

# *Dennettia tripetala* Combats Oxidative Stress, Protein and Lipid Dyshomeostasis, Inflammation, Hepatic Injury, and Glomerular Blockage in Rats

Sylvia Oghogho Oimage<sup>1</sup>, Noghayin E. Jerry Orhue<sup>1</sup>, and Kingsley Oimage<sup>2</sup>

<sup>1</sup>Department of Biochemistry, Faculty of Life Sciences, University of Benin, Benin City, Edo State 300213, Nigeria

<sup>2</sup>Department of Biochemistry, College of Health Sciences, School of Basic Medical Sciences, Igbinedion University Okada, Benin City, Edo State 302110, Nigeria

**ABSTRACT:** *Dennettia tripetala*, better known as ‘pepperfruit’, is a medicinal plant consumed in West Africa. *D. tripetala* possesses strong antioxidant properties and contains uvariopsine, an alkaloid which improves bile secretion and liver function. In the present study, the ethanolic extract of *D. tripetala* fruits was tested for its ability to alleviate pathophysiological conditions bordering on oxidative stress, including protein and lipid dyshomeostasis, inflammation, and hepatic and glomerular injury. Male albino Wistar rats were administered carbon tetrachloride twice a week for two weeks, and the ethanolic extract of *D. tripetala* fruits was administered from days 8~14. The serum, liver, and kidneys of the rats were then subjected to biochemical assays and imaging. The extract restored the activities of liver marker enzymes in serum and the concentrations of lipids and proteins in both circulation and the liver to normal. The extract also restored the activities of antioxidant enzymes in liver and kidneys, and the concentrations of urea and creatinine in the blood. The extract also repaired the altered structures of the liver and kidney. Overall, *D. tripetala* elicited strong medicinal effects in rats. This study showed that the fruits of *D. tripetala* contain substances that could be extracted or synthesized for use in drugs for the treatment of liver and kidney disease.

**Keywords:** antioxidant, carbon tetrachloride, *Dennettia tripetala*, hepatorenal damage, oxidative stress

## INTRODUCTION

*Dennettia tripetala* (pepperfruit) is a fruit eaten in the Western parts of Africa. *D. tripetala* possesses a spicy taste, which endears it to the inhabitants of this region, who both consume it as a fruit and use it to season foods including *zobo*, a local drink made from the flowers of *Hibiscus sabdariffa* (Ihemeje et al., 2013). *D. tripetala* is also consumed for medicinal purposes and used for food preservation (Okolie et al., 2014; Iseghohi, 2015). A number of scientific experiments have characterized the properties and potentials of *D. tripetala*, which have been extensively reviewed by Iseghohi (2015). Other experiments on *D. tripetala* have since been conducted, a number of which are mentioned below.

In 2016, the components of the essential oil of *D. tripetala* fruits were characterized using gas chromatography mass spectrometry (Okoh et al., 2016). The authors also studied the antioxidant and bactericidal properties of the

oil to show that it possesses antioxidant properties *in vitro* and exhibits strong bacteriostatic effects on common multi-drug resistant bacterial strains (Okoh et al., 2016). In 2017, methanol extracts and methanol fractions of *D. tripetala* seeds were shown to exert hypoglycemic and anti-hyperlipidemic effects in rats challenged with streptozotocin (Anioke et al., 2017). However, another study showed that the extract can trigger increases in gastric acid secretion in rats (Bright et al., 2017).

In 2017, *D. tripetala* powder was shown to be tolerated by rats when incorporated into their diet as a form of botanical pesticide (Nwosu et al., 2017). The aqueous and petroleum ether extracts were also found to elicit inhibitory effects on the activity of CYP3A enzymes *in vitro* (in rat liver and intestinal microsomes) and *in vivo* in rats (Nduka et al., 2017). In 2018, ethanolic extracts of *D. tripetala* fruits were shown to enhance the testosterone levels as well as the number and motility of sperm in rats (Obiandu and Green, 2018).

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Correspondence to Sylvia Oghogho Oimage, Tel: +234-526-005-53, E-mail: sylvia.iseghohi@uniben.edu

Author information: Sylvia Oghogho Oimage (Researcher), Noghayin E. Jerry Orhue (Professor), Kingsley Oimage (Researcher)

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More recently, we showed that the powder and aqueous and ethanolic extracts of *D. tripetala* fruits contain flavonoids, tannins, phenols, saponins, and alkaloids, and possess strong antioxidant properties (comparable to that of rutin) against 2,2-diphenyl-1-picrylhydrazyl, hydrogen peroxide, and ferric ions (Omage et al., 2018). Furthermore, we showed that the aqueous and ethanolic extracts of *D. tripetala* are not toxic to the liver, kidney, or heart of rats at doses of up to 5,000 mg/kg body weight (bw; acute exposure) and 1,000 mg/kg bw (28-day exposure). In other studies, the essential oils of *D. tripetala* seeds, particularly its  $\beta$ -phenylnitroethane component, exhibited memory-enhancing abilities (Oyemitan et al., 2019). The properties of *D. tripetala* have also been examined in humans. For example, the seeds have been found to reduce intraocular pressure of normotensive emmetropic humans (Timothy and Okere, 2008), and to elicit bronchiodilatory effects (Omgie et al., 2017).

Carbon tetrachloride (CCl<sub>4</sub>) is a commonly used chemical for induction of hepatorenal injuries in laboratory animals; its mechanism of action is very well understood. CCl<sub>4</sub> is metabolized by cytochrome P450 enzymes, leading to production of free radicals that trigger oxidative stress and damage cellular constituents, including lipids, proteins, and genetic material, eventually resulting in injury to organs including the liver (Weber et al., 2003) and kidneys (Sanzgiri et al., 1997). The toxicity of CCl<sub>4</sub> is related to its dose and the duration of the exposure; higher doses and longer exposures tend to cause more serious and permanent effects (Weber et al., 2003).

In 2002, *D. tripetala* was shown to contain the alkaloid uvariopsine, which improves bile secretion and liver function (López-Martín et al., 2002). Our research group has also previously shown that *D. tripetala* extracts can prevent liver and kidney damage *in vivo* in rats subsequently exposed to a single administration of CCl<sub>4</sub> (Iseghohi and Orhue, 2015; Iseghohi et al., 2017). In the present study, we tested the ability of ethanolic extracts of *D. tripetala* fruits to do the reverse, i.e., attenuate already existing liver and kidney lesions. These injuries were inflicted by repeated exposure to CCl<sub>4</sub>, in order to cause more severe damage than that induced by a single exposure to the toxin.

## MATERIALS AND METHODS

### Plant materials

Ripe *D. tripetala* fruits were purchased from a market in Benin City, Nigeria. The fruits were identified by a botanist in the Department of Plant Biology and Biotechnology, University of Benin as *D. tripetala* (family: Annonaceae), and a plant voucher number (UBHD360) was allocated. The fruits were diced, dried in the sun, and pul-

verized into a powdery form. Then, 500 g of the powder was soaked in 4 L of ethanol for two days with regular agitation. The resulting filtrate (obtained with the aid of a cheese cloth) was freeze dried and reconstituted in distilled water for administration to animals.

### Phytochemical content, *in vitro* antioxidant properties, and toxicity study of the freeze-dried extract

The phytochemical content, *in vitro* antioxidant properties and toxicity of the extract have been previously published (Omage et al., 2018).

### Animals

Male albino Wistar rats of approximately 5 weeks of age and weighing 100~140 g were used in experiments. Rats were bred in wooden cages covered with barbed wire and allowed free access to feed (Growers' mash, Bendel Feed and Flour Mill Ltd., Ewu, Edo State, Nigeria) and water. The comfort of the rats was taken into consideration throughout the duration of the experiment. All animal experiments were carried out in accordance with the NIH guide for the care and use of laboratory animals (NIH publication No. 8023, revised 1978) and with permission from the Animal Ethics committee of the Faculty of Life Sciences, University of Benin, Nigeria (approval number LS16106). After acclimatizing for two weeks, rats were randomized into five groups of five. The experimental design is shown in Table 1.

Rats in the control group (group 1) were only administered feed and water. Rats in groups 2~5 were administered CCl<sub>4</sub> dissolved in olive oil (1:1 w/v) at 3 mL/kg bw twice a week for two weeks. Plant extract was dissolved in ethanol (200 mg/mL) and administered at doses of 250, 500, and 1,000 mg/kg bw to groups 3~5, respectively, daily for seven days from day 8 of CCl<sub>4</sub> administration. All experimental substances were administered orally using a gavage. On day 14, rats were subjected to an overnight fast, and sacrificed on day 15 using chloroform anaesthesia. Blood (serum), liver, and kidneys were collected for biochemical assays and histopathology.

**Table 1.** Experimental design

Groups	Treatment
1	Control
2	CCl <sub>4</sub>
3	CCl <sub>4</sub> + ETDT 250
4	CCl <sub>4</sub> + ETDT 500
5	CCl <sub>4</sub> + ETDT 1000

ETDT, ethanolic extract of *Dennettia tripetala*. *D. tripetala* was administered in doses of 250, 500, and 1,000 mg/kg bw (n=5).

**Biochemical assays**

Rat serum was assayed for aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP),  $\gamma$ -glutamyltransferase (GGT), total cholesterol, triglyceride, total protein, albumin, globulin, urea, and creatinine. Livers and kidneys were assayed for superoxide dismutase (SOD), catalase, and malondialdehyde (MDA). All but four assays were conducted using kits from Randox Laboratories (Crumlin, UK), following the manufacturer’s protocol. SOD, catalase, and MDA assays were conducted using reagents purchased from Pyrex-Ig Science/laboratory (Benin City, Nigeria), following methods described by Misra and Fridovich (1972), Góth (1991), and Buege and Aust (1978) for SOD, catalase, and MDA assays, respectively. ALP was assessed using kits from Teco Diagnostics (Anaheim, CA, USA), following manufacturer’s instructions.

**Histopathology**

Portions of the liver and kidneys were fixed in 10% neutral buffered formalin for histopathological analysis. A Leica TP1020 automatic tissue processor (Leica Biosystems, Wetzlar, Germany) was used to process the tissues, which were stained with hematoxylin and eosin and viewed under a light microscope using both 10 $\times$  and 40 $\times$  magnification.

**Statistics**

Data were analyzed using one-way ANOVA and Tukey’s post-hoc test and presented as mean $\pm$ standard error of

the mean (SEM). GraphPad Prism version 7 software (GraphPad Software, San Diego, CA, USA) was used for data analysis.

**RESULTS**

**Effect on liver marker enzymes in serum**

CCl<sub>4</sub> treatment induced significant increases in the activities of the liver marker enzymes AST and ALT in serum, and a non-significant elevation of ALP (Table 2). The ethanolic extract of *D. tripetala* fruits helped alleviate damage induced by CCl<sub>4</sub>, by lowering the activities of ALT, AST, and ALP in serum of the rats administered CCl<sub>4</sub> and extracts vs. rats administered CCl<sub>4</sub> alone.

**Effect on lipid homeostasis**

Furthermore, CCl<sub>4</sub> significantly elevated serum total cholesterol and low-density lipoprotein (LDL)-cholesterol and significantly reduced serum high-density lipoprotein (HDL)-cholesterol and triacylglycerol (TAG) (Table 3). The ethanolic extract of *D. tripetala* showed low potential for alleviating the distortions in lipid concentrations that were altered by CCl<sub>4</sub>. In the liver, CCl<sub>4</sub> elevated the concentration of cholesterol and TAGs (Table 4), and caused steatosis (fatty accumulation) in liver cells (Fig. 1 and Fig. 2). *D. tripetala* extracts lowered the concentration of these lipids in the liver (Table 4, Fig. 1, and Fig. 2).

**Table 2.** Effect of *Dennettia tripetala* on serum activity of liver marker enzymes in rats administered CCl<sub>4</sub> (unit: U/L)

Groups	AST	ALT	ALP
Control	242.10 $\pm$ 6.55 <sup>a</sup>	52.50 $\pm$ 4.79 <sup>a</sup>	39.15 $\pm$ 5.88 <sup>a</sup>
CCl <sub>4</sub>	809.00 $\pm$ 15.6 <sup>c</sup>	486.70 $\pm$ 6.67 <sup>b</sup>	45.70 $\pm$ 1.59 <sup>a</sup>
ETDT 250 + CCl <sub>4</sub>	493.50 $\pm$ 19.64 <sup>b</sup>	460.00 $\pm$ 48.99 <sup>b</sup>	58.06 $\pm$ 5.11 <sup>b</sup>
ETDT 500 + CCl <sub>4</sub>	465.30 $\pm$ 6.36 <sup>b</sup>	336.70 $\pm$ 38.44 <sup>b</sup>	44.81 $\pm$ 3.15 <sup>a</sup>
ETDT 1000 + CCl <sub>4</sub>	520.00 $\pm$ 1.16 <sup>b</sup>	362.50 $\pm$ 34.73 <sup>b</sup>	43.63 $\pm$ 2.72 <sup>a</sup>

The values presented are the mean $\pm$ SEM (n=5). Values with different letters (a-c) within the same column are significantly different at *P*<0.05. ETDT, ethanolic extract of *D. tripetala*; AST, aspartate aminotransferase; ALT, alanine aminotransferase; ALP, alkaline phosphatase. *D. tripetala* was administered in doses of 250, 500, and 1,000 mg/kg bw.

**Table 3.** Effect of *Dennettia tripetala* on serum lipid concentration in rats administered CCl<sub>4</sub> (unit: mg/dL)

Groups	Total cholesterol	HDL-cholesterol	LDL-cholesterol	TAG
Control	38.28 $\pm$ 1.93 <sup>a</sup>	25.45 $\pm$ 2.20 <sup>b</sup>	13.75 $\pm$ 0.95 <sup>a</sup>	127.40 $\pm$ 25.10 <sup>c</sup>
CCl <sub>4</sub>	47.85 $\pm$ 1.08 <sup>b</sup>	7.24 $\pm$ 0.64 <sup>a</sup>	41.07 $\pm$ 0.94 <sup>b</sup>	84.30 $\pm$ 4.43 <sup>b</sup>
ETDT 250 + CCl <sub>4</sub>	47.09 $\pm$ 0.61 <sup>b</sup>	28.57 $\pm$ 0.24 <sup>b</sup>	23.08 $\pm$ 3.12 <sup>a</sup>	46.62 $\pm$ 4.91 <sup>a</sup>
ETDT 500 + CCl <sub>4</sub>	47.40 $\pm$ 2.10 <sup>b</sup>	24.89 $\pm$ 2.82 <sup>b</sup>	32.33 $\pm$ 3.65 <sup>b</sup>	72.00 $\pm$ 6.79 <sup>b</sup>
ETDT 1000 + CCl <sub>4</sub>	42.84 $\pm$ 1.39 <sup>ab</sup>	9.57 $\pm$ 0.97 <sup>a</sup>	38.30 $\pm$ 0.58 <sup>b</sup>	49.23 $\pm$ 2.22 <sup>a</sup>

The values presented are the mean $\pm$ SEM (n=5). Values with different letters (a-c) within the same column are significantly different at *P*<0.05. ETDT, ethanolic extract of *D. tripetala*; HDL, high-density lipoprotein; LDL, low-density lipoprotein; TAG, triacylglycerols. *D. tripetala* was administered in doses of 250, 500, and 1,000 mg/kg bw.

**Table 4.** Effect of *Dennettia tripetala* administration on liver lipid concentration in rats administered CCl<sub>4</sub> (unit: mg/dL)

Groups	Total cholesterol	TAG
Control	41.68±0.49 <sup>a</sup>	16.88±2.11 <sup>a</sup>
CCl <sub>4</sub>	51.44±7.59 <sup>b</sup>	28.84±6.01 <sup>ab</sup>
ETDT 250 + CCl <sub>4</sub>	44.06±0.97 <sup>a</sup>	7.74±0.70 <sup>a</sup>
ETDT 500 + CCl <sub>4</sub>	44.46±0.23 <sup>a</sup>	11.96±0.70 <sup>a</sup>
ETDT 1000 + CCl <sub>4</sub>	46.44±0.33 <sup>ab</sup>	16.35±5.05 <sup>a</sup>

The values presented are the mean±SEM (n=5). Values with different letters (a,b) within the same column are significantly different at  $P<0.05$ . ETDT, ethanolic extract of *D. tripetala*; TAG, triacylglycerols. *D. tripetala* was administered in doses of 250, 500, and 1,000 mg/kg bw.

#### Effect on serum protein profile

CCl<sub>4</sub> significantly elevated the total protein and globulin concentrations in serum and significantly reduced serum albumin levels and the albumin : globulin ratio (Table 5). Therefore, we concluded that *D. tripetala* ethanolic extract shows potential for restoring normal concentrations of serum proteins.

#### Effect on recruitment of immune cells in the liver

Histopathology images revealed that CCl<sub>4</sub> caused immune cells to be recruited to the sites of damage in the liver (Fig. 1 and Fig. 2).

#### Effect on antioxidant enzyme activities in liver and kidneys

CCl<sub>4</sub> induced a reduction in the activities of the antioxidant enzymes SOD and catalase in both the liver and the kidneys (Table 6 and Table 7). In addition, CCl<sub>4</sub> significantly elevated the concentration of the lipid peroxidation product malondialdehyde in both the liver and kidneys. Interestingly, *D. tripetala* ethanolic extract significantly elevated the activities of the antioxidant enzymes and reduced malondialdehyde levels in the liver and kidneys of rats administered CCl<sub>4</sub> and the extract compared with rats administered CCl<sub>4</sub> alone.

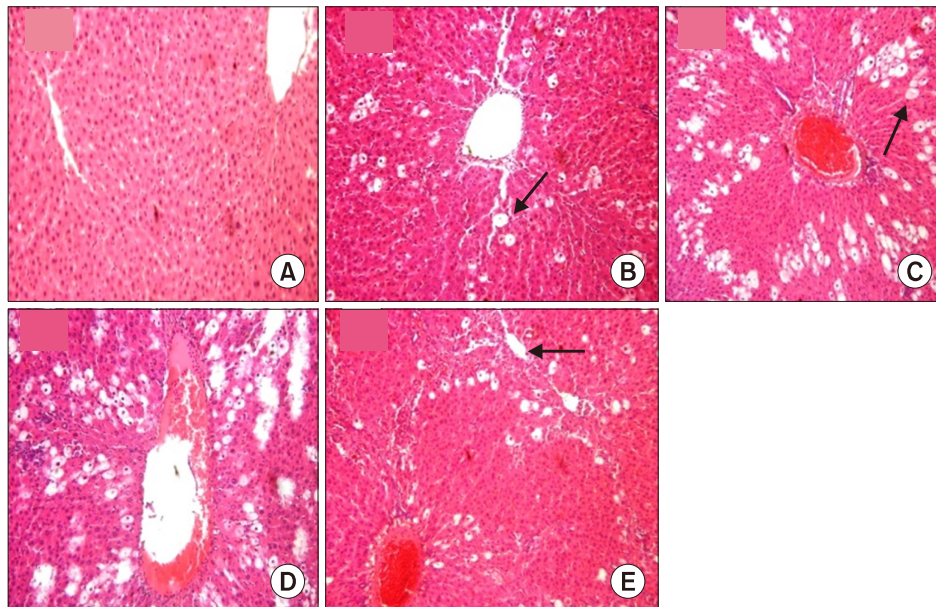
#### Effect on serum urea and creatinine concentrations

CCl<sub>4</sub> elevated the concentration of urea and creatinine in serum (Table 8). This observation was supported by histopathology results (Fig. 3 and Fig. 4) showing that CCl<sub>4</sub> severely altered the structure of the kidneys. In addition, *D. tripetala* ethanolic extract reduced urea and creatinine concentrations in the serum of rats administered CCl<sub>4</sub> and the extracts compared with rats administered CCl<sub>4</sub> alone; the observation further supported by histopathology results (Fig. 3 and Fig. 4).

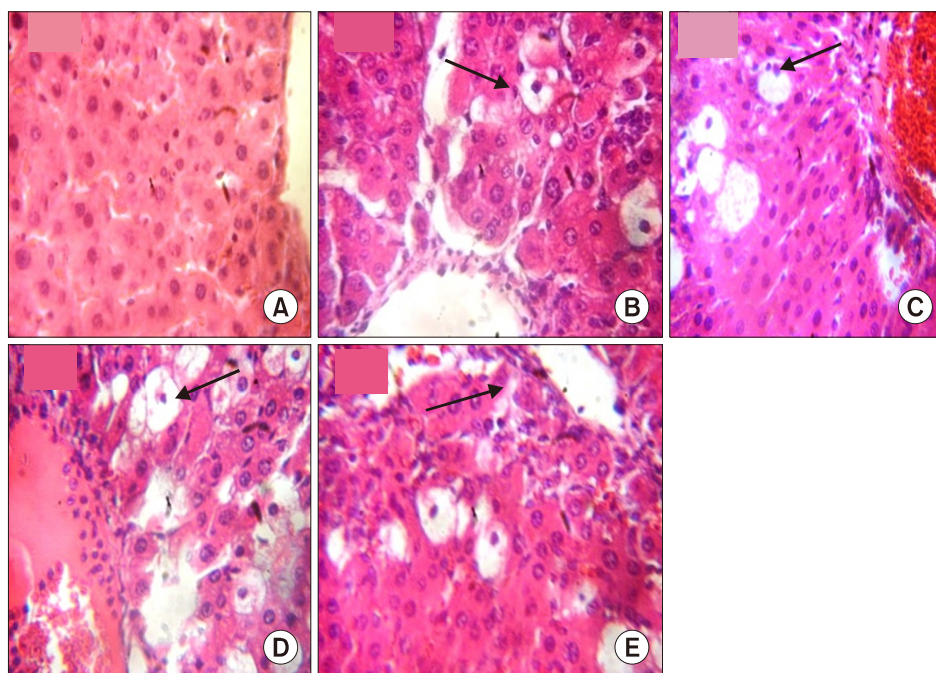
## DISCUSSION

#### Effect on liver marker enzymes in serum

ALT and AST are normally localized in the cells of the



**Fig. 1.** Photomicrographs of liver sections from (A) control rats showing normal liver histology: clear centrioles with well fenestrated sinusoidal space and distinct hepatocytes; (B) rats treated with 250 mg/kg bw ETDT and CCl<sub>4</sub>, showing very mild fatty changes with clear centrioles; (C) rats treated with 500 mg/kg bw ETDT and CCl<sub>4</sub>, showing fatty changes and little hydropic degeneration of the hepatocytes, with centrioles appearing congested surrounded by neutrophilic infiltrates; (D) rats treated with 1,000 mg/kg bw ETDT and CCl<sub>4</sub>, showing fatty changes with partially clear centrioles surrounded by visible multifocal neutrophilic infiltrates; (E) rats treated with CCl<sub>4</sub> only, showing fatty changes (macrovesicular steatosis) and hydropic degeneration of the hepatocytes with multifocal distortion in liver histology, congestion of the central vein, with neutrophilic infiltrates. The liver sections were stained with hematoxylin and eosin and observed with a 10× objective. ETDT, *D. tripetala* ethanolic extract.



**Fig. 2.** Photomicrographs of liver sections from (A) control rats showing normal liver histology: clear centrioles with well fenestrated sinusoidal space and distinct hepatocytes with well differentiated nucleus; (B) rats treated with 250 mg/kg bw ETDT and CCl<sub>4</sub>, showing very mild fatty changes although with clear centrioles; (C) rats treated with 500 mg/kg bw ETDT and CCl<sub>4</sub>, showing fatty changes and little hydropic degeneration of the hepatocytes, with centriole appearing congested surrounded by neutrophilic infiltrates; (D) rats treated with 1,000 mg/kg bw ETDT and CCl<sub>4</sub>, showing fatty changes with partially clear centrioles surrounded by visible multifocal neutrophilic infiltrates; (E) rats treated with CCl<sub>4</sub> only showing fatty changes (macrovesicular steatosis) and hydropic degeneration of the hepatocytes with multifocal distortion in liver histology, congestion of the central vein with neutrophilic infiltrates. The liver sections were stained with hematoxylin and eosin and observed with a 40× objective. ETDT, *D. tripetala* ethanolic extract.

**Table 5.** Effect of *Dennettia tripetala* on serum protein concentration in rats administered CCl<sub>4</sub>

Groups	Total protein (g/dL)	Albumin (g/dL)	Globulin (g/dL)	Albumin : globulin ratio
Control	5.94±0.18 <sup>a</sup>	3.24±0.04 <sup>b</sup>	2.68±0.09 <sup>a</sup>	1.13±0.04 <sup>c</sup>
CCl <sub>4</sub>	21.04±1.45 <sup>c</sup>	2.96±0.03 <sup>a</sup>	14.05±1.67 <sup>b</sup>	0.18±0.02 <sup>a</sup>
ETDT 250 + CCl <sub>4</sub>	13.11±0.95 <sup>b</sup>	2.71±0.05 <sup>a</sup>	10.42±0.91 <sup>b</sup>	0.26±0.02 <sup>a</sup>
ETDT 500 + CCl <sub>4</sub>	8.69±1.05 <sup>a</sup>	3.38±0.02 <sup>b</sup>	6.12±1.34 <sup>a</sup>	0.31±0.10 <sup>a</sup>
ETDT 1000 + CCl <sub>4</sub>	9.35±0.25 <sup>a</sup>	2.82±0.07 <sup>a</sup>	6.06±0.45 <sup>a</sup>	0.46±0.05 <sup>b</sup>

The values presented are the mean±SEM (n=5). Values with different letters (a-c) within the same column are significantly different at *P*<0.05. ETDT, ethanolic extract of *D. tripetala*. *D. tripetala* was administered in doses of 250, 500, and 1,000 mg/kg bw.

**Table 6.** Effect of *Dennettia tripetala* on liver antioxidant enzyme activity and lipid peroxidation status in rats administered CCl<sub>4</sub> (unit: units/g wet tissue)

Groups	SOD	Catalase	MDA
Control	1,225±3 <sup>c</sup>	4,683±11 <sup>c</sup>	0.08±0.01 <sup>a</sup>
CCl <sub>4</sub>	744.0±2 <sup>a</sup>	4,326±51 <sup>a</sup>	0.17±0.01 <sup>c</sup>
ETDT 250 + CCl <sub>4</sub>	891.7±36 <sup>b</sup>	4,386±31 <sup>a</sup>	0.09±0.01 <sup>a</sup>
ETDT 500 + CCl <sub>4</sub>	1,500±25 <sup>d</sup>	4,518±8 <sup>b</sup>	0.10±0.01 <sup>ab</sup>
ETDT 1000 + CCl <sub>4</sub>	1,125±52 <sup>c</sup>	4,656±45 <sup>c</sup>	0.09±0.01 <sup>a</sup>

The values presented are the mean±SEM (n=5). Values with different letters (a-d) within the same column are significantly different at *P*<0.05. ETDT, ethanolic extract of *D. tripetala*; SOD, superoxide dismutase; MDA, malondialdehyde. *D. tripetala* was administered in doses of 250, 500, and 1,000 mg/kg bw.

**Table 7.** Effect of *Dennettia tripetala* on kidney antioxidant enzyme activity and lipid peroxidation status in rats administered CCl<sub>4</sub>

Groups	SOD [units/g wet tissue ( $\times 10^{-2}$ )]	Catalase (units/g wet tissue)	MDA (units/g wet tissue)
Control	873 $\pm$ 28 <sup>a</sup>	5,076 $\pm$ 294 <sup>b</sup>	0.20 $\pm$ 0.01 <sup>a</sup>
CCl <sub>4</sub>	644 $\pm$ 28 <sup>a</sup>	3,984 $\pm$ 18 <sup>a</sup>	0.25 $\pm$ 0.01 <sup>b</sup>
ETDT 250 + CCl <sub>4</sub>	1,130 $\pm$ 346 <sup>a</sup>	4,749 $\pm$ 11 <sup>b</sup>	0.21 $\pm$ 0.01 <sup>a</sup>
ETDT 500 + CCl <sub>4</sub>	1,027 $\pm$ 208 <sup>a</sup>	4,815 $\pm$ 26 <sup>b</sup>	0.19 $\pm$ 0.01 <sup>a</sup>
ETDT 1000 + CCl <sub>4</sub>	1,030 $\pm$ 79 <sup>a</sup>	4,827 $\pm$ 7 <sup>b</sup>	0.23 $\pm$ 0.01 <sup>ab</sup>

The values presented are the mean $\pm$ SEM (n=5).

Values with different letters (a,b) within the same column are significantly different at  $P < 0.05$ .

ETDT, ethanolic extract of *D. tripetala*; SOD, superoxide dismutase; MDA, malondialdehyde.

*D. tripetala* was administered in doses of 250, 500, and 1,000 mg/kg bw.

**Table 8.** Effect of *Dennettia tripetala* on urea and creatinine concentrations in rats administered CCl<sub>4</sub> (unit: mg/dL)

Groups	Urea	Creatinine
Control	6.43 $\pm$ 0.83 <sup>a</sup>	1.54 $\pm$ 0.01 <sup>a</sup>
CCl <sub>4</sub>	10.73 $\pm$ 0.04 <sup>b</sup>	2.15 $\pm$ 0.02 <sup>b</sup>
ETDT 250 + CCl <sub>4</sub>	8.31 $\pm$ 0.63 <sup>b</sup>	1.36 $\pm$ 0.03 <sup>a</sup>
ETDT 500 + CCl <sub>4</sub>	7.53 $\pm$ 0.72 <sup>ab</sup>	1.48 $\pm$ 0.14 <sup>a</sup>
ETDT 1000 + CCl <sub>4</sub>	7.37 $\pm$ 0.87 <sup>ab</sup>	1.36 $\pm$ 0.07 <sup>a</sup>

The values presented are the mean $\pm$ SEM (n=5).

Values with different letters (a,b) within the same column are significantly different at  $P < 0.05$ .

ETDT, ethanolic extract of *D. tripetala*.

*D. tripetala* was administered in doses of 250, 500, and 1,000 mg/kg bw.

liver, but can drain into the blood and cause noticeable elevations following damage to the membranes of hepatocytes, as is the case with the metabolites of CCl<sub>4</sub> (Singh et al., 2011). This readily occurs in the liver, which contains a high amount of cytochrome P450 enzymes responsible for metabolizing drugs and toxins. In the present study, the ethanolic extract of *D. tripetala* fruits alleviated damage induced by CCl<sub>4</sub>, observed by lower activities of ALT, AST, and ALP in the serum of the rats administered extracts and CCl<sub>4</sub> compared with CCl<sub>4</sub> alone. *D. tripetala* contains flavonoids and phenols that elicit antioxidant effects. In addition, *D. tripetala* elicits *in vitro* antioxidant effects (Omage et al., 2018). Therefore, *D. tripetala* may have alleviated the damage by combating lipid peroxidation caused by trichloromethyl radicals produced during CCl<sub>4</sub> metabolism in the liver, thus protecting hepatocyte membranes from distortion that could cause spillage of enzymes into the blood.

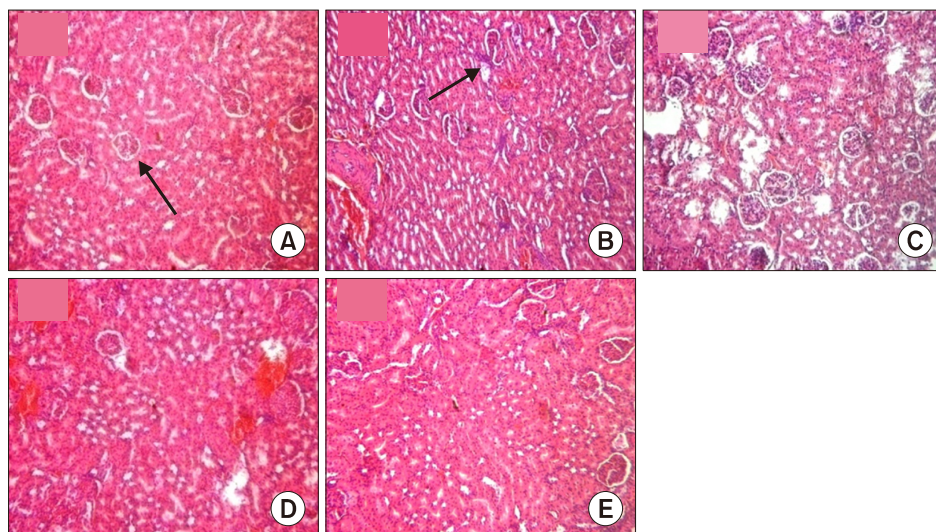
### Effect on lipid homeostasis

Dyslipidemia is caused by increases in total cholesterol and LDL-cholesterol and decreases HDL-cholesterol in serum, and is a risk factor for atherosclerosis and other diseases of the heart and blood vessels (Mahdy Ali et al., 2012). Certain extents of damage to the liver have been shown to alter its ability to synthesize, package, and se-

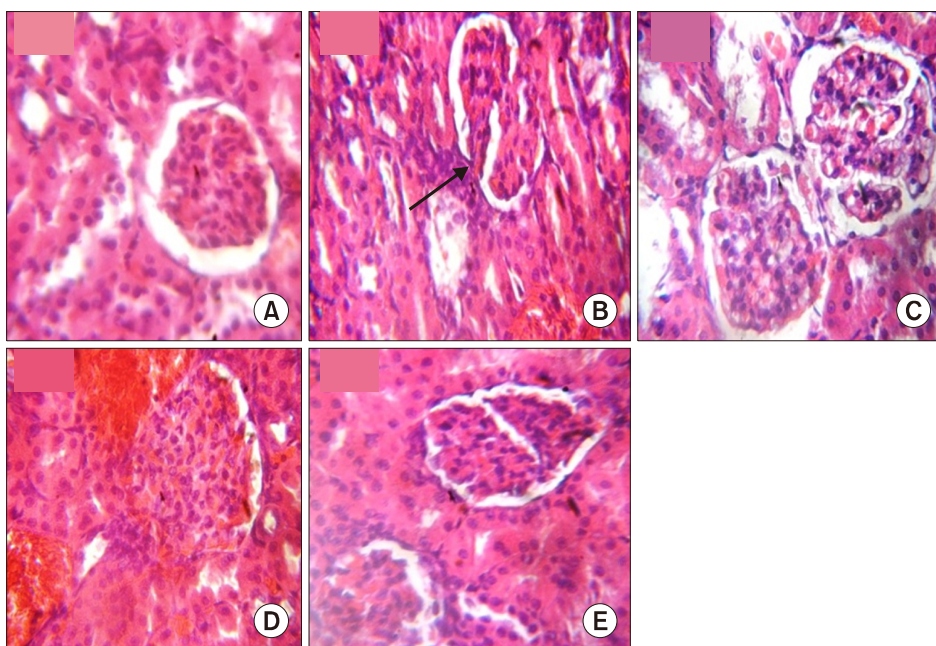
crete lipids into the blood (Fromenty and Pessayre, 1995; Boll et al., 2001; Khalaf et al., 2009; Al-Yahya et al., 2013; Hamed et al., 2016). Therefore, it was unsurprising that recurrent challenges with CCl<sub>4</sub> induced dyslipidemia. However, *D. tripetala* ethanolic extract showed low potential to alleviate the distortions in lipid concentrations altered by CCl<sub>4</sub>. The mechanism of action of *D. tripetala* extract may include restoration of the liver's ability to metabolize these lipids. Our findings also indicated that CCl<sub>4</sub> elevates the concentration of cholesterol and TAGs in the liver, which supports the hypothesis that CCl<sub>4</sub> may alter the ability of the liver to metabolize lipids. In this case, CCl<sub>4</sub> most likely increased synthesis of cholesterol in the liver, which may have elevated the concentration of total cholesterol in the serum. On the other hand, since TAGs accumulated in the liver, secretion of TAGs by the liver may have been impaired, leading to a noticeable drop in serum TAG levels. This result is supported by histopathology images of the liver (Fig. 1 and Fig. 2) that show steatosis (fatty accumulation) in the liver cells. *D. tripetala* extracts lowered the concentration of these lipids in the liver (Table 4, Fig. 1, and Fig. 2).

### Effect on serum protein profiles

The elevated total protein in the serum may comprise that which leaked out of the injured liver. The decrease in albumin may indicate that the liver had difficulty synthesizing normal amounts of this protein following injury induced by CCl<sub>4</sub>. The ethanolic extract of *D. tripetala* showed potential in restoring the concentrations of serum proteins to normal. It is unsurprising that the extract reduced the concentrations of total proteins since the extract reduced the amount of enzymes that leaked out of the liver in the first place. In the case of albumin, the extract may have restored (to some extent) the synthetic capacity of the liver, leading to concentrations similar to those of the control group. In general, the *D. tripetala* extracts altered the albumin:globulin ratio towards that recorded in the control group.



**Fig. 3.** Photomicrographs of kidney sections from (A) control rats showing normal histological features. The section indicates a detailed cortical parenchyma and the renal corpuscles appear as dense rounded structures; (B) rats treated with 250 mg/kg bw ETDT and CCl<sub>4</sub>, showing a varying degree of distortion and disruption in microanatomy of the renal cortex (atrophy), when compared to the control although with prominent renal corpuscles; (C) rats treated with 500 mg/kg bw ETDT and CCl<sub>4</sub>, showing some degree of recovery in microanatomy of the renal cortex, including queried edema, when compared to the control with some atrophied renal corpuscles; (D) rats treated with 1,000 mg/kg bw ETDT and CCl<sub>4</sub>, showing some degree of distortion in microanatomy of the renal cortex, including queried edema, when compared to the control; (E) rats treated with CCl<sub>4</sub> only, showing severe distortion and disruption in microanatomy of the renal cortex, including queried edema. The kidney sections were stained with hematoxylin and eosin and observed with a 10× objective. ETDT, *D. tripetala* ethanolic extract.



**Fig. 4.** Photomicrographs of kidney sections from (A) control rats showing normal histological features. The section indicates a detailed cortical parenchyma and the renal corpuscles appear as dense rounded structures; (B) rats treated with 250 mg/kg bw ETDT and CCl<sub>4</sub> showing some degree of distortion and disruption in microanatomy of the renal cortex (atrophy), when compared to the control, although with prominent renal corpuscles; (C) rats treated with 500 mg/kg bw ETDT and CCl<sub>4</sub> showing some varying degree of recovery in microanatomy of the renal cortex, including queried edema, when compared to the control with some atrophied renal corpuscles; (D) rats treated with 1,000 mg/kg bw ETDT and CCl<sub>4</sub> showing some varying degree of distortion in microanatomy of the renal cortex, including queried edema, when compared to the control; (E) rats treated with CCl<sub>4</sub> only showing severe distortion and disruption in microanatomy of the renal cortex, including queried edema. The kidney sections were stained with hematoxylin and eosin and observed with a 40× objective. ETDT, *D. tripetala* ethanolic extract.

**Effect on recruitment of immune cells in the liver**

Histopathology images revealed that CCl<sub>4</sub> caused immune cells to be recruited to the sites of damage in the liver

(Fig. 1 and Fig. 2). This is unsurprising since CCl<sub>4</sub> is a foreign substance and is capable of evoking an immune response *in vivo* by inducing Kupffer cells to release cyto-

kines such as tumor necrosis factor- $\alpha$ , transforming growth factor- $\beta$ , nitric oxide, and various interleukins (Weber et al., 2003). *D. tripetala* extract did not noticeably alter infiltration of immune cells in the liver. Therefore, under the conditions of this experiment, *D. tripetala* extract did not majorly influence the ability of the local immune system of the liver to deal with the toxicant CCl<sub>4</sub>.

#### Effect on antioxidant enzyme activities in livers and kidneys

Metabolism of CCl<sub>4</sub> caused oxidative stress in the liver and kidneys by decreasing SOD and catalase activities and increasing the concentration of the lipid peroxidation product malondialdehyde. CCl<sub>4</sub> radical formed from the breakdown of CCl<sub>4</sub> can form DNA adducts as well as protein adducts, which may lead to impairment of the function of certain genes and proteins (Weber et al., 2003). In this study, it is possible that the metabolites of CCl<sub>4</sub> reduced antioxidant enzymes by randomly inhibiting their formation and function at both the genetic and protein levels. Our results show that in the context of this experiment, *D. tripetala* used an antioxidant mechanism to alleviate damage to organs caused by CCl<sub>4</sub>.

#### Effect on serum urea and creatinine concentrations

Urea and creatinine are waste products of metabolism that are normally filtered by the kidneys into the urine. However, alterations to the structure of the kidneys may affect its ability to efficiently filter these waste products, leading to their elevation in the blood (Ravnskov, 2005). In this study, CCl<sub>4</sub> increased the concentrations of urea and creatinine in serum, while *D. tripetala* extract restored the concentrations to normal. These results in combination with the histopathology images (Fig. 3 and Fig. 4) suggest that *D. tripetala* extracts can largely restore the architecture of the kidneys following treatment with CCl<sub>4</sub>.

In conclusion, our study provides evidence that the ethanolic extract of *D. tripetala* fruits alleviate damage to the organs of rats caused by oxidative stress resulting from repeated exposures to CCl<sub>4</sub>. Going forward, it may be possible to extract or semi-synthesize drugs for the treatment of liver and kidney diseases from *D. tripetala*.

#### AUTHOR DISCLOSURE STATEMENT

The authors declare no conflict of interest.

#### AUTHOR CONTRIBUTIONS

SOO and NEJO designed the study. SOO and KO carried out the experiments. SOO analyzed the data and wrote the manuscript. All authors approved the final copy of the

manuscript.

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