GSH, biomarkers in the kidney when compared with those in the normal control animals (Table 3). 200 and 400 mg/kg doses of both aqueous and methanol extracts of *D. edulis* stem bark avoided these perturbations caused by CCl_4 administration.

Table 3: *Effect of D. edulis Stem Bark on Antioxidant and Lipid Per-oxidation Status in the Kidney of Treated Rats*

Groups	GSH (units/gwet tissue)	CAT (units/gwet tissue)	SOD (units/gwet tissue)	GPx (units/gwet tissue)	MDA (units/gwet tissue)
Control	0.72±0.001 e	0.0032±0.0001 a	0.0121±0.004 a	0.032±0.001 a	0.040±0.002 a
CCl4 only	0.30±0.001 b	0.0055±0.0001 c	0.0162±0.001 d	0.073±0.002 d	0.086±0.001 e
AQSB 100mg/kg	0.27±0.000 a	0.0055±0.0001 c	0.0166±0.003 d	0.078±0.001 d	0.081±0.001 e
AQSB 200mg/kg	0.29±0.001 a	0.0059±0.0002 c	0.0162±0.001 d	0.071±0.001 d	0.073±0.002 d
AQSB 400mg/kg	0.44±0.001 c	0.0045±0.0001 b	0.0152±0.001 c	0.052±0.001 c	0.060±0.002 c
MTSB 100mg/kg	0.26±0.001 a	0.0051±0.0002 c	0.0163±0.001 d	0.077±0.003 d	0.086±0.001 e
MTSB 200mg/kg	0.38±0.001 b	0.0047±0.0003 b	0.0150±0.001 c	0.053±0.002 c	0.053±0.001 b
MTSB 400mg/kg	0.55±0.001 d	0.0042±0.0004 b	0.0144±0.001 b	0.048±0.003 b	0.043±0.002 a

Results are presented as mean ± SEM with n = 7. Values with different superscripts along the same column are significantly different at $p<0.05$.

Legend: AQSB = aqueous extract of *D. edulis* stembark, MTSB = Methanol extract of *D. edulis* stem bark, GSH = Glutathione, CAT = Catalase, SOD = Superoxide Dismutase, MDA = Malondialdehyde, GPx = Glutathione Peroxidase

[DOI: 10.61186/pbp.5.2.100]

Hepatoprotective Phase Effect of Extracts of D. edulis Seed on Oxidative Stress Biomarkers in the Kidney of CCl_4-induced Rats

Treatment of animals with Carbon tetrachloride caused a significant ($p<0.05$) increase in the activities of CAT, SOD, GPx, as well as MDA but significantly decreased the concentration of GSH, in the kidney when compared with those in the normal control animals (Table 4). Different doses of 200mg/kg aqueous and 200 and 400mg/kg methanol extracts of *D. edulis* seed were able to prevent these perturbations in the kidneys of treated animals.

Table 4: *Effect of D. edulis Seed on Antioxidant and Lipid Per-oxdiation Status in the Kidney of Treated Rats*

Groups	GSH (units/gwet tissue)	CAT (units/gwet tissue)	SOD (units/gwet tissue)	GPx (units/gwet tissue)	MDA (units/gwet tissue)
Control	0.78±0.001 e	0.0036±0.0002a	0.0124±0.002a	0.044±0.002a	0.048±0.002a
CCl4 only	0.35±0.001 a	0.0058±0.0002cd	0.0162±0.001e	0.071±0.001d	0.081±0.002e
AQSD 100mg/kg	0.31±0.002 a	0.0051±0.0001c	0.0162±0.001e	0.073±0.001d	0.075±0.001d
AQSD 200mg/kg	0.39±0.002 ab	0.0046±0.0002b	0.0159±0.002d	0.064±0.002c	0.066±0.002c
AQSD 400mg/kg	0.41±0.003 b	0.0043±0.0003b	0.0147±0.002c	0.058±0.002b	0.052±0.001b
MTSD 100mg/kg	0.32±0.003 a	0.0062±0.0001e	0.0160±0.002e	0.071±0.002d	0.071±0.001d
MTSD 200mg/kg	0.49±0.003 bc	0.0049±0.0002b	0.0152±0.003d	0.062±0.001c	0.062±0.001c
MTSD400mg/kg	0.51±0.002 d	0.0042±0.00024b	0.0135±0.002b	0.045±0.001a	0.045±0.002a

Results are presented as mean ± SEM (n =7). Values with different superscripts along the same column are significantly different at $p<0.05$.

Legend: AQSD = Aqueous extract of *D. edulis* Seed, MTSD = Methanol extract of *D. edulis* Seed, GSH = Glutathione, CAT = Catalase, SOD = Superoxide Dismutase, MDA = Malondialdehyde, GPx = Glutathione Peroxidase

Hepatoprotective Phase Effect of Extracts of D. edulis Seed on Red Blood Cell Parameters of CCl_4-induced Rats

Exposure of animals to carbon tetrachloride resulted in a significant ($p<0.05$) decrease in the RBC, HBG, HCT, MCV, and MCH when compared with those in the control animals (Table 5). Prophylactic treatment of animals with 400 mg/kg, aqueous as well as 200 and 400 mg/kg methanol

[DOI: 10.61186/pbp.5.2.100]

extracts of *D. edulis* seed, was able to stop the reduction in RBC caused by CCl_4 administration. Similarly, pretreatment of animals with 400 mg/kg aqueous and methanol extracts of *D. edulis* seed was able to prevent the alteration in hemoglobin concentration caused by CCl_4 administration. In the same vein, prophylactic treatment of animals with 200 and 400 mg/kg methanol and aqueous extracts of *D. edulis* seed was able to alleviate the reduction in HBG, HCT, MCH, and MCV caused by CCl_4 exposure.

Table 5: *Effect of Extracts of D. edulis Seed on Red Blood Cell Parameters of CCl_4-induced Rats*

Groups	RBC (X109/L)	HGB (g/dl)	HCT (%)	MCV (fl)	MCH (pg)
Control	16.15±0.92d	15.65±2.21e	65.19±1.44e	52.12±2.34d	32.11±5.23d
CCl4 only	5.78±1.20a	4.55±0.83b	43.29±1.39c	30.35±3.5b	10.23±3.12a
AQSD 100 mg/kg	6.13±1.68a	2.00±1.04a	23.88±3.31a	28.43±3.99a	11.23±4.13a
AQSD 200 mg/kg	7.42±1.36ab	3.89±1.55a	27.50±0.87a	35.64±4.12b	17.45±7.11b
AQSD 400 mg/kg	11.82±1.09c	7.64±1.56c	37.67±0.76b	39.56±2.66b	22.43±6.21c
MTSD 100 mg/kg	6.51±1.74a	3.13±1.54a	24.22±1.20a	32.14±1.88b	13.45±5.24a
MTSD 200 mg/kg	8.09±1.17b	5.30±1.82b	34.95±2.1b	37.13±2.00b	19.22±6.9b
MTSD 400 mg/kg	12.26±0.87cd	9.64±2.08d	52.38±1.95d	44.85±3.8c	17.34±2.11b

Results are presented as mean ± SEM with n = 7. Values with different superscripts along the same column are significantly different at $p<0.05$.

Legend: AQSD = Aqueous extract of *D. edulis* Seed, MTSD = Methanol extract of *D. edulis* Seed, RBC = Red Blood Cell, HGB = Hemoglobin, HCT = Hematocrit, MCV = Mean Corpuscular Volume, MCH = Mean Corpuscular Hemoglobin

Hepatoprotective Phase Effect of Extracts of D. edulis Seed on White Blood Cell Parameters of CCl_4-induced Rats

Exposure of animals to carbon tetrachloride resulted in a significant ($p<0.05$) decrease in the WBC, platelet, monocyte, lymphocyte, and granulocyte counts when compared with those in the control animals (Table 6). Prophylactic treatment of animals with 400 mg/kg aqueous as well as 200 and 400 mg/kg methanol extracts of *D. edulis* seed was able to ameliorate the reduction in granulocyte count caused by CCl_4 administration. Similarly, pre-treatment of animals with 200 and 400 mg/kg of aqueous and methanol extracts of *D. edulis* seed was used to ameliorate the reduction in WBC,

[DOI: 10.61186/pbp.5.2.100]

lymphocyte, monocyte count, and platelet counts caused by CCl_4 administration.

Table 6: Effect of Extracts of D. edulis Seed on White Blood Cell Parameters of CCl_4-induced Rats

Groups	WBC (X109/L)	LYM (%)	MON (%)	GRA (%)	PLT (X109/L)
Control	22.94±1.28c	27.92±1.89d	15.45±2.42c	95.59±6.88d	398.07±8.39d
CCl4 only	11.04±1.17a	11.22±5.78a	8.07±1.42a	70.23±8.39a	235.36±8.70a
AQSD 100mg/kg	14.74±0.54a	11.30±3.86a	7.47±1.59a	79.99±5.83ab	234.14±5.70a
AQSD 200mg/kg	17.76±1.95b	14.85±7.08b	11.06±2.31b	79.78±7.14ab	248.32±3.87b
AQSD 400mg/kg	19.43±0.77b	15.94±3.82b	12.12±1.65b	82.17±4.39c	322.09±11.42c
MTSD 100mg/kg	14.33±1.80a	10.21±4.12a	10.92±1.75b	78.96±4.52ab	233.40±7.43a
MTSD 200mg/kg	19.29±1.95b	16.18±7.01b	14.92±1.90c	81.82±7.67c	257.35±7.14b
MTSD 400mg/kg	20.75±1.27c	18.04±1.56bc	17.67±2.29c	85.26±6.93c	337.45±7.58c

Results are presented as mean ± SEM with n = 7. Values with different superscripts along the same column are significantly different at $p<0.05$.

Legend: AQSD = Aqueous extract of *D. edulis* Seed, MTSD = Methanol extract of *D. edulis* Seed, WBC = White Blood Cell Count, PLT = Platelet Count, LYM = Lymphocyte Count, MON = Monocyte Count, GRA = Granulocyte Count

Hepatoprotective Phase Effect of Extracts of D. edulis Stembark on Red Blood Cell Parameters of CCl_4-induced Rats

Exposure of animals to carbon tetrachloride resulted in a significant ($p<0.05$) decrease in the red blood cell parameters of treated animals when compared with those in the normal control animals (Table 7). 200 and 400mg/kg doses of both aqueous and methanol extracts of *D. edulis* stem bark significantly hinder this decrease in RBC, HGB, HCT, MCV, and MCH of treated animals with 400 mg/kg of both extracts having more effect.

[DOI: 10.61186/pbp.5.2.100]

Table 7: *Effect of Extracts of D. edulis Stembark on Red Blood Cell Parameters of CCl_4-induced Rats*

Groups	RBC ($X10^9$/L)	HGB (g/dl)	HCT (%)	MCV (fl)	MCH (pg)
Control	19.64±1.06^{e}	15.42±1.06^{d}	68.16±0.99^{d}	56.24±2.13^{c}	32.28±3.24^{e}
CCl_4 only	6.91±0.0.99^{a}	6.16±0.64^{b}	48.12±1.01^{ab}	33.05±1.24^{a}	12.15±4.13^{a}
AQSB 100 mg/kg	7.12±0.96^{a}	4.21±1.12^{a}	44.32±0.86^{a}	30.15±1.87^{a}	12.56±3.87^{a}
AQSB 200 mg/kg	11.76±1.68^{b}	8.32±1.01^{b}	49.14±1.36^{ab}	45.65±1.56^{b}	13.07±4.11^{a}
AQSB 400 mg/kg	13.4±1.40^{c}	10.12±0.91^{c}	59.36±2.46^{c}	44.47±2.11^{b}	18.35±3.45^{c}
MTSB 100 mg/kg	7.08±1.00^{a}	6.91±0.86^{b}	47.22±0.99^{ab}	35.25±2.08^{a}	15.48±2.93^{b}
MTSB 200 mg/kg	10.14±1.36^{b}	9.17±1.14^{bc}	51.14±1.20^{b}	43.35±1.98^{b}	21.51±2.55^{d}
MTSB 400 mg/kg	15.2±1.32^{d}	12.66±1.00^{d}	62.23±1.36^{cd}	55.11±2.11^{c}	25.73±3.14^{d}

Results are presented as mean ± SEM with n = 7. Values with different superscripts along the same column are significantly different at $p<0.05$.

Legend: AQSB = Aqueous extract of *D. edulis* stembark, MTSB = Methanol extract of *D. edulis* stembark, RBC = Red Blood Cell, HGB = Hemoglobin, HCT = Hematocrit, MCV = Mean Corpuscular Volume, MCH = Mean Corpuscular Hemoglobin

Hepatoprotective Phase Effect of Extracts of D. edulis Stembark on White Blood Cell Parameters of CCl_4-induced Rats

Treatment of animals with carbon tetrachloride resulted in a significant ($p<0.05$) decrease in the white blood cell parameters of animals when compared with those in the normal control animals (Table 8). 200 and 400 mg/kg doses of both aqueous and methanol extracts of *D. edulis* stembark significantly inhibit this decrease in WBC, platelet, lymphocyte, monocyte, and Granulocyte counts of treated animals.

[DOI: 10.61186/pbp.5.2.100]

Table 8: *Effect of Extracts of D. edulis Stembark on White Blood Cell Parameters of CCl_4-induced Rats*

Groups	WBC(X10^9/L)	LYM (%)	MON (%)	GRA (%)	PLT(X10^9/L)
Control	22.54±1.18^c	24.61±1.32^c	17.77±2.43^c	92.19±5.33^d	364.28±6.81^e
CCl_4 only	10.18±0.82^a	11.56±0.86^a	7.92±2.29^a	76.84±4.83^a	280.51±3.16^{bc}
AQSB 100mg/kg	12.18±1.10^a	17.36±1.10^b	8.67±1.66^a	72.08±5.07^a	212.16±2.14^a
AQSB 200mg/kg	21.94±1.42^c	16.48±1.68^b	12.93±2.39^b	81.11±4.59^b	294.25±1.68^{bc}
AQSB 400mg/kg	25.16±0.86^{cd}	19.33±3.41^b	15.66±2.09^b	89.87±6.17^c	332.14±2.47^d
MTSB 100mg/kg	11.99±1.52^a	12.14±1.42^a	9.29±2.23^a	78.52±4.56^a	262.32±3.14^b
MTSB 200mg/kg	17.88±0.99^b	20.81±0.91^c	13.90±2.23^b	82.59±3.89^b	322.14±3.01^d
MTSB 400mg/kg	22.48±1.36^c	26.89±1.36^c	16.19±3.23^b	87.01±6.66^{bc}	346.36±2.41^{de}

Results are presented as mean ± SEM with n = 7. Values with different superscripts along the same column are significantly different at $p<0.05$.

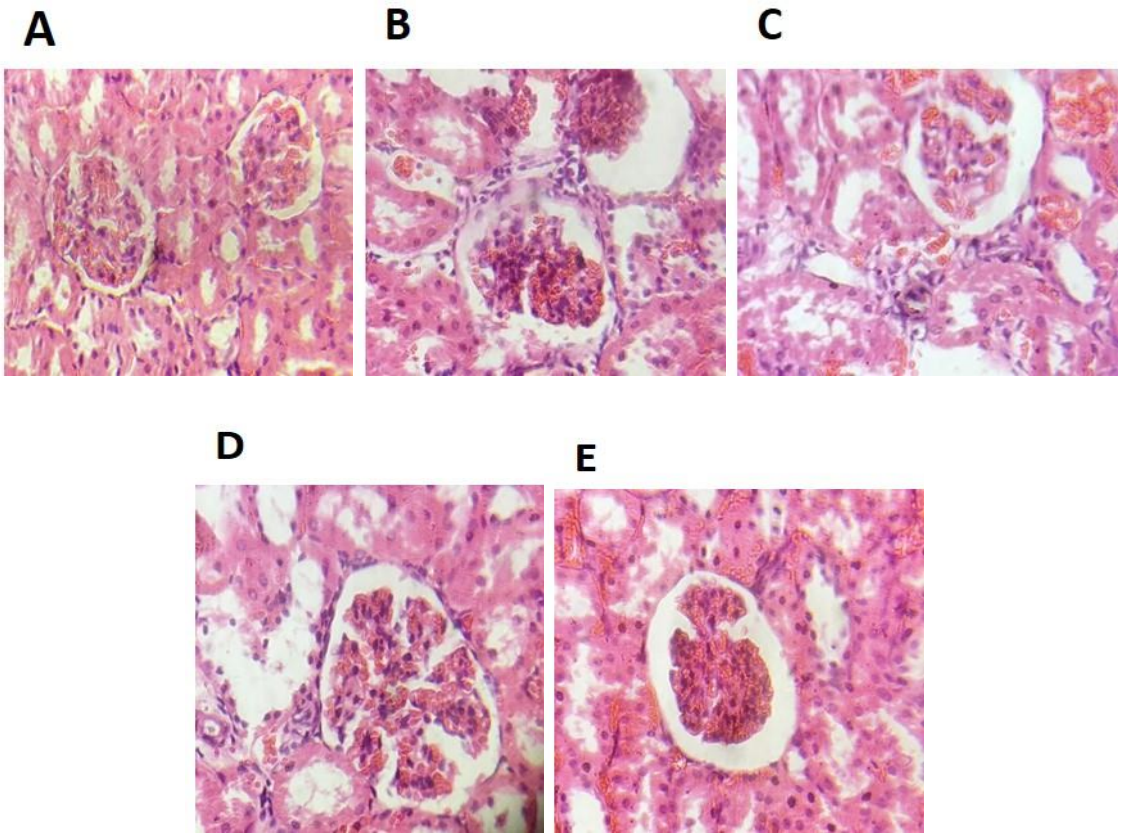
Legend: AQSB = Aqueous extract of *D. edulis* stem bark, MTSB = Methanol extract of *D. edulis* stem bark, WBC = White Blood Cell Count, PLT = Platelet Count, LYM = Lymphocyte Count, MON = Monocyte Count, GRA = Granulocyte Count

Histopathological examination of rat kidneys administered with aqueous extracts of D. edulis stem bark.

The kidneys of rats in group A showed visible renal corpuscle and interstitial space and tubules, and rats in group B showed visible atrophied renal corpuscle and interstitial space with diffused mononuclear infiltrates and necrosed tubules, indicative of renal damage. Rats in group C showed visible atrophied renal corpuscle and interstitial space with focal mononuclear infiltrates and mildly necrosed tubules, while rats in group D showed visible renal corpuscle and interstitial space with diffused mononuclear infiltrates and mildly necrosed tubules and E revealed visible renal corpuscle and interstitial space and mildly necrosed tubules as shown in Fig. 1 A-E.

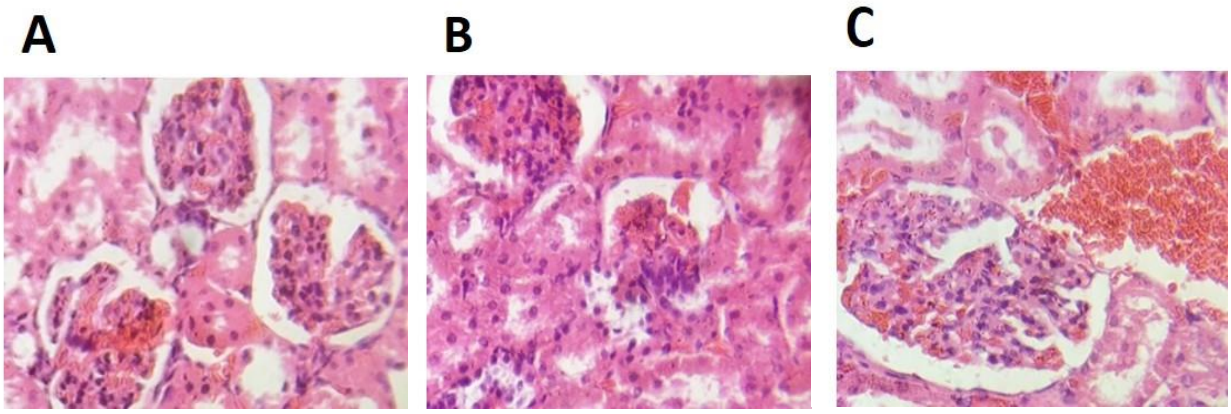
Fig. 1. Kidney section of rats administered with aqueous extracts of *D. edulis* stem bark. *(A) Control kidney rat, (B) rat kidney administered with only CCl_4, (C) rat kidney administered with 100 mg/kg b.w aqueous stem bark of D. edulis (D) rat kidney administered with 200 mg/kg b.w aqueous stem bark of D. edulis. (E) rat kidney administered with 400 mg/kg b.w aqueous stem bark of D. edulis. Sections were stained with hematoxylin and eosin and viewed using 10X and 40X objectives.*

[DOI: 10.61186/pbp.5.2.100]

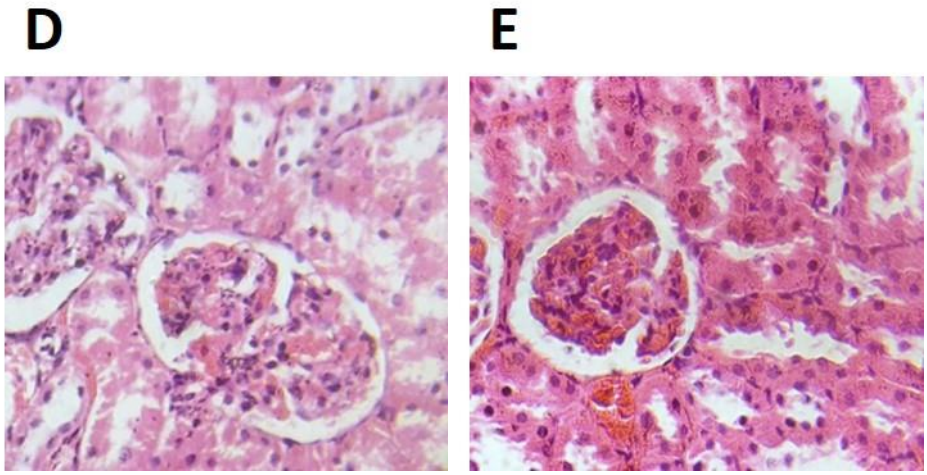


Histopathological examination of rat kidneys administered with methanol extracts of D. edulis stem bark.

The kidneys of rats in group A showed visible renal corpuscle and interstitial space and tubules, and rats kidneys in group B showed visibly distorted renal corpuscle and interstitial space with focal mononuclear cells and mildly necrosed tubules, while rats kidneys in group C showed visibly distorted renal corpuscle and interstitial space and less prominent tubules, rats kidneys in group D showed visible renal corpuscle and interstitial space and less prominent tubules and those in group E showed visible renal corpuscle and interstitial space and tubules as shown in Fig. 2A-E.

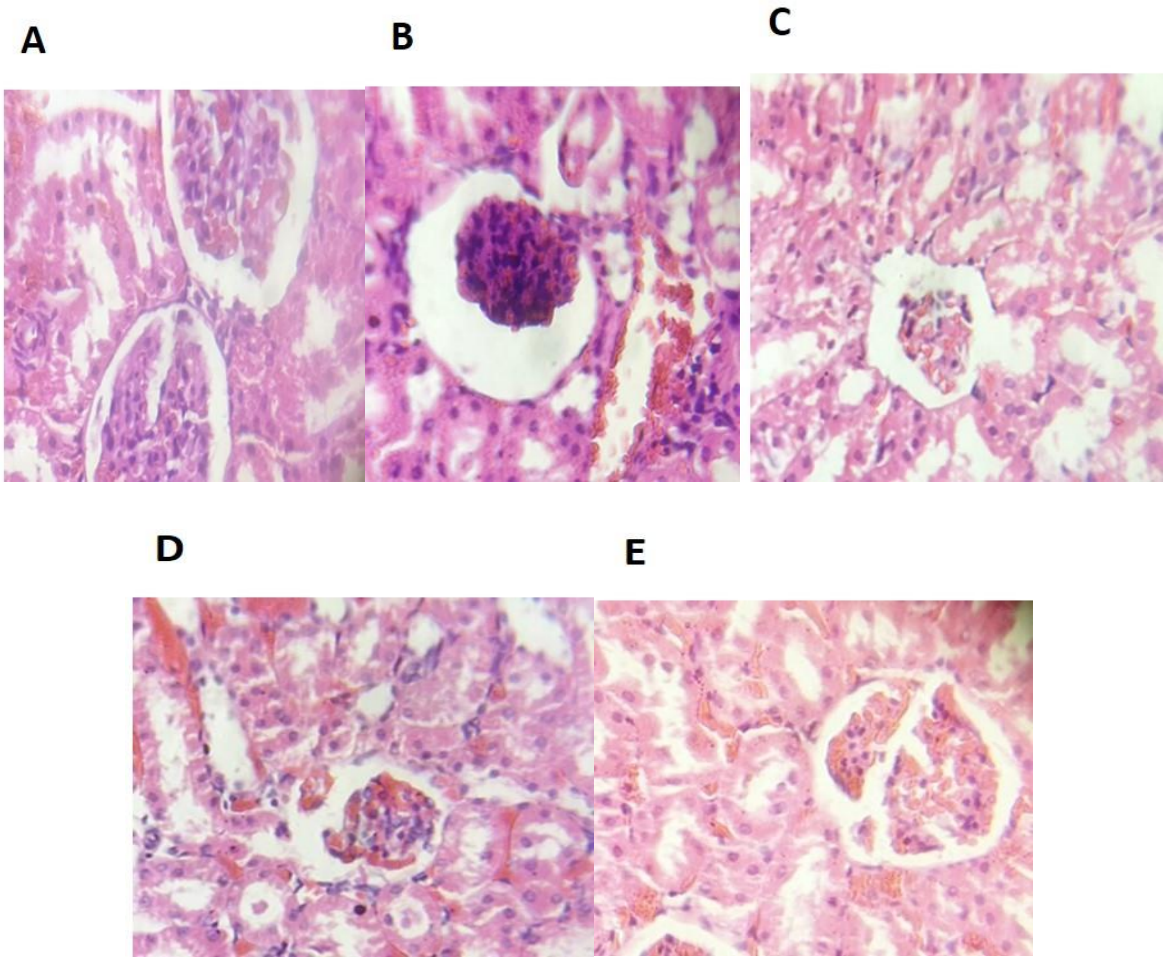


[DOI: 10.61186/pbp.5.2.100]



Histopathological examination of rat kidneys administered with aqueous extracts of D. edulis seeds.

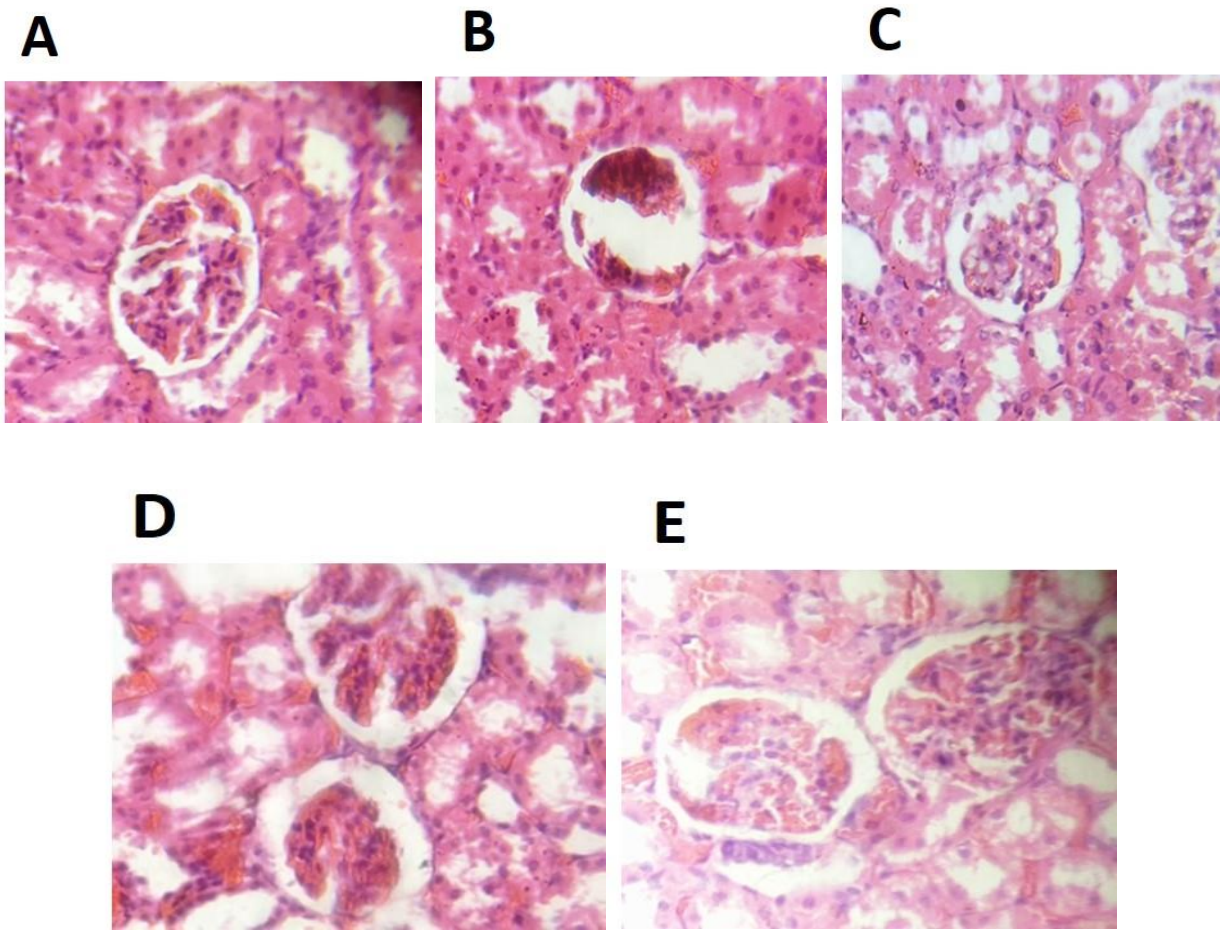
Rats kidneys in group A showed visible renal corpuscle and interstitial space and tubules, those in group B showed visible renal corpuscle with atrophied glomerulus and interstitial space with focal mononuclear exudates and less prominent tubules, and the rats in group C showed visible atrophied renal corpuscle and interstitial space and tubular necrosis, while those in group D showed mildly atrophied renal corpuscle and interstitial space and tubules and group E showed visible renal corpuscle and interstitial space and mild tubules necrosis as shown in Fig. 3A-E.



[DOI: 10.61186/pbp.5.2.100]

Histopathological examination of rat kidneys administered with methanol extracts of D. edulis seeds.

Rats in group A showed visible renal corpuscle and interstitial space and tubules, group B showed distorted renal corpuscle and interstitial space and tubules with prominent necrosis, and group C showed less prominent renal corpuscle and distorted interstitial space and tubular necrosis, while those of group D showed visible renal corpuscle and interstitial space and mild tubular necrosis and group E showed visible renal corpuscle and interstitial space and tubules as shown in Fig. 4A-E.



Discussion

The kidneys are important organs that play very important roles in the formation of urine, through which toxic wastes such as urea and uric acid are excreted. They also function in regulating water/electrolyte balance and control of blood pH [14]. Damage to the kidneys by any means results in serious trouble in how the body handles the metabolic wastes, resulting in the accumulation of electrolytes, urea, and creatinine in the blood and alteration of blood pH [15]. The serum concentration of creatinine is an important marker for glomerular filtration rate (GFR) used as an index of renal function in clinical practice [16]. Serum creatinine concentration is determined by how well the kidneys can filter it out, and it is a function of the functional status of the kidneys. Alterations in the metabolism and filtration of creatinine may have a profound impact on the plasma concentration of creatinine, and it's constant among individuals over time when the rate of creatinine production is equal to its excretion rate. Plasma creatinine concentration is inversely proportional to GFR so each halving of the GFR results in a doubling of the plasma creatinine concentration [17]. According to a report by Mandell et al [18], exogenous creatinine clearance decreased with a 10-fold increase of creatinine concentration in the blood by infusion. The decrease was thought to be due to saturation of the tubular secretory mechanism because the inulin clearance was not

[DOI: 10.61186/pbp.5.2.100]

affected by the exogenous increase of the creatinine concentration in the blood [18]. Creatinine reabsorption during low rates of urine flow is thought to result from its passive back diffusion from the lumen to the blood. Thus, when the urine flow rate is very low, passive reabsorption of creatinine might result in a lower creatinine clearance and a higher concentration of serum creatinine than what one would expect solely based on the glomerular filtration rate [16]. Therefore, serum urea and creatinine levels are indications of kidney function both in men and in rodents. Dietary protein deficiency leads to negative nitrogen balance and loss of muscle mass, thereby decreasing creatinine production. Less severe alterations in the diet, however, also may have important effects on the size of the creatine pool and creatinine excretion, which are independent of nitrogen balance and muscle mass. In this study, a significant increase was observed in serum urea and creatinine concentrations of animals administered with CCl4 when compared to those of the control animals. This may be attributed to kidney damage caused by the administered CCl4. CCl4 causes serious damage to the kidneys, resulting in altered creatinine excretion [19]. This agrees with the findings of Iseghohi and Orhue [1] who reported an increase in serum concentration of urea and creatinine due to the effect of CCl4 administered to rats. The report is in line with the findings of Ohwokevwo and Ogunka-Nnoka [22] conveyed a significant increase in the serum urea and creatinine concentrations of animals exposed to CCl4 when compared to rats in the untreated group. In this study, we demonstrated that treatment of CCl4-treated rats with extracts from the seeds and stembark of D. edulis resulted in a decrease of the elevated levels of serum creatinine and urea caused by CCl4 exposure, especially at a high dose of 400 mg/kg (Tables 1 and 2). This suggested that the extracts contained bioactive compounds that protected the kidneys from damage by CCl4. A report on D. edulis by Duru et al [20] indicated that it contained bioactive phytochemicals that may have exerted the protective effects recorded in our study. Our report agrees with the findings of Okolo et al [21] who reported that extract of D. edulis fruit reduced serum levels of urea and creatinine in alloxan-treated rats.

CCl4 administration resulted in a significant increase in plasma potassium (K), sodium (Na), and chloride (Cl) when compared with those in the control group (Tables 1 and 2). CCl4-induced Na elevation could lead to the retention of water in the renal tubules due to the transfer of water from intercellular to extracellular space. The link between sodium and hypertension is based on the capacity of the electrolyte to “attract” water. When the plasma sodium level is high, the body retains water in a bid to reduce the plasma sodium concentration, thereby causing the blood pressure, which depends in part on blood volume, to increase. Our report agrees with the findings of Ullah et al [23] but contradicts that of Ohwokevwo and Ogunka-Nnoka [22] who reported a non-significant difference in the serum concentrations of electrolytes in animals exposed to CCl4 when compared with those in the control group. Inorganic electrolytes occur in large quantities in both extracellular and intracellular fluids, and because they can dissociate readily into their constituent ions or radicals, they constitute the most important factor in the transfer and movement of water and electrolytes between the extracellular and intracellular components. Serum chloride is an important electrolyte used to assess renal functions. In this study, we recorded an increase in plasma chloride caused by CCl4 damage to the kidneys. Sodium and potassium concentrations are usually maintained by the activity of Na/K ATPase in the renal tubules, such that when one is high, the other reduces. CCl4 caused an increase in plasma concentrations of both sodium and potassium which could result in alteration of osmotic pressure of body fluid and affect the blood pressure. Administration of both 200 and 400 mg/kg aqueous and methanol D. edulis significantly reversed this alteration in the activity of Na/K ATPase in the renal tubules thereby inhibiting the negative effects that could have resulted from CCl4-induced oxidative damage.

The extensive damage to tissues in free radical-mediated lipid peroxidation (LPO) results in membrane damage and subsequently decreases the membrane fluid content. D. edulis pretreatment significantly reversed these alterations causing a significant decrease in MDA levels, suggesting its protective effects against CCl4-induced oxidative damage. This is consistent with the study of Omonhinmin and Agbara (2013) who assessed the In vivo antioxidant properties of D. edulis and Ficusexasperata as anti-malaria plants. It is also in agreement with the report of Miguel et al. (2020) who reported that the extract of D. edulis prevented the increased lipid peroxidation occasioned by stress in Wistar rats. Reduced glutathione level in animals treated with CCl4 only relates to CCl4-induced oxidative stress and direct conjugation of GSH with reactive intermediates of CCl4

[DOI: 10.61186/pbp.5.2.100]

oxidation. This might be due to enhanced substrate utilization by glutathione peroxidase, as there is a direct correlation between GSH depletion and enhanced lipid peroxidation (Al-Shabanah et al., 2000). This result agrees with the finding of Omonhinmin & Agbara (2013) who reported that CCl4 administration caused a significant reduction in the glutathione levels in different livers of animals. It also corresponds to the findings of Alayunt et al., (2019) and Ohwokevwo & Ogunka-Nnoka (2019) who independently recorded a significant decrease in the concentration of GSH following CCl4 administration in Wistar rats. Pretreatment of animals with 100 mg/kg of both aqueous and methanolic extracts of D. edulis stembark was unable to prevent the effect of CCl4 on renal glutathione concentrations. However, animals pretreated with 200 and 400 mg/kg of aqueous and methanolic extracts of D. edulis stembark had significantly higher glutathione concentrations when compared with those induced with CCl4 without pretreatment with the groups treated with 400 mg/kg having the highest effect. This is an indication that the effect of the extracts on hepatic and renal glutathione concentration is dose-dependent. The significant increase in the glutathione levels in the kidney of D. edulis pre-treated rats CCl4- when compared with those given only CCl4 may be due to the direct ROS scavenging effect of D. edulis at these doses or an increase in GSH synthesis. The antioxidant potential of D. edulis seed reported by Ogunmoyole et al., (2012) suggested that this could be responsible for this potency. This is consistent with the finding of Omonhinmin & Agbara (2013) who reported that extracts of D. edulis prevented the reduction in the concentration of hepatic GSH sequel to CCl4-induction.

A significant increase was observed in the activity of catalase in the kidneys of animals induced with CCl4 without pretreatment when compared with those in the control group. This increase might not be unconnected with the increased generation of free radicals sequel to CCl4 exposure. This is consistent with the findings of Omonhinmin & Agbara (2013) who reported that CCl4 induction led to an elevation in the activities of antioxidant enzymes (CAT, SOD, and GPx) in Wistar rats. Elevation in the generation of free radicals by toxicants could lead to tissue damage (Megwas et al., 2021b). Thus, exposure of animals to CCl4 in this present study might have compromised the integrity of the kidney. The increase in the activities of antioxidant enzymes following CCl4 exposure is not surprising because the cell may attempt to counteract the effects of radicals generated by activating genes responsible for encoding antioxidant enzymes (Birben et al., 2012). It is also a known fact that certain reactive oxygen species can activate stress-induced transcription factors and these transcription factors may include those that are critical to the expression of antioxidant enzymes (Iseghohi & Orhue, 2017). Prophylactic treatment of animals with both aqueous and methanolic extracts of D. edulis stembark and seed was able to ameliorate these perturbations with 400 mg/kg methanolic extract showing a more promising effect. The action of the extract could be attributed to its high phytochemical content and antioxidant potential (Ogunmoyole et al., 2012). This result corresponds with the study of Ononamadu et al. (2019) who observed that methanol-extract/fractions of Dacryodes edulis was able to ameliorate the effect of diabetes-induced oxidative stress in the activity of CAT, SOD, GPx in streptozotocin-induced diabetic Wistar rats.

Anemia increases in prevalence and severity as renal function decreases, it becomes much more common at a reduced glomerular filtration rate (Airaodion et al., 2019o). In this study, a significant decrease was observed when the levels of erythrocyte parameters: red blood cell (RBC), hematocrit (HCT), and hemoglobin (HBG) of CCl4-treated animals were compared with those of the control group at $P<0.05$ as presented in Table 5-8. This agrees with the findings of Saba et al., (2010) and Amer et al., (2015) who independently reported a significant decrease in red blood parameters of animals following CCl4 exposure. However, pre-treatment of animals with both aqueous and methanolic extracts of D. edulis stem bark was able to ameliorate this effect, especially at high doses of 400 mg/kg. This was evident as a significant increase was observed in the red blood parameters of animals pretreated with 200 and 400 mg/kg of both aqueous and methanolic extracts of D. edulis stembark when compared with those in animals induced with CCl4 without pretreatment. The increase in the levels of erythrocyte parameters observed in this study may suggest that D. edulis stem bark and seed has the potential to enhance erythropoietin release from the kidneys, which is the hormonal regulator of RBC production and also affect the oxygen-carrying capacity of the blood and the amount of oxygen delivered to the tissues since red blood cells and hemoglobin (Hb) are very important in transferring

[DOI: 10.61186/pbp.5.2.100]

respiratory gases (Oyedeji et al., 2013; Airaodion et al., 2019o). Moreover, a significant decrease in the white blood cell parameters and platelets of animals exposed to CCl4 was seen when compared with those of the control group at $P<0.05$ as presented in Table 5-8. This corresponds to the findings of Ohwokevwo & Ogunka-Nnoka (2019) who reported a significant decrease in white blood cell parameters of animals exposed to CCl4 when compared with those in the control group. These perturbations were minimized when animals were pretreated with both aqueous and methanolic extracts of D. edulis stembark for 21 days before CCl4 induction. This might suggest that D. edulis stembark could inhibit the action of CCl4 on WBCs and increase its production. This suggestion is in line with Ufelle et al., (2015) who reported a significant increase in the WBC of animals treated with D. edulis when compared with the control. In addition, histological examination of CCl4-treated rats showed that it caused serious damage to the kidneys, but treatment with both aqueous and methanol seed and stembark mostly at 400mg/kg D. edulis inhibits its toxic effects (Fig. 1-4).

Conclusion

Treatments of 200 and 400mg/kg aqueous and methanolic D. edulis (seed and stem bark) extracts prevented kidney damage caused by CCl4 in a dose-dependent manner. However, 400mg/kg methanol of both extracts possessed a stronger protective effect on the kidneys against CCl4 damaging activity, than the aqueous extracts thereby showing good nephron-protective potentials. These data suggest that both the aqueous and methanol extracts from the seeds and stembark of D. edulis possessed protective activities against CCl4-induced kidney damage.

Funding/Support

No funding/support was given.

Acknowledgment

I wish to say a very big thank you to the Department of Pharmacology and Toxicology for their massive support during this study and Mr. Philip Obarisiagbon for his support during all this work.

Authors' Contribution

Patricia N. Akunne and Noghayin E. J. Orhue conceptualized and designed the study, Patricia N. Akunne carried out the experiments, collected data, and analyzed the data. Patricia N. Akunne, Chidube A. Alagbaoso, and Noghayin EJ Orhue drafted the manuscript, reviewed it, and approved the final version.

Conflict of Interest

The authors have no conflict of interest to declare.

References

1. Iseghohi SO, Orhue NEJ. Aqueous extract of Dennettia tripetala ameliorates liver and kidney damage caused by multiple exposures to carbon tetrachloride. Clin Phytoscie 2017; 3(4):1-8. doi 10.1186/s40816-017-0043-x
2. Okoro RN, Farate VT. The use of nephrotoxic drugs in patients with chronic kidney disease. Int J Clin Pharm 2019; 41 (3):767-775. doi: 10.1007/s11096-019-00811-9.
3. Giannini EG, Testa R, Savarino V. Liver enzyme alteration: a guide for clinicians. CMAJ 2005; 172:367-379. doi: 10.1503/cmaj.1040752
4. Visen P, Saraswat B, Dhawan B. Curative effect of picroliv on primary cultured rat hepatocytes against different hepatotoxins: an *in vitro* study. J. Pharmacol. Toxicol Methods 1998; 40: 173-179. doi: 10.1016/s1056-8719(98)00052-5
5. Leakey R, Atangana E, Kengnni A. Characterization of genetic variation and Domestication of *Dacryodes edulis* in West and Central Africa. J Tree livelihoods 2002; 12:57-71. doi.org/10.1080/14728028.2002.9752410
6. Okunomo K, Egho EO. Economic importance of some underexploited tree species in Nigeria: Urgent need for separate research centers, Continental J Biol Sci 2010; 3:16-32.
7. Chimaobi J, Ononamadu AJ, Alhassan AI, Abdullahi AI, Godwin O, Ihegboro TA, et al. Methanol-Extract/Fractions of *Dacryodes edulis* Leaves Ameliorate Hyperglycemia and Associated Oxidative Stress in Streptozotocin-Induced Diabetic Wistar Rats. J Evidence-Based

[DOI: 10.61186/pbp.5.2.100]

Integrative Med 2019; 24: 1-12. doi: 10.1177/2515690X19843832

8. NAS. Guide for the Care and Use of Laboratory Animals. Eighth Edition. 2011.
9. Böhme A. Spectral Behavior and Proton Effects of the Type IV Broad-Band Continua. Solar Phys 1972; 25: 478-488. doi: 10.1007/BF00192344
10. Weatherburn MW. Phenol-hypochlorite reaction for determination of ammonia. Anal Chem 1967; 39: 971. doi.org/10.1021/ac60252a045
11. Tietz NW, White WL, Mosby CO, St Louis P, Young DS, Henry RJ. (1964). Determination of serum chloride. Chem 1964; 10: 533.
12. Terri AE, Sesin PG. Determination of serum potassium by using the sodium tetraphenylboro method. American J Clin Pathol 1958; 29 (1): 86-90. doi: 10.1093/ajcp/29.1_ts.86
13. Maruna RFl. Clin Chem Acta 1958; 2: 581.
14. Ogbuagu EO, Airaodion AI, Okoroukwu VN, Ogbuagu U, Ekenjoku JA. Effect of Monosodium Glutamate on Body Weight and Alanine Aminotransferase Activity in Wistar Rats. International Research J Gastroenterol Hepatol 2019; 2 (2):1-8.
15. Shittu OK, Lawal B, Alozieuwa BU, Haruna GM, Abubakar AN, Berinyuy EB. Alteration in biochemical indices following chronic administration of methanolic extract of Nigeria bee *Propolis* in Wistar rats. Asian Pac J Trop Dis 2015; 5(8): 654-657. https://doi.org/10.1016/S2222-1808(15)60907-0
16. Airaodion AI, Ogbuagu EO, Ewa O, Ogbuagu U, Awosanya OO, Adekale OA. Ameliorative efficacy of methanolic extract of *Corchorus olitorius* leaves against acute ethanol-induced oxidative stress in Wistar rats. Asian J Biochem Genetics Molecular Biol 2019; 7 (6):1-9. doi:10.9734/ajbgmb/2019/v2i230054
17. Kassirer JP. Clinical evaluation of kidney function-glomerular function. N Engl J Med 2001; 285: 385-389. doi: 10.1056/NEJM197108122850706
18. Mandell EE, Jones F, Willis MJ, Cargill WH. Renal excretion of creatinine and inulin in man. J Lab Clin Med 2003; 42: 621-637.
19. Airaodion AI, Alabi OJ, Ogbuagu EO, Atiba FA, Olawoyin DS. Nephro and hepatotoxic effect of air-freshener in Wistar rats. Asian J Res Nephrol 2020; 3 (2):1-9.
20. Duru M, Amadi C, Ugbogu A, Eze A, Amadi B. Phytochemical, vitamin, and proximate composition of *Dacryodes edulis* fruit at different stages of maturation. Asian J Plant Sci Res 2012; 2 (4):437-441.
21. Okolo CA, Ejere VC, Chukwuka CO, Ezeigbo II, Nwibo DD, Okorie AN. Hexane extract of *Dacryodes edulis* fruits possesses anti-diabetic and hypolipidaemic potentials in alloxan diabetes of rats. African Journal of Traditinal and Complement Altern Med 2016; 13(4):132-144. doi: 10.21010/ajtcam.v13i4.18
22. Ohwokevwo OA, Ogunka-Nnoka CU. Effect of ethanol leaf extract of *Spilanthes filicaulis* on liver indices and selected biochemicals on carbon tetrachloride induced hepatic damage in Wistar rats. Medicinal and Aromatic Plants. 2019; 8(6):1-9. doi:10.35248/2167-0412.19.8.342
23. Ullah H, Khan A, Baig MW, Ullah N, Ahmed N, Tipu MK, et al. (2020). Poncirin attenuates CCL4-induced liver injury through inhibition of oxidative stress and inflammatory cytokines in mice. BMC Complementary Med Thera 20 (115):1-14. doi: 10.1186/s40360-020-00458-7
24. Zandecki, M., Genevoeve, F. and Gerard, J. *et al*, (2007). Spurious counts and spurious results on hematology analyzers: a review. Part 1: platelets. Int J lab Hematol 29:1. doi: 10.1111/j.1365-2257.2006.00871.x.
25. Chapman, M. (2000). Hematology analyzers offer new technology and user-friendliness. Lab Med 31, 3. https://doi.org/10.1309/AXXV-56JD-0F64-F5LD
26. Airaodion AI, Ekenjoku JA, Ogbuagu EO, Okoroukwu VN, Ogbuagu U. Hematopoietic Propensity of Ethanolic Leaf Extract of *Colocasia esculenta* Linn.In Wistar Rats. Am J Med Pharmacy Res 2019; 01(3):1-8. doi: 10.34297/AJBSR.2019.05.000917.
27. Al-Shabanah OA, Alam K, Nagi MN, Al-Rikabi AC, Al- Bekairi AM. Protective effect of

[DOI: 10.61186/pbp.5.2.100]

aminoguanidine, a nitric oxide synthase inhibitor, against carbon tetrachloride induced hepatotoxicity in mice. Life Sci 2000; 66: 265-270. doi: 10.1016/s0024-3205(99)00589-5.

28. Alayunt ON, Aksoy L, Karafakioğlu YS, Sevimli S. Assessment of anti-inflammatory and antioxidant properties of Safranal on CCl_4-induced oxidative stress and inflammation in rats. Annals Braz Academy Sci 2019; 91(2):1-11. doi: 10.1590/0001-3765201920181235
29. Amer MA, EL-missiry MA, EL-nabi AA. The Role of *Ficus carica* leaf extract in modulation of the experimentally induced hepatotoxic damage in male rats. Int J Adv Res 2015; 3 (12): 572-585.
30. Buege JA, Aust SD. Microsomal lipid peroxidation. Methodr Enzymol 1978; 52: 302-310. https://doi.org/10.1016/S0076-6879(78)52032-6.
31. Birben E, Sahiner UM, Sackesen C, Erzurum S, Kalayci O. Oxidative stress and antioxidant defense. WAO J 2012; 2012:9. doi: 10.1097/WOX.0b013e3182439613
32. Ellman GL Tissue Sulfhydryl Groups. Archives of Biochem Biophys 1959; 82: 70-77. doi: 10.1016/0003-9861(59)90090-6
33. Goth L. A simple method for determination of serum catalase activity and revision of refernce raange. Clin Chem Acta *1991;* 196: 143:151. doi: 10.1016/0009-8981(91)90067-m.
34. Iseghohi SO, Orhue NEJ. Aqueous extract of *Dennettia tripetala* ameliorates liver and kidney damage caused by multiple exposures to carbon tetrachloride. Clin Phytosci 2017; 3 (4):1-8. doi 10.1186/s40816-017-0043-35.
35. Koudou JP, Edou LC, Obame I, Bassole H, Figueredo G. Volatile components, antioxidant and antimicrobial properties of the essential oil of Dacryodes edulis G. Don from Gabon. J Appl Sci 2004; 8: 3532 – 3535. doi: 10.3923/jas.2008.3532.3535
36. Leudeu BC, Tchiégang C, Dzondo-Gadet M, Barbé F, Nicolas B, Sokeng S, Moundipa-Fewou P, Kapseu C, Parmentier M, Guéant JL. Effect of Canariumschweinfurthii Oils on Blood Lipids, Lipid Peroxidation and Oxidative Stress in Rats. J Food Technol 2006; 4 (4): 275-282.
37. Megwas AU, Njoku OC, Akunne PN, Oladosu NO, Airaodion AI. Ameliorative Potential of Bambara Nuts (*Vigna subterranea* (L.) Verdc) Extract against Acute Ethanol-Induced Oxidative Stress in Wistar Rats. Int J Health, Safety and Environment 2021; 7 (02):789-800.
38. Miguel LM, N'jilo DG, Bolanga RB, Mokondjimobe E, Moukassa D, Longo-Mbenza B, Abena AA, Mabiala GM, Muanda HN. Effect of *Dacryodes edulis* pulp oil treatment on restraint stress-induced behavioral and biochemical alterations in mice. Int J Health Sci Res *2020;* 10 (1):127-134.
39. Misra HP, Fridovich I The role of superoxide anions in the autoxiation of epinephrine and simple assay for superoxide dismutase. J Biol Chem 1972; 247: 3170-3175.
40. Nyman M. Uber Haptoglobinbestimmung im Serum, Normalkonzentration und Verhältnis zu Smithies Serumgruppen. Clin Chim Acta 1958; 3 (1):111–113.
41. Ogunmoyole T, Kade IJ, Johnson OD, Makun OJ. Effect of boiling on the phytochemical constituents and antioxidant properties of African pear *Dacryodes edulis* seeds *in vitro.* Afr J Biochem Res 2012; 6(8):105-114. doi: 10.5897/AJBR12.023
42. Ohwokevwo OA, Ogunka-Nnoka CU. Effect of ethanol leaf extract of *Spilanthes filicaulis* on liver indices and selected biochemicals on carbon tetrachloride induced hepatic damage in Wistar rats. Medicinal & Aromatic Plants 2019; 8(6):1-9. doi: 10.35248/2167-0412.19.8.342
43. Okwu DE, Nnamdi FU. Evaluation of the chemical composition of Dacryodes edulis check for this species in other resources and raphia hookeri mann and wendl exudates used in herbal medicine in South Eastern Nigeria, Afr J Tradit Complement Altern Med 2008; 5: 194-200.
44. Omonhinmin AC, Agbara IU. Assessment of In vivo antioxidant properties of Dacryodes edulis and Ficus exasperate as anti-malaria plants. Asian Pacific Journal of Tropical Disease 2013; 3(4): 294-300. https://doi.org/10.1016/S2222-1808(13)60072-9

[DOI: 10.61186/pbp.5.2.100]

45. Ononamadu CJ, Alhassan AJ, Ibrahim A, Imam AA, Ihegboro GO, Owolarafe TA, Sule MS. Methanol-extract/fractions of *Dacryodes edulis* leaves ameliorate hyperglycemia and associated oxidative stress in streptozotocin-induced diabetic wistar rats. J Evidence-Based Integr Med 2019; 1-12. doi: 10.1177/2515690X19843832
46. Orhue NEJ, Adaikpoh MA, Odude O, Iseghohi SO. Prevention of carbon tetrachloride-induced hepatic steatosis and cellular damage by aqueous extract of *Dacryodes edulis* seeds in Wistar rats. Bio-Res 2015; 13: 885 – 888. doi:10.4314/br.v13i1.144795
47. Saba AB, Oyagbemi AA, Azeez OI. Amelioration of carbon tetrachloride-induced hepatotoxicity and haemotoxicity by aqueous leaf extract of *Cnidoscolus aconitifolius* in rats. Nigerian J Physiological Sci 2010; 139-147.
48. Ufelle S, Ukaejiofo E, Achukwu P, Eluke B, Ghasi S, Neboh E. Potential Haemopoietic Effects of *Dacryodes Edulis* Seeds Extract in Wistar Rats. Int J Healthcare Sci 2015; 3(1):413-416.

[DOI: 10.61186/pbp.5.2.100]