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Dennettia tripetala Relieves Chronic Hepatorenal Injuries in Rats by Altering *fas*, *sod-1*, and *tnf-α* Expression

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ABSTRACT: The effectiveness of *Dennettia tripetala* extracts was compared to that of the standard drug, silymarin, in reducing chronic liver and kidney anomalies. Male albino Wistar rats were grouped in tens. Carbon tetrachloride was dissolved in olive oil (1:4) and administered to specific groups at a dose of 3 mL/kg body weight (bw) twice a week for six weeks. From week five, the extracts and silymarin were administered in distilled water daily for two weeks at doses of 250 mg/kg bw and 6 mg/kg bw, respectively, to specific groups. All administrations were carried out using a gavage, with appropriate controls. These results showed that the plant extracts decreased the serum activity of liver marker enzymes, restored the liver and serum lipid profiles as well as serum protein profile, reduced serum, urea, and creatinine, and restored superoxide dismutase and catalase activities in the liver and kidneys, which carbon tetrachloride had altered. The extracts also decreased steatosis and centriole congestion in the liver as well as necrosis and structural damage in the kidneys, which carbon tetrachloride caused, and the extracts proved to be as potent as silymarin. The extracts also decreased the expression of *fas* ($P<0.05$), *sod-1* ($P<0.05$), and *tnf-α* ($P>0.05$) in the liver, which carbon tetrachloride had increased. Conclusively, *D. tripetala* reduced chronic liver and kidney damage induced by carbon tetrachloride; it reduced the expression of *fas*, *sod-1*, and *tnf-α* in the liver to levels similar to that of the control group, and it was as effective as silymarin.

Keywords: antioxidant, *Dennettia tripetala*, dose-response, hormesis, liver disease

INTRODUCTION

The liver and kidneys are responsible for many important metabolic processes that support life. One of such vital function of the liver is drug detoxification (Al-Yahya et al., 2013). Interestingly, the detoxification of toxic substances can cause the liver to be susceptible to injury. Cytochrome P-450 (CYP450) enzymes are also present in the kidneys, and they help to further break down toxic substances, thereby predisposing the kidneys to injury (Al-Yahya et al., 2013). Diseases of the liver and kidneys can be life-threatening, and the options for managing these diseases are currently very expensive. There is, therefore, a need to find medicines that effectively heal these organs and are highly affordable.

Several researchers are now turning to plants, in the search for active medicinal substances. This is because plants contain powerful chemicals that elicit medicinal actions in humans and animals. Also, numerous drugs currently used for treating patients have their starting compounds from medicinal plants. Silymarin, for example, is a standard drug for managing liver injury, and it is an extract of the milk thistle plant, also known as *Silybum marianum* (Karimi et al., 2011).

Dennettia tripetala is a spicy plant native to West Africa. It is also known as "Mmimi", "Ata Igbere", and "Ako" in Igbo, Yoruba, and Edo languages, respectively. It is consumed as a fruit as well as for medicinal purposes, including the management of cough, diabetes, fever, diarrhea, sore throat, and nausea (Iseghohi, 2015). It possesses analgesic and anti-inflammatory properties (Oyemitan et al., 2008) as well as anti-diabetic properties (Anaga and Asuzu, 2010).

Carbon tetrachloride was previously used as a refrigerant, an industrial solvent, and in fire extinguishers until it was found to be hepatotoxic and carcinogenic. The mech-

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anisms by which it exerts its toxicity are well-known. Cytochrome P450 enzymes in the liver and kidneys break down carbon tetrachloride into its free radical metabolites: trichloromethyl and trichloromethyl peroxy radicals, causing adduct formation and lipid peroxidation, respectively, to macromolecular constituents of the cell (Weber et al., 2003). This causes injury to the cells of the liver and kidney. The injury is usually in the form of lipid accumulation in hepatocytes, loss of calcium sequestration, oxidative stress, scar tissue formation (in hepatocytes), increased membrane permeability, and necrosis, among others (Weber et al., 2003).

A previous finding by a Spanish group (López-Martín et al., 2002) noting that the essential oil of *D. tripetala* contains uvariopsine (an alkaloid that aids bile secretion and improves liver health), prompted the evaluation of the potential of the extracts of this plant to ease hepatorenal injuries *in vivo*. In this study, carbon tetrachloride was used to cause chronic liver and kidney injury in rats, and the aqueous and ethanolic extracts of *D. tripetala* were tested for their potency in alleviating such chronic damage. The effectiveness of the plant extracts was compared to a standard drug for managing liver damage (silymarin) and observed differences in the selected genes' expression in the liver to identify some mechanisms by which the plant extracts work.

MATERIALS AND METHODS

Plant extracts

Ripe *D. tripetala* fruits were harvested on a farm in the Ikpoba-okha area of Benin City, Nigeria. The plant name was checked with http://www.theplantlist.org. A Botanist identified the fruits in the Department of Plant Biology and Biotechnology, University of Benin, Nigeria, and a voucher specimen number (UBH_D360) was allocated. The fruits were diced, sun dried, and pulverized to a fine powdery form. For the aqueous extract, four liters of distilled water was used to soak 500 g of the powder, while for the ethanolic extract, four liters of ethanol was used to soak 500 g of the powder. The soaking lasted for two days with regular stirring, followed by sieving using a clean cloth and concentration using a freeze dryer. Finally, the freeze-dried extracts were reconstituted in distilled water for administration to animals.

Table 1. Groups of animals and treatments administered

Group	Treatment
1	Control
2	CCl_4
3	CCl_4 + AQDT
4	CCl_4 + ETDT
5	CCl_4 + silymarin
6	AQDT
7	ETDT
8	Silymarin

AQDT, aqueous extract of *Dennettia tripetala*; ETDT, ethanolic extract of *D. tripetala*.

Experimental design

Eighty healthy male albino rats of Wistar strain were purchased from the animal house of the Department of Anatomy, University of Benin. The animals were acclimatized for two weeks and then distributed randomly into eight groups of ten rats each. Table 1 shows the groups and what they were administered. The experimental substances were orally administered using a gavage. CCl_4 was dissolved in olive oil in a ratio of 1:4 and administered at a dose of 3 mL/kg body weight (bw). The plant extracts were administered at 250 mg/kg bw dose, and silymarin was dissolved in distilled water and administered at a dose of 6 mg/kg bw. The administration period of each experimental substance is described in Fig. 1. The safety and comfort of the animals were taken into special consideration throughout the experiment, and the Animal Ethics Committee of the Faculty of Life Sciences, University of Benin approved all experimental protocols (approval number: LS16106). The experiments were conducted following the NIH guidelines for the care and use of laboratory animals (NIH Publications No. 8023, revised 1978).

CCl_4 was administered twice a week for six weeks. From week five until week six, *D. tripetala* was administered daily to the respective groups. Silymarin was also administered to the respective groups of rats daily from week five until week six. The rats in the control group were given feed and water only. After an overnight fast, the experiment was terminated by sacrificing the animals using chloroform anesthesia. The blood, liver, and kidney were collected for biochemical, molecular, and histopathological analyses. The blood was centrifuged at 3,500 rpm for ten minutes after clotting, and serum was retrieved and stored at 4°C. Weighed portions of the liver and kidney were also homogenized in normal saline and centrifuged at 4,000 rpm for 15 min, and the supernatant was stored at −20°C.

Biochemical assays

All reagent kits used in this study were purchased from

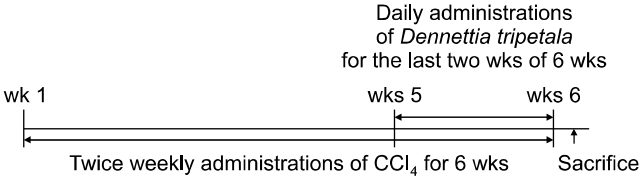

Fig. 1. Duration of administration of CCl_4 and *Dennettia tripetala*.

Randox Laboratories Ltd. (Crumlin, UK). The manufacturer's protocols were strictly followed. For malodialdehyde, superoxide dismutase (SOD), and catalase assays, the methods used are those of Buege and Aust (1978), Misra and Fridovich (1972), and Góth (1991), in the respective order. The chemicals used for these three assays were purchased from Pyrex chemicals, Benin City, Nigeria. They were pure and of analytical grade. The sera were assayed for the activities of aspartate transaminase (AST), alanine transaminase (ALT), and alkaline phosphatase (ALP), as well as the concentrations of total cholesterol, triglyceride, total protein, and albumin. The liver and kidney homogenates were assayed for the activities of the antioxidant enzymes SOD and catalase as well as the lipid peroxidation product, malondialdehyde.

Molecular assays

A weighed portion of liver was cut from rats in each group and stored in an Invitrogen RNA stabilization solution before RNA extraction. RNA was extracted from the liver samples using ISOLATE II RNA mini kit (Meridian Bioscience, Memphis, TN, USA), and the manufacturer's protocols were adhered to strictly. A Nanodrop (NanoDrop ND-1000, Thermo Fisher Scientific, Waltham, MA, USA) was used to measure the purity and quantity of the isolated RNA. The integrity of the isolated RNA was also confirmed using agarose gel electrophoresis. The isolated RNA was converted to complementary DNA (cDNA) using SensiFAST cDNA synthesis kit (Bioline, London, UK), and the manufacturer's protocols were adhered to strictly. Real time-polymerase chain reaction (PCR) was conducted on the cDNA obtained to observe the differences in the expression of some selected genes (*fas*, *sod-1*, and *tnf-α*; *β-actin* was used as an internal PCR control). The primers were synthesized by Inqaba Biotechnical Industries (Pty) Ltd. (Pretoria, South Africa), and their sequences are shown in Table 2.

Table 2. Primers used for polymerase chain reaction

Gene	Primer (5′→3′)
β-actin	
Forward	CGTGACATCAAAGAGAAGCTGTGC
Reverse	GCTCAGGAGGAGCAATGATCTTGAT
fas	
Forward	CTCCAGACATTGTTC
Reverse	CGCCTATGGTTGTTGACC
sod-1	
Forward	GCAGGACCTCATTTTAATCCTCACT
Reverse	AGGTCTCCAACATGCCTCTCTTC
tnf-α	
Forward	ACAGAAAGCATGATCCGC
Reverse	CTCGGACCCCTGGACGTA

The thermocycler used was a Labnet MultiGene mini thermocycler (Sigma-Aldrich Co., St. Louis, MO, USA), and a UV transilluminator from Biologix (Camarillo, CA, USA) was also used. Images were captured using an Accuris Smart Doc imaging system and camera. Image J (U.S. National Institutes of Health, Bethesda, MD, USA) was used to quantify the light intensity from the bands, and GraphPad Prism version 7.0 (GraphPad Software, San Diego, CA, USA) was used to analyze the data.

Histopathology

Portions of the liver and kidney were fixed in 10% neutral buffered formalin for histopathological analysis. A Leica TP2010 automatic tissue processor (Leica Biosystems, Wetzlar, Germany) was used to process the tissues stained with hematoxylin and eosin and viewed under a light microscope using 40× magnification.

Statistical analysis

GraphPad Prism version 7.0 (GraphPad Software, San Diego, CA, USA) was used to analyze the data. The data were subjected to a one-way ANOVA, and the results were presented as mean±standard error of mean.

RESULTS

The results show that chronic exposure to carbon tetra-

Table 3. Effect of *Dennettia tripetala* extracts on liver marker enzymes in the serum of rats chronically exposed to carbon tetrachloride (unit: U/L)

Group	Aspartate transaminase	Alanine transaminase	Alkaline phosphatase
Control	236.42±6.21[a]	59.61±4.71[a]	37.60±2.71[a]
CCl_4	812.73±4.17[d]	462.90±14.22[d]	48.59±2.03[b]
AQDT 250 mg/kg bw + CCl_4	520.62±12.23[c]	245.80±8.62[c]	46.24±5.12[b]
ETDT 250 mg/kg bw + CCl_4	546.50±18.64[c]	257.30±7.73[c]	48.61±1.62[b]
Silymarin + CCl_4	340.40±16.91[b]	101.50±16.12[b]	39.12±4.59[a]
AQDT 250 mg/kg bw	236.60±2.38[a]	62.23±15.19[a]	36.12±2.88[a]
ETDT 250 mg/kg bw	237.14±1.12[a]	65.72±13.12[a]	36.44±7.45[a]
Silymarin	242.71±3.27[a]	61.60±6.74[a]	37.84±4.50[a]

The values are presented as mean±SEM (n=10).
Different letters (a-d) in the same column are significantly different at $P<0.05$.
AQDT, aqueous extract of *D. tripetala*; ETDT, ethanolic extract of *D. tripetala*; bw, body weight.

chloride, increased the serum activities of AST, ALT, and ALP, and both plant extracts dampened this elevation significantly, but they were not as potent as the standard drug, silymarin (Table 3). Chronic exposure to carbon tetrachloride also increased the serum concentration of cholesterol and decreased the serum triglyceride concentration. Both plant extracts significantly dampened these distortions just as effectively as silymarin (Table 4). Chronic exposure to carbon tetrachloride increased liver cholesterol and triglyceride concentrations, and both plant extracts significantly dampened the increases but not as effectively as silymarin (Table 5).

Table 6 shows that chronic exposure to carbon tetrachloride significantly increased the total protein concentration and globulin concentration in the serum with a concomitant decrease in serum albumin concentration.

Table 4. Effect of *Dennettia tripetala* extracts on serum lipid profile of rats chronically exposed to carbon tetrachloride (unit: mg/dL)

Group	Total cholesterol	Triglyceride
Control	39.41±1.12^{a}	129.51±3.64^{c}
CCl_4	50.24±3.59^{c}	85.96±2.81^{a}
AQDT 250 mg/kg bw + CCl_4	44.52±1.15^{b}	117.42±3.72^{b}
ETDT 250 mg/kg bw + CCl_4	43.12±1.68^{b}	119.38±1.19^{b}
Silymarin + CCl_4	43.68±1.24^{b}	116.54±4.43^{b}
AQDT 250 mg/kg bw	39.82±2.76^{a}	130.12±3.91^{c}
ETDT 250 mg/kg bw	40.24±0.32^{a}	127.64±2.24^{c}
Silymarin	40.10±2.54^{a}	128.67±2.42^{c}

The values are presented as mean±SEM (n=10).
Different letters (a-c) in the same column are significantly different at $P<0.05$.
AQDT, aqueous extract of *D. tripetala*; ETDT, ethanolic extract of *D. tripetala*; bw, body weight.

Table 5. Effect of *Dennettia tripetala* extracts on liver lipid profile of rats chronically exposed to carbon tetrachloride (unit: mg/dL)

Group	Total cholesterol	Triglyceride
Control	45.64±1.64^{a}	15.45±0.54^{a}
CCl_4	56.28±0.38^{c}	31.64±2.13^{c}
AQDT 250 mg/kg bw + CCl_4	49.83±1.91^{b}	21.20±3.18^{b}
ETDT 250 mg/kg bw + CCl_4	50.21±0.69^{b}	20.98±0.69^{b}
Silymarin + CCl_4	47.22±0.24^{a}	18.69±5.02^{a}
AQDT 250 mg/kg bw	44.23±3.62^{a}	14.78±1.71^{a}
ETDT 250 mg/kg bw	44.98±1.02^{a}	16.21±1.24^{a}
Silymarin	46.12±2.12^{a}	15.21±1.13^{a}

The values are presented as mean±SEM (n=10).
Different letters (a-c) in the same column are significantly different at $P<0.05$.
AQDT, aqueous extract of *D. tripetala*; ETDT, ethanolic extract of *D. tripetala*; bw, body weight.

Table 6. Effect of *Dennettia tripetala* extracts on serum protein profile of rats exposed chronically to carbon tetrachloride (unit: g/dL)

Group	Total protein	Albumin	Globulin
Control	7.92±0.43^{a}	3.72±0.03^{c}	4.42±0.19^{a}
CCl_4	23.45±0.91^{c}	2.10±0.09^{a}	20.61±0.24^{c}
AQDT 250 mg/kg bw + CCl_4	12.75±3.02^{b}	3.10±0.08^{b}	8.71±0.49^{b}
ETDT 250 mg/kg bw + CCl_4	14.12±1.61^{b}	3.23±0.13^{b}	10.92±0.61^{b}
Silymarin + CCl_4	10.64±0.74^{b}	3.24±0.04^{b}	7.42±0.13^{b}
AQDT 250 mg/kg bw	8.69±0.84^{a}	3.79±0.14^{c}	4.53±0.30^{a}
ETDT 250 mg/kg bw	7.93±0.50^{a}	3.82±0.06^{c}	4.22±0.12^{a}
Silymarin	8.32±0.33^{a}	3.61±0.02^{c}	4.41±0.61^{a}

The values are presented as mean±SEM (n=10).
Different letters (a-c) in the same column are significantly different at $P<0.05$.
AQDT, aqueous extract of *D. tripetala*; ETDT, ethanolic extract of *D. tripetala*; bw, body weight.

Table 7. Effect of *Dennettia tripetala* extracts on antioxidant enzyme activity, and lipid peroxidation status in liver of rats exposed chronically to carbon tetrachloride (unit: unit/g wet tissue)

Group	Superoxide dismutase	Catalase	Malodialdehyde
Control	1,540.1±5.2^{a}	4,120.1±34.2^{a}	0.09±0.01^{ns}
CCl_4	1,991.2±17.5^{b}	4,549.0±16.2^{b}	0.11±0.02
AQDT 250 mg/kg bw + CCl_4	1,521.1±13.9^{a}	4,144.4±8.9^{a}	0.10±0.01
ETDT 250 mg/kg bw + CCl_4	1,570.3±6.2^{a}	4,178.2±10.4^{a}	0.10±0.02
Silymarin + CCl_4	1,566.3±8.9^{a}	4,171.3±7.7^{a}	0.09±0.01
AQDT 250 mg/kg bw	1,573.2±12.2^{a}	4,186.1±15.7^{a}	0.09±0.02
ETDT 250 mg/kg bw	1,776.4±22.5^{ab}	4,205.0±67.2^{a}	0.08±0.01
Silymarin	1,599.2±5.4^{a}	4,197.3±9.9^{a}	0.08±0.01

The values are presented as mean±SEM (n=10).
Different letters (a,b) in the same column are significantly different at $P<0.05$.
^{ns}Not significant.
AQDT, aqueous extract of *D. tripetala*; ETDT, ethanolic extract of *D. tripetala*; bw, body weight.

Both plant extracts significantly dampened these distortions just as effectively as silymarin. Table 7 shows that chronic exposure to carbon tetrachloride increased the activities of SOD and catalase in the liver. The increase in the lipid peroxidation marker (malondialdehyde) was not significant under the conditions of this experiment. The plant extracts worked as effectively as silymarin to bring the activities of the antioxidant enzymes and the malondialdehyde concentration in the liver to normal levels. Table 8 shows that chronic exposure to carbon tetrachloride increased serum urea and creatinine concentrations. Both plant extracts restored these metabolites to normal levels. Table 9 shows that chronic exposure to carbon tetrachloride increased the activities of SOD and catalase in the kidney. The increase in the lipid peroxidation marker (malondialdehyde) was not significant under the conditions of this experiment. The plant extracts significantly dampened the changes in the activities of the antioxidant enzymes and the malondialdehyde concentration in the kidney.

Fig. 2 shows that chronic exposure to carbon tetrachloride severely distorted the liver architecture, including but not limited to: fatty accumulation, severe congestion of the centrioles, dilated sinusoidal spaces, and inflammatory changes. Both extracts reduced the severity of the distortions to similar extents as silymarin could. Fig. 3 shows that chronic exposure to CCl_4 distorted the kidney architecture, including severe disruption in the microanatomy of the renal cortex and edema. Both extracts reduced the severity of the distortions to similar extents as silymarin did.

Fig. 4 shows that chronic exposure to CCl_4 significantly increased the expression of *fas* in the liver. Both extracts of *D. tripetala* significantly reduced *fas* expression to levels similar to control. The aqueous extract was slightly more potent than silymarin, while the ethanolic extract was just as effective as silymarin. The administration of either extract alone did not significantly change *fas* expression compared to the control group.

Fig. 5 shows that a six-week CCl_4 administration significantly increased the expression of *sod-1* in the liver of rats. The administration of both extracts of *D. tripetala* for two weeks significantly reduced *sod-1* expression to levels not significantly different from the control group. The aqueous extract was slightly more potent than silymarin, while the ethanolic extract was as potent as silymarin. Administration of the aqueous extract alone did not significantly alter *sod-1* expression compared to the control group. Conversely, the ethanolic extract seemed to increase the level of *sod-1* slightly compared to the control group, although this was not statistically significant.

Fig. 6 shows that a six-week carbon tetrachloride administration slightly increased *tnf-α* expression, although this was not statistically significant. A two-week administration of *D. tripetala* aqueous extract tended to return the level to normal, just as silymarin did, while the ethanolic extract did not seem to offer any remedy. In all cases however, the observed differences were not statistically significant.

Table 8. Effect of *Dennettia tripetala* extracts on urea and creatinine concentration in the serum of rats exposed chronically to carbon tetrachloride (unit: mg/dL)

Group	Urea	Creatinine
Control	7.74±0.50[a]	1.32±0.12[a]
CCl_4	12.69±0.46[b]	3.19±0.45[b]
AQDT 250 mg/kg bw + CCl_4	8.26±0.62[a]	1.92±0.62[a]
ETDT 250 mg/kg bw + CCl_4	7.97±0.41[a]	1.68±0.35[a]
Silymarin + CCl_4	8.13±0.57[a]	1.40±0.06[a]
AQDT 250 mg/kg bw	7.85±0.19[a]	1.41±0.10[a]
ETDT 250 mg/kg bw	7.20±0.72[a]	1.39±0.08[a]
Silymarin	7.82±0.21[a]	1.34±0.04[a]

The values are presented as mean±SEM (n=10).
Different letters (a,b) in the same column are significantly different at $P<0.05$.
AQDT, aqueous extract of *D. tripetala*; ETDT, ethanolic extract of *D. tripetala*; bw, body weight.

Table 9. Effect of *Dennettia tripetala* extracts on antioxidant enzyme activity, and lipid peroxidation status in the kidney of rats exposed chronically to carbon tetrachloride

Group	Superoxide dismutase [units/g wet tissue ($\times10^{-2}$)]	Catalase (unit/g wet tissue)	Malodialdehyde (unit/g wet tissue)
Control	552.1±15.2[a]	4,118.1±9.2[a]	0.24±0.02[ns]
CCl_4	974.0±12.6[c]	5,023.4±14.3[c]	0.26±0.01
AQDT 250 mg/kg bw + CCl_4	865.4±6.7[b]	4,465.2±1.4[b]	0.25±0.01
ETDT 250 mg/kg bw + CCl_4	834.2±8.7[b]	4,421.1±12.6[b]	0.25±0.02
Silymarin + CCl_4	582.0±25.3[a]	4,294.0±22.7[a]	0.24±0.01
AQDT 250 mg/kg bw	563.4±4.5[a]	4,154.0±4.3[a]	0.24±0.02
ETDT 250 mg/kg bw	570.3±3.8[a]	4,139.2±5.1[a]	0.23±0.01
Silymarin	581.0±12.2[a]	4,172.3±13.2[a]	0.23±0.01

The values are presented as mean±SEM (n=10).
Different letters (a-c) in the same column are significantly different at $P<0.05$.
[ns]Not significant.
AQDT, aqueous extract of *D. tripetala*; ETDT, ethanolic extract of *D. tripetala*; bw, body weight.

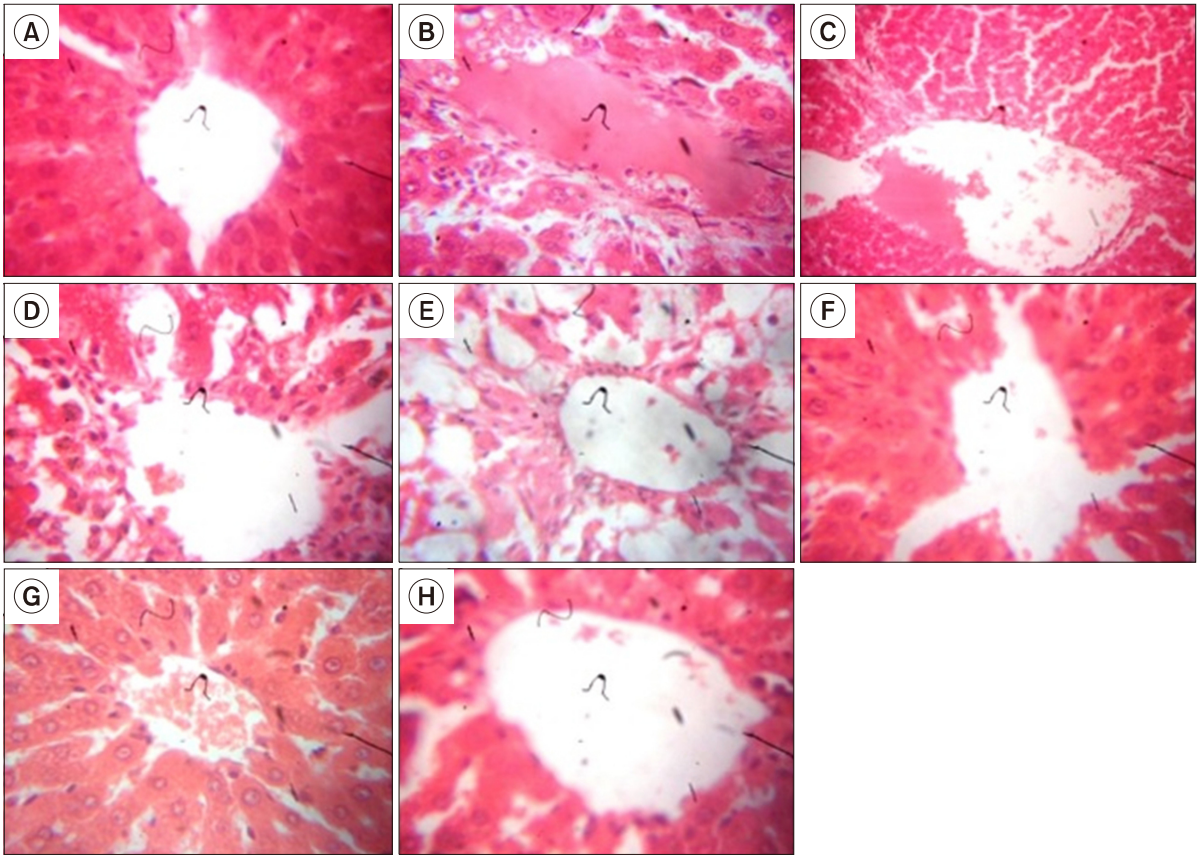

Fig. 2. Effect of *Dennettia tripetala* extracts on liver morphology. (A) Liver section from control rat. (B) Liver section from rat treated with CCl_4 showing highly congested centriole, fatty accumulation, hydropic changes, dilated sinusoidal spaces, focal inflammatory changes. (C) Liver section from rats treated with 250 mg/kg body weight (bw) AQDT and CCl_4 showing remarkable recovery from steatosis and centriole congestion with scanty inflammatory cells surrounding the centriole and mild sinusoidal space dilation. (D) Liver section from rats treated with 250 mg/kg bw ETDT and CCl_4, showing remarkable recovery from centriole congestion although with fatty accumulation. (E) Liver section from rats treated with silymarin and CCl_4, showing remarkable recovery from centriole congestion with fatty accumulation, dilated sinusoidal spaces and inflammatory changes. (F) Liver section from rats treated with AQDT, showing normal liver histology. (G) Liver section from rats treated with ETDT, showing congested centriole but otherwise normal liver histology. (H) Liver section from rats treated with silymarin, showing normal liver histology. The liver sections were stained with H&E and observed with a 40× magnification. AQDT, aqueous extract of *D. tripetala*; ETDT, ethanolic extract of *D. tripetala*.

DISCUSSION

Carbon tetrachloride, a well-known hepatorenal toxicant, was used to induce experimental damage to the liver and kidneys of rats. Its metabolism leads to the formation of trichloromethyl radicals that reacts with oxygen to form trichloromethyl peroxy radicals that cause peroxidation of the lipid components of membranes, thus increasing the permeability of the cell (Weber et al., 2003). Therefore, it becomes easy for enzymes to leak out of their cells, thus resulting in an increased serum activity of liver marker enzymes, such as ALT and AST during CCl_4 poisoning. The mechanism by which the extracts of *D. tripetala* caused this relief, may revolve around stabilizing the cell membranes, preventing CCl_4 metabolism into radicals by inhibiting the CYP450 activity, or preventing peroxidation of the lipids of the membranes by itself acting as an antioxidant. The first hypothesis is highly possible as *D. tripetala* contains tannins (Iseghohi, 2015; Omage et al., 2018) that can "coat" and protect membranes, increasing the resistance to stress and limiting its labilization (de Jesus et al., 2012). The second hypothesis is also possible, as numerous plants inhibit CYP450 (Ashour et al., 2017), and the last hypothesis is also very likely as *D. tripetala* has several antioxidant components and elicits *in vitro* antioxidant properties (Iseghohi, 2015; Omage et al., 2018).

Carbon tetrachloride also alters the metabolism of lipids (Weber et al., 2003), and in this study, it caused an accumulation of cholesterol and triglycerides in the liver. It also elevated serum cholesterol but a drop in serum triglyceride. A previous work has revealed some mechanisms by which the concentration of lipids in the liver and serum may be distorted; these mechanisms include the increased synthesis of lipids in the liver which leads to the accumulation of these lipids (Boll et al., 2001), inhibition of the β-oxidation of fatty acids in the liver which leads to the accumulation of triglycerides (Fromenty and

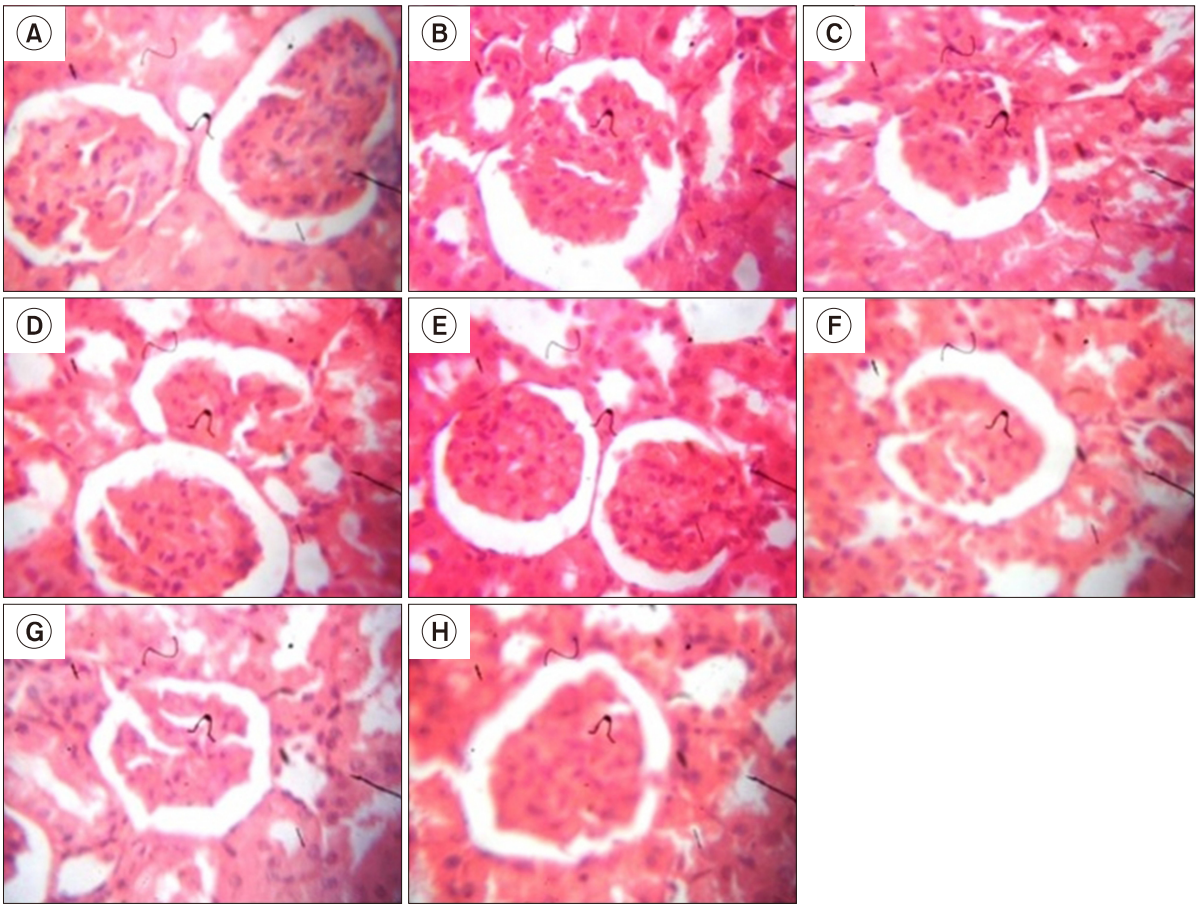

Fig. 3. Effect of *Dennettia tripetala* extracts on kidney morphology. (A) Kidney section from control rat. (B) Kidney section from rat treated with CCl_4, severe distortion and disruption in microanatomy of the renal cortex, including queried edema, when compared to the control. (C) Kidney section from rats treated with 250 mg/kg body weight (bw) AQDT and CCl_4, showing some varying degree of distortion and disruption in microanatomy of the renal cortex. (D) Kidney section from rats treated with 250 mg/kg bw ETDT and CCl_4, showing some varying degree of distortion and disruption in microanatomy of the renal cortex. (E) Kidney section from rats treated with silymarin and CCl_4, showing some varying degree of distortion and disruption in microanatomy of the renal cortex. (F) Kidney section from rats treated with AQDT, showing normal kidney histology. (G) Kidney section from rats treated with ETDT, showing normal kidney histology. (H) Kidney section from rats treated with silymarin, showing normal kidney histology. The kidney sections were stained with H/E and observed with a 40× magnification. AQDT, aqueous extract of *D. tripetala*; ETDT, ethanolic extract of *D. tripetala*.

Pessayre, 1995), reduction of the liver's ability to secrete lipids which leads to the accumulation of these lipids (Fromenty and Pessayre, 1995), inhibition of the activity of lysosomal acid triglyceride lipase which causes the accumulation of triglycerides in the liver (Kato and Nakazawa, 1987; Khalaf et al., 2009), reduction in the ability of the liver to remove cholesterol from the blood which causes cholesterol to accumulate in the blood (Owen, 1990), and reduction in the activity of peripheral lipase which causes triglycerides to accumulate in the blood (Jahn et al., 1985).

In this study, some mechanisms stated above may have worked singly or in concert to distort lipid concentration in the liver and serum, and in the case of serum triglyceride, the concentration may have reduced because the liver may have had difficulty secreting triglycerides into the blood. This hypothesis is supported by the histological images (Fig. 2), which showed lipid accumulation in the hepatocytes, and the results of biochemical assays (Table 5), which showed triglyceride accumulation in the liver. The mechanisms by which *D. tripetala* alleviated the lipid dyshomeostasis induced by carbon tetrachloride probably revolved around preventing more sporadic damage caused by CCl_4 to the types of machinery involved in maintaining lipid homeostasis. More specifically the plant may have prevented the formation of more adducts formed from the trichloromethyl radical breakdown product of CCl_4.

In this study, carbon tetrachloride also increased the serum total protein and globulin concentration. The proteins that leaked out of the liver likely contributed to the increased protein concentrations measured in the serum. It is also important to note that most of the proteins measured in the serum were globulins since the globulin level increased and the albumin level reduced drastically. Albumin is usually used to study the synthetic capacity of the liver since it is the most abundant protein synthesized by the liver. Therefore, carbon tetrachloride must

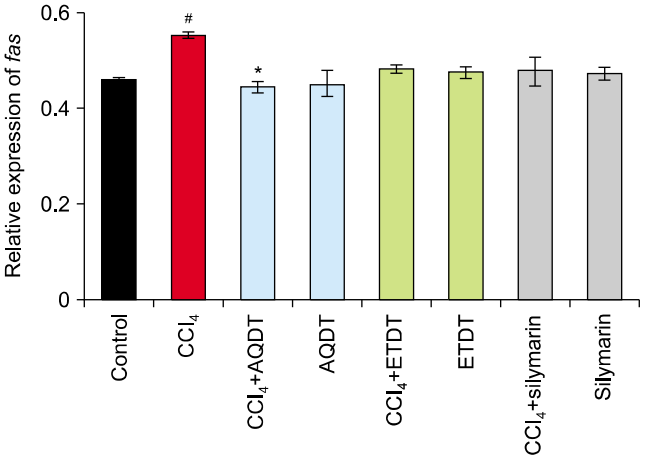

Fig. 4. Effect of *Dennettia tripetala* extracts on *fas* RNA expression in the liver of rats exposed chronically to CCl_4. Significantly different from the control group at $^{\#}P<0.05$ and the CCl_4 group at $^{*}P<0.01$. The results depicted were normalized to β-actin. AQDT, aqueous extract of *D. tripetala*; ETDT, ethanolic extract of *D. tripetala*.

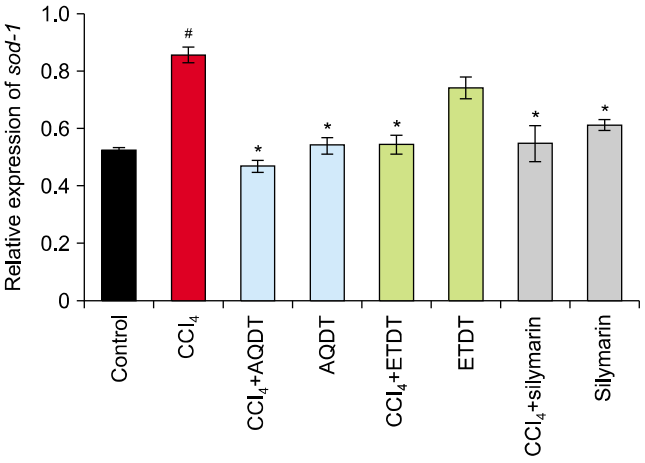

Fig. 5. Effect of *Dennettia tripetala* extracts on *sod-1* RNA expression in the liver of rats exposed chronically to CCl_4. Significantly different from the control group at $^{\#}P<0.0001$ and the CCl_4 group at $^{*}P<0.01$. The results depicted were normalized to β-actin. AQDT, aqueous extract of *D. tripetala*; ETDT, ethanolic extract of *D. tripetala*.

have affected the liver so badly that the capacity for albumin synthesis dropped drastically in this study. The extracts of *D. tripetala* may have worked by preventing the destruction carbon tetrachloride caused to the liver, including necrosis, and this hypothesis is backed up by the gene expression results (Fig. 4), which showed that *D. tripetala* extracts reduced the expression of the pro-apoptotic gene *fas*, in the liver.

In this study, carbon tetrachloride caused the activity of the antioxidant enzymes: SOD and catalase to increase in the liver. This means that prolonged exposure to carbon tetrachloride may have caused a form of "adaptation" in which the expression of antioxidant enzymes increased to combat the free radical damage that was caused by CCl_4. This result is supported by the gene expression results (Fig. 5) in which CCl_4 increased the expression of *sod-1* in the liver. *D. tripetala* intervention helped to restore the activity of SOD and catalase in the liver and kidneys as well as the expression of *sod-1* in the liver to normal. In our opinion, the response of rat organs to carbon tetrachloride likely varies with the duration of exposure. Results from other labs and previous experiments in our lab, showed that fewer exposures to CCl_4 reduces antioxidant enzyme activity (Zhang et al., 2013; Hafez et al., 2014; Adewale and Orhue, 2015; Iseghohi et al., 2017; Iseghohi and Orhue, 2017; Omage et al., 2021) but in this study, a longer exposure caused the opposite phenomenon. This could mean that a few encounters with carbon tetrachloride may at the onset start to overpower the antioxidant systems that ensure homeostasis (leading to a reduced antioxidant enzyme activity), but if the exposure continues for a longer period, it may start to sensitize the organ to subsequent carbon tetrachloride exposure (a form of adaptative response, increasing the antioxidant enzyme expression and activity). A time eventually arises after a very prolonged exposure to carbon tetrachloride, when the system eventually becomes overwhelmed once more, reducing the antioxidant enzyme activity, with a resulting sinusoidal dose-response curve. Therefore, termination of an experiment before the onset of adaptation is likely to lower the antioxidant enzyme activity. Suppose an experiment is allowed to continue till after adaptation has kicked in, then there is the possibility of observing an increase in antioxidant enzyme activity, and if the experiment is prolonged for several months, a reduction in antioxidant enzymes will not be out of place. Interestingly, our observation from this study, was similar to that of other researchers such as Ozturk et al. (2003) and Uzma et al. (2011), who have observed significant increases in antioxidant enzyme expression and activity following exposure to an external

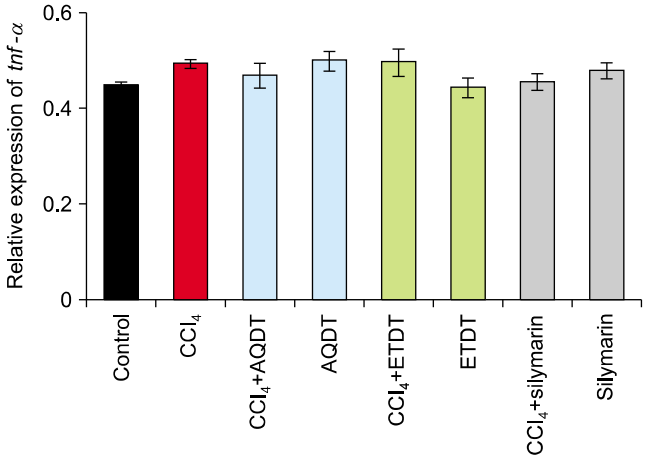

Fig. 6. Effect of *Dennettia tripetala* extracts on *tnf-α* RNA expression in the liver of rats exposed chronically to CCl_4. The results depicted were normalized to β-actin. AQDT, aqueous extract of *D. tripetala*; ETDT, ethanolic extract of *D. tripetala*.

source of free radicals.

In this study, CCl_4 significantly increased the concentration of urea and creatinine in the serum. This is not surprising, as CCl_4 altered the normal architecture of the glomeruli of the kidneys which are normally responsible for filtering waste substances from the blood into the urine. This alteration in their structures very likely altered their functional capacity to filter the blood properly, and this resulted in accumulation of waste substances such as urea and creatinine in the blood. *D. tripetala* protected the kidneys from structural and functional damage caused by carbon tetrachloride and hence the waste substance urea and creatinine did not accumulate in the blood of the rats that received the plant intervention.

From the results of this experiment (Fig. 4), one can infer that the induction of apoptosis is a mechanism by which CCl_4 causes liver injury. Zhang et al. (2013) and Hafez et al. (2014) made similar observations. *fas* is a gene that triggers apoptosis in cells. Under conditions of poisoning, such as is the case with CCl_4, the consequences may increase *fas* expression, triggering the cell to die by suicide. It is reasonable to find that *D. tripetala* worked by reducing *fas* expression in the liver, thereby preventing apoptosis, hence buying more time for the hepatocytes to recover rather than dying by suicide. Another piece of evidence backing up the apoptosis we that was concluded on the basis of *fas* expression, is the fact that as CCl_4 was triggering apoptosis, or more specifically "necrosis", these triggered immune cells to migrate to the site of the necrotic hepatocytes. This is why in the histology images (Fig. 2), there were many immune cells surrounding the centrioles in the liver of the rats poisoned with CCl_4.

From the results of this study (Fig. 5), it is obvious that a six-week exposure to CCl_4 increased in the expression of *sod-1* (which encodes the antioxidant enzyme, SOD) in the liver. This means that prolonged CCl_4 administration caused an adaptation, perhaps a form of "hormetic" effect, as explained earlier, making the liver to start synthesizing more antioxidant enzymes to combat the harmful effects of CCl_4. Perhaps a longer exposure to CCl_4 (longer than six weeks) may have ultimately resulted in a situation where the free radicals produced from CCl_4, eventually overpowers the antioxidant defense system of the body, causing more destruction to antioxidant enzymes that have already been produced, and sporadically reducing the expression of more antioxidant genes. In this experiment, the *D. tripetala* extracts restored homeostasis by reducing *sod-1* expression, which CCl_4 had increased. Perhaps, the plant itself acted as a supply of antioxidants, reducing the "pressure" on the liver to cause the expression of more antioxidants as a way of adapting to, and combating the effects of CCl_4.

Finally, under these experimental conditions, CCl_4 barely increased the *tnf-α* expression in the liver (Fig. 6). *tnf-α* is a gene involved in the immune response (Parameswaran and Patial, 2010; Zimmermann et al., 2012). It triggers apoptosis or cell recovery depending on the scenario at that body site (Weber et al., 2003). It is upstream of several genes that cause the recruitment of immune cells to a site of damage. In this experiment, the plant extracts barely returned the *tnf-α* expression to normal. The non-significant results recorded was unexpected since the histology results showed that CCl_4 massively recruited immune cells to the site of damaged necrotic liver tissue; but perhaps under these experimental conditions, CCl_4 worked through other genes either downstream of *tnf-α* or genes that function in pathways that do not directly involve *tnf-α* but are highly involved in the immune response. In subsequent experiments, it will be interesting to determine what other genes of the immune system, CCl_4, and *D. tripetala* extracts, may have been working through.

Conclusively, a six-week CCl_4 administration induced chronic liver and kidney damage at the biochemical and histological level. CCl_4 also increased the expression of the pro-apoptotic gene *fas* ($P<0.05$), the antioxidant gene *sod-1* ($P<0.05$), and a gene involved in the immune response *tnf-α* ($P>0.05$) in the liver. A two-week intervention using extracts of the medicinal plant, *D. tripetala*, reduced liver and kidney damage and restored the expression of these genes to normal. Also, the plant extracts proved to be as potent as the standard drug, silymarin. In the future, perhaps *D. tripetala* may serve as a starting material for the synthesis/semi-synthesis or, at the very least, the extraction of drugs to manage hepatorenal diseases.

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AUTHOR DISCLOSURE STATEMENT

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

NEJO and SOO designed the experiments. SOO and KO carried out the experiments. SOO analyzed the data and

drafted the manuscript. All authors edited and approved the final version of the manuscript.

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