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Aqueous extract of *Dennettia tripetala* ameliorates liver and kidney damage caused by multiple exposures to carbon tetrachloride

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Abstract

Background: *Dennettia tripetala* is a medicinal plant with in vitro antioxidant activities. It is capable of protecting the liver and kidney of rats from damage when administered prior to a single exposure of carbon tetrachloride. The aim of this study was to document the possible ameliorative effect of the aqueous extract of *Dennettia tripetala* fruits on rats subjected to multiple exposures of carbon tetrachloride.

Methods: Carbon tetrachloride was administered orally to male albino rats of Wistar strain four times over a 2 week period. The aqueous extract of *Dennettia tripetala* fruits was administered daily for 7 days starting on the 8th day after carbon tetrachloride administration had commenced.

Results: Carbon tetrachloride caused increases in serum ALT, AST and ALP, serum total cholesterol, serum LDL-cholesterol, liver total cholesterol and triglyceride, serum total protein, globulin, urea, creatinine as well as liver and kidney malondialdehyde levels. Carbon tetrachloride also caused significant reductions in serum HDL-cholesterol, serum triglyceride, serum albumin:globulin ratio as well as liver and kidney SOD and catalase activities. The plant extract was able to restore the biochemical parameters to levels comparable to those of the control group in all instances. Further evidence in support of these results was derived from histopathological analysis.

Conclusion: Taken together, the results of this study show that the aqueous extract of *Dennettia tripetala* fruits is able to ameliorate liver and kidney damage caused by multiple exposures to carbon tetrachloride probably via an antioxidant-dependent mechanism.

Keywords: Carbon tetrachloride, *Dennettia tripetala*, Liver, Kidney, Antioxidant

Background

A considerable number of people all over the world suffer from liver disease, with a large percentage unable to afford sophisticated treatment. There is therefore the need for effective and affordable drugs with fewer side effects. Many affordable drugs currently in use have their starting source from medicinal plants. Examples of plants that have shown promise in alleviating liver damage in experimental animals include: *Silybum marianum* [1], *Curcuma longa* [2, 3], *Dacryodes edulis* [4, 5] amongst others.

In this report, *Dennettia tripetala* was investigated for its possible hepatoprotective ability. The plant is widely consumed in West Africa for its spicy taste and medicinal value. It is used traditionally for the management of fever, diabetes, diarrhea, toothache, sore throat, nausea, among other ailments [6]. Research has shown that *Dennettia tripetala* possesses anti-microbial properties [7–9], analgesic/anti-inflammatory properties [10], antihyperglycemic properties [11], anti-cancer properties [12] as well as in vitro antioxidant properties [13–15].

Recently, we established the fact that pre-administration of the aqueous and ethanolic extracts of *Dennettia tripetala* can protect the liver and kidney of rats from damage arising

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from a single administration of carbon tetrachloride [16, 17].

In the present study, we investigated the ability of the aqueous extract of *Dennettia tripetala* fruits to ameliorate liver and kidney damage induced by pre-exposure of Wistar albino rats to carbon tetrachloride multiple times. It is a well known fact that when the liver is damaged by free radicals (such as those produced during the metabolism of a toxic substance like carbon tetrachloride), the cell membrane can be a target [18]. This causes certain liver marker enzymes to leak out of the cell. Lipid homeostasis is also affected by free radicals eventually leading to lipid accumulation in the cells of the liver [18]. The kidney is also known to be damaged by free radicals resulting from carbon tetrachloride metabolism [19]. In a similar vein as the liver, free radicals can attack the nephron and eventually lead to problems with filtration of waste such as urea and creatinine from the blood into the urine. Therefore the levels of these substances tend to be higher than normal in a situation where there is kidney damage. In this report, we evaluated the activity of liver marker enzymes as well as the concentration of lipids and proteins in the blood and hepatocytes in order to determine the level of carbon tetrachloride-induced liver injury as well as the level of hepatoprotection offered by the aqueous extract of *Dennettia tripetala*. We also evaluated the levels of urea and creatinine in the blood in order to assess the extent of compromise in kidney function. We measured the activity of antioxidant enzymes as well as the lipid peroxidation status of the liver and kidneys in order to determine the extent of damage and restoration provided by carbon tetrachloride and *Dennettia tripetala* respectively.

Methods

Plant extracts

Mature fruits of *Dennettia tripetala* were purchased from a local market in Benin City, Nigeria. The fruits were sliced, sun-dried and blended into fine powder. A weighed portion (500 g) of the powder was soaked in 4 L of distilled water for 48 h with regular stirring. The extract was sieved with a clean cheese cloth and concentrated using a freeze dryer. A stock solution containing 200 mg/ml of the freeze dried extract was prepared by dissolving the appropriate weight of freeze-dried sample in the requisite volume of distilled water. This stock solution was used in the subsequent treatment of the animals as required.

Animals and experimental design

Twenty five male albino rats of Wistar strain weighing 120 ± 20 g were acclimatized for 2 weeks and fed *ad libitum* with standard pellets and tap water. The animals were housed in wooden cages with barbed wire netting. The experimental procedures performed on the animals were approved by the Animal Ethics Committee of the Faculty of Life sciences, University of Benin, Nigeria. The animals were randomized into five groups (A-E) of five rats. Group A (control) rats received only feed and water. Groups B-E received CCl_4 in olive oil. Groups B, C, and D in addition, received 250, 500 and 1000 mg/kg bw respectively of *Dennettia tripetala* aqueous fruit extract. The CCl_4 was diluted with olive oil in a 1:1 ratio and the CCl_4: olive oil mixture was administered at a dose of 3 ml/kg b.w. The CCl_4 as well as the plant extracts were administered orally by gavage. CCl_4 was administered to the rats twice a week for 2 weeks, while the plant extract was administered daily from the 8^{th} to the 14^{th} day. On the 14^{th} night, the rats were fasted overnight and sacrificed under chloroform anaesthesia on day 15. Blood was collected from the heart, allowed to clot and then centrifuged at 3500 rpm for 10 min. The serum obtained was used for the required biochemical assays. The liver was also harvested and weighed. A portion of the liver was homogenized in normal saline (1:5 w/v). The homogenate was centrifuged at 4000 rpm for 15 min to obtain supernatant which was also used for the relevant biochemical assays.

Histology

Portions of the liver and kidney were fixed in 10% neutral buffered formalin for histology. Thin sections of the liver were dissected and processed using Leica TP2010 automatic tissue processor for 18 h. The processor passed the tissues through fixation, dehydration, dealcoholisation and paraffination. Ultra-thin sections of 5 μm were sliced from the paraffinated sections using a Thermo scientific semi-automated rotary microtome. The tissues were then subjected to hematoxylin and eosin staining and viewed under a microscope using 10 X and 40 X magnification.

Reagents

The following test kits were obtained from Randox Laboratories, United Kingdom: Alanine transaminae (ALT), Apartate transaminae (AST), Triglyceride (TRIG), Cholesterol (CHOL), HDL-Cholesterol (HDL-CHOL), Albumin and Total protein. Alkaline phosphatase (ALP) test kit was obtained from Teco, USA. The manufacturer's protocols were strictly followed in all instances. Reagents for Superoxide dismutase (SOD), Catalase (CAT) and Malondialdehyde (MDA) assays were all of analytical grade. The method of Misra and Fridovich [20], Goth [21] and Buege and Aust, [22] were used for SOD, catalase and malondialdehyde assays respectively.

Table 1 Effect of aqueous extract of *Dennettia tripetala* on liver marker enzymes in serum of rats exposed to carbon tetrachloride

Groups	AST (U/L)	ALT (U/L)	ALP (U/L)
Control	242.50 ± 31.98^{a}	52.50 ± 4.79^{a}	39.15 ± 5.88^{a}
AQDT 250 + CCl_4	743.30 ± 73.33^{b}	236.70 ± 40.96^{ab}	54.26 ± 2.88^{a}
AQDT 500 + CCl_4	643.30 ± 123.3^{ab}	472.00 ± 25.96^{b}	49.03 ± 2.01^{a}
AQDT 1000 + CCl_4	516.70 ± 89.50^{ab}	454.00 ± 82.86^{b}	44.34 ± 4.60^{a}
CCl_4	807.50 ± 200.10^{b}	486.70 ± 6.67^{b}	45.70 ± 1.59^{a}

Values are presented as mean ± SEM. Values with different superscripts are significantly different from one another at $P < 0.05$. Values with the same superscript are not significantly different from one another. $n = 5$ rats per group. AQDT means aqueous extract of *Dennettia tripetala*

Statistics

Data were subjected to one-way ANOVA using GraphPadPrism version 7. The data are presented as mean +/- SEM. Statistical significance was set at $P < 0.05$.

Results

Effect of aqueous extract of *Dennettia tripetala* on liver marker enzymes in serum of rats exposed to carbon tetrachloride

The results of this study showed that carbon tetrachloride caused the activities of AST, ALT and ALP in serum to increase ($P < 0.05$, $P < 0.05$, $P > 0.05$ respectively) compared to those of the control animals (Table 1). The aqueous extract of *Dennettia tripetala* fruits reduced the levels of these enzymes to different extents at different doses of the extract.

Effect of aqueous extract of *Dennettia tripetala* on serum and liver lipid profile of rats exposed to carbon tetrachloride

The results from this study showed that carbon tetrachloride caused a significant increase ($P < 0.05$) in serum total cholesterol which was restored to normal by the plant extract in a non-dose-dependent manner (Table 2). Carbon tetrachloride also caused a significant ($P < 0.05$) decrease in serum HDL-cholesterol concentration as well as a significant ($P < 0.05$) increase in serum LDL-cholesterol concentration. The plant extract was able to counter the effects of CCl_4 in both cases to different extents at different doses of the extract. Carbon tetrachloride also caused a 34% reduction in serum triglyceride levels. The highest dose of the plant extract barely countered this decrease in serum triglyceride levels brought about by carbon tetrachloride. In the liver, carbon tetrachloride caused increases in cholesterol as well as triglyceride levels which the plant extract was able to lower to different extents at different doses of the extract (Table 3).

Effect of aqueous extract of *Dennettia tripetala* on serum protein profile of rats exposed to carbon tetrachloride

Table 4 shows that administration of carbon tetrachloride induced significant elevations ($P < 0.05$) in the levels of serum total protein and globulin with a concomitant decrease in albumin and the albumin: globulin ratio. The aqueous extract of *Dennettia tripetala* significantly ($P < 0.05$) restored the levels of these parameters.

Effect of aqueous extract of *Dennettia tripetala* on antioxidant enzyme activity and lipid peroxidation status in liver of rats exposed to carbon tetrachloride

The results in Table 5 reveal that carbon tetrachloride caused a significant reduction in the activity of superoxide dismutase ($P < 0.05$) as well as catalase ($P < 0.05$) in the liver. The activities of these enzymes were significantly ($P < 0.05$) restored to normal levels by the plant extract. Lipid peroxidation status was measured in form of malondialdehyde (MDA) levels. Carbon tetrachloride caused a significant ($P < 0.05$) increase in MDA levels which the plant extract was able to reduce to levels comparable with the control group.

Effect of aqueous extract of *Dennettia tripetala* on urea and creatinine concentration in the serum of rats exposed to carbon tetrachloride

Table 6 shows that carbon tetrachloride caused a 40% increase in serum urea levels compared to control. The plant extract was able to restore the urea concentration to levels similar to that of the control group. Carbon

Table 2 Effect of aqueous extract of *Dennettia tripetala* on serum lipid profile of rats exposed to carbon tetrachloride

Groups	Total CHOL (mg/dl)	HDL-CHOL (mg/dl)	LDL-CHOL (mg/dl)	TAG (mg/dl)
Control	38.28 ± 1.93^{a}	25.45 ± 2.20^{a}	13.75 ± 1.95^{a}	127.4 ± 25.1^{a}
AQDT 250 + CCl_4	37.60 ± 0.68^{a}	15.15 ± 2.99^{ab}	22.22 ± 3.59^{a}	43.70 ± 6.51^{c}
AQDT 500 + CCl_4	41.93 ± 0.83^{ab}	12.88 ± 1.11^{b}	31.18 ± 3.75^{b}	60.00 ± 10.78^{c}
AQDT 1000 + CCl_4	43.52 ± 3.42^{ab}	12.94 ± 2.69^{b}	17.65 ± 5.05^{a}	98.3 ± 26.27^{b}
CCl_4	47.85 ± 1.08^{b}	7.24 ± 0.64^{b}	41.07 ± 0.94^{b}	84.30 ± 3.43^{b}

Values are presented as mean ± SEM. Values with different superscripts are significantly different from one another at $P < 0.05$. Values with the same superscript are not significantly different from one another. $n = 5$ rats per group. AQDT means aqueous extract of *Dennettia tripetala*

Table 3 Effect of aqueous extract of *Dennettia tripetala* on liver lipid profile of rats exposed to carbon tetrachloride

Groups	Total CHOL (mg/dl)	TAG (mg/dl)
Control	41.68 ± 0.49^{a}	16.88 ± 2.11^{a}
AQDT 250 + CCl_4	39.30 ± 0.49^{a}	24.62 ± 4.61^{ab}
AQDT 500 + CCl_4	38.90 ± 1.05^{a}	36.57 ± 4.28^{b}
AQDT 1000 + CCl_4	52.00 ± 2.42^{b}	16.88 ± 2.11^{a}
CCl_4	51.44 ± 7.59^{b}	28.84 ± 6.01^{ab}

Values are presented as mean ± SEM. Values with different superscripts are significantly different from one another at $P < 0.05$. Values with the same superscript are not significantly different from one another. $n = 5$ rats per group. AQDT means aqueous extract of *Dennettia tripetala*

tetrachloride also caused a 28% increase in serum creatinine levels compared to control. The plant extract was able to restore the level of creatinine to normal to different extents at different doses of the extract.

Effect of aqueous extract of *Dennettia tripetala* on antioxidant enzyme activity and lipid peroxidation status in kidney of rats exposed to carbon tetrachloride

The results in Table 7 reveal that carbon tetrachloride caused a 26% reduction in the activity of superoxide dismutase in the kidney. Carbon tetrachloride also caused a significant reduction in the activity of catalase ($P < 0.05$) in the kidney. The activities of these enzymes were significantly ($P < 0.05$) restored to normal levels by the plant extract. Carbon tetrachloride caused a significant ($P < 0.05$) increase in MDA levels which the plant extract was able to reduce to levels comparable with the control group.

Histopathological examination of the liver and kidney

The biochemical results were supported by the outcome of the histopathological examination of the liver and kidney. Figures 1 and 2 are sections of the liver from a representative rat in each of the five groups. Figures 1 and 2, show that under the conditions of this experiment, carbon tetrachloride caused damage to the liver mainly by inducing macrovesicular steatosis and congestion of the centrioles. Other damages that were less conspicuous include: erosion of the centriole, alterations in the parenchyma and general architecture of the liver, as well as infiltration of the liver by immune cells. The plant extract was able to ameliorate the harmful effects of carbon tetrachloride to varying degrees; with the highest dose seemingly the least effective. The different doses of the plant extract also drew the attention of large numbers of immune cells to the regions of the liver damaged by carbon tetrachloride to varying degrees.

Figures 3 and 4 are sections of the kidney from a representative rat in each of the five groups. They show that under the conditions of this experiment, carbon tetrachloride caused damage to the kidney mainly by inducing inflammation and swelling of the tubules, and necrosis of the tubular lining. There was also congestion. The plant extract was able to ameliorate the harmful effects of carbon tetrachloride to varying degrees.

Discussion

Our earlier studies showed that extracts of *Dennettia tripetala* fruits can prevent liver and kidney damage when administered prior to a single dose of carbon tetrachloride in rats [16, 17]. The aim of the present study was to investigate the potentials of *Dennettia tripetala* fruits in restoring the health of the liver and kidney when considerable damage (caused by multiple exposures to carbon tetrachloride) had already occurred. Our results reveal that the aqueous extract of *Dennettia tripetala* fruits can ameliorate liver and kidney damage posed by multiple exposures to carbon tetrachloride.

ALT, AST and ALP are markers of liver damage. They are majorly localized within hepatocytes. Damage to the membrane of hepatocytes can cause these enzymes to leak out thereby leading to their elevation in the serum [23]. In this study, ALT, AST and ALP which were elevated by carbon tetrachloride were significantly reduced by the plant extract indicating hepatoprotection.

The distribution of lipids in the body is a good indicator of health status. Dyslipidemia which refers to a distortion in plasma lipid pattern is often characterized by a profound drop in HDL-cholesterol levels and a concomitant rise in LDL-cholesterol levels and signals severe

Table 4 Effect of aqueous extract of *Dennettia tripetala* on serum protein profile of rats exposed to carbon tetrachloride

Groups	Total protein (g/dl)	Albumin (g/dl)	Globulin (g/dl)	Albumin: Globulin ratio
Control	5.94 ± 0.18^{a}	3.24 ± 0.04^{a}	2.68 ± 0.09^{a}	1.13 ± 0.04^{a}
AQDT 250 + CCl_4	15.29 ± 0.29^{b}	3.09 ± 0.10^{a}	12.11 ± 0.32^{b}	0.26 ± 0.01^{b}
AQDT 500 + CCl_4	12.39 ± 0.23^{b}	3.07 ± 0.08^{a}	8.74 ± 0.48^{c}	0.35 ± 0.02^{b}
AQDT 1000 + CCl_4	12.48 ± 0.66^{b}	3.13 ± 0.07^{a}	9.36 ± 0.70^{c}	0.31 ± 0.02^{b}
CCl_4	21.04 ± 1.45^{c}	2.96 ± 0.15^{b}	14.05 ± 1.67^{b}	0.18 ± 0.02^{b}

Values are presented as mean ± SEM. Values with different superscripts are significantly different from one another at $P < 0.05$. Values with the same superscript are not significantly different from one another. $n = 5$ rats per group. AQDT means aqueous extract of *Dennettia tripetala*

Table 5 Effect of aqueous extract of *Dennettia tripetala* on antioxidant enzyme activity and lipid peroxidation status in liver of rats exposed to carbon tetrachloride

Groups	SOD (units/g wet tissue)	Catalase (units/g wet tissue)	MDA (units/g wet tissue)
Control	1225 ± 137.70^{a}	4683 ± 10.82^{a}	0.08 ± 0.01^{a}
AQDT 250 + CCl_4	1033 ± 155.70^{ab}	4610 ± 57.93^{a}	0.12 ± 0.01^{a}
AQDT 500 + CCl_4	975 ± 17.68^{ab}	4554 ± 63.00^{a}	0.11 ± 0.01^{a}
AQDT 1000 + CCl_4	1350 ± 94.65^{a}	4316 ± 49.40^{b}	0.11 ± 0.01^{a}
CCl_4	744 ± 62.40^{b}	4326 ± 51.14^{b}	0.17 ± 0.01^{b}

Values are presented as mean ± SEM. Values with different superscripts are significantly different from one another at $P < 0.05$. Values with the same superscript are not significantly different from one another. $n = 5$ rats per group. AQDT means aqueous extract of *Dennettia tripetala*

Table 7 Effect of aqueous extract of *Dennettia tripetala* on antioxidant enzyme activity and lipid peroxidation status in kidney of rats exposed to carbon tetrachloride

Groups	SOD (units/g wet tissue)	Catalase (units/g wet tissue)	MDA (units/g wet tissue)
Control	873 ± 28.00^{a}	5076 ± 293.6^{a}	0.20 ± 0.01^{a}
AQDT 250 + CCl_4	2265 ± 742.0^{b}	4493 ± 40.31^{ab}	0.20 ± 0.01^{a}
AQDT 500 + CCl_4	923 ± 86.00^{a}	4569 ± 3.00^{a}	0.17 ± 0.01^{b}
AQDT 1000 + CCl_4	1570 ± 317.0^{ab}	4595 ± 55.66^{a}	0.23 ± 0.01^{ac}
CCl_4	644 ± 286.0^{a}	3984 ± 18.25^{b}	0.25 ± 0.01^{c}

Values are presented as mean ± SEM. Values with different superscripts are significantly different from one another at $P < 0.05$. Values with the same superscript are not significantly different from one another. $n = 5$ rats per group. AQDT means aqueous extract of *Dennettia tripetala*

problems with lipid metabolism. Such distortions in plasma lipid patterns could be predictive of artherosclerosis and associated cardiovascular complications [24]. In this study, the plant extract was able to dampen the dangerous trends in the levels of HDL-and LDL-cholesterol caused by carbon tetrachloride. The plant also dampened the trends in the levels of serum total cholesterol and triglyceride caused by carbon tetrachloride.

In the liver of the rats that were exposed to carbon tetrachloride, our data revealed an apparent accumulation of lipids. This was also evident from the histopathological evaluations. Other researchers have reported this pathology of lipid accumulation in the liver of animals following carbon tetrachloride administration [25–27]. The possible mechanism for this lipid accumulation may include: carbon tetrachloride-induced decrease in the secretion of lipids from the liver [28] as well as increase in the synthesis of lipids in the liver [29]. In the present study, the plant extract greatly reduced the accumulation of lipids in the liver as supported by both biochemical and histological evidences.

In this study, there was a large increase in serum total protein in rats treated with carbon tetrachloride. This may be partly due to the leakage of enzymes and other proteins from the liver and possibly other organs of the body into serum. There was also a decrease in serum albumin as well as the albumin: globulin ratio indicating possible compromise in the synthetic capacity of the liver [18]. *Dennettia tripetala* extract was able to prevent this and boost both the structural and functional integrity of the liver, including its synthetic capacity.

Table 6 Effect of aqueous extract of *Dennettia tripetala* on urea and creatinine concentration in the serum of rats exposed to carbon tetrachloride

Groups	Urea (mg/dl)	Creatinine (mg/dl)
Control	6.43 ± 0.83^{a}	1.54 ± 0.01^{a}
AQDT 250 + CCl_4	7.69 ± 1.54^{a}	2.25 ± 0.03^{b}
AQDT 500 + CCl_4	6.43 ± 0.68^{a}	1.69 ± 0.04^{ab}
AQDT 1000 + CCl_4	6.94 ± 0.75^{a}	1.56 ± 0.25^{a}
CCl_4	10.71 ± 2.48^{a}	2.15 ± 0.16^{b}

Values are presented as mean ± SEM. Values with different superscripts are significantly different from one another at $P < 0.05$. Values with the same superscript are not significantly different from one another. $n = 5$ rats per group. AQDT means aqueous extract of *Dennettia tripetala*

Urea and creatinine are commonly used as markers of kidney function. When the levels of these metabolites increase in serum, it indicates that there is a problem with the kidney [26, 30]. This is because the kidney is responsible for filtration of the blood, and when there is a problem with the kidney, urea and creatinine which are supposed to be filtered into the urine accumulates in the blood [26, 30]. In the present study, the concentration of urea in the serum of rats administered carbon tetrachloride increased by 40%. The histopathology also revealed critical changes in kidney architecture characteristic of acute tubular necrosis. Our data also showed a 28% rise in serum creatinine concentration in the rats treated with carbon tetrachloride. In the present study, *Dennettia tripetala* extract ameliorated kidney damage as shown by restoration of the normal kidney architecture. At the biochemical level, the plant extract lowered the serum urea and creatinine concentration to levels very similar to those of the control group.

Antioxidant enzymes are natural intrinsic enzymes which living organisms use to combat reactive oxygen species (ROS) produced during normal metabolism. They act by scavenging free radicals and by terminating any free radical-induced chain reaction. When an animal is exposed to an external source of free radicals (e.g., as a result of exposure to a toxic chemical) the animal's antioxidant defense mechanism steps in and tries to control the rate of deleterious bio-oxidation and molecular damage [31].

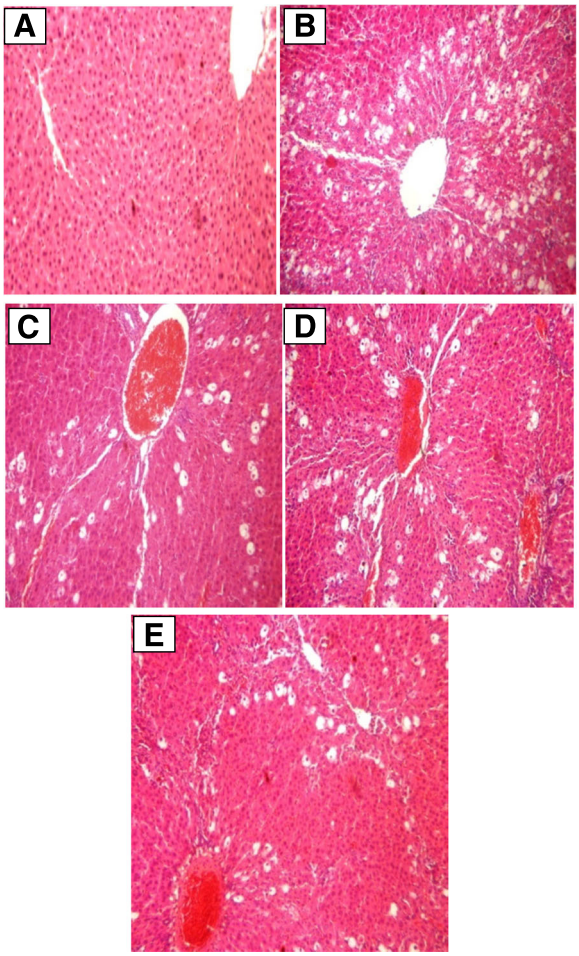

Fig. 1 Photomicrographs of liver sections from (**a**) Control rats showing normal liver histology: clear centrioles with well fenestrated sinusoidal space, (**b**) rats treated with 250 mg/kg b.w DT and CCl_4 showing fatty accumulation although with clear centrioles (**c**) rats treated with 500 mg/kg bw. DT and CCl_4 showing less fatty accumulation although the centriole appears congested with neutrophilic infiltrates around it, (**d**) rats treated with 1000 mg/kg bw DT and CCl_4 showing mild fatty accumulation with congested centrioles amidst distinct hepatic nuclei, neutrophilic infiltrates are also present (**e**) rats treated with CCl_4 only showing fatty accumulation and multifocal distortion in liver histology and congestion of the central vein. The liver sections were stained with H/E and observed with a 10X objective. DT stands for *Dennettia tripetala*

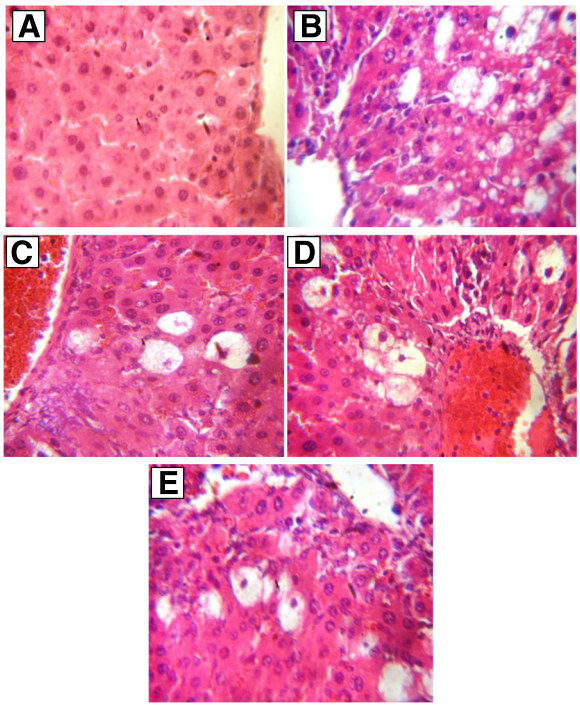

Fig. 2 Photomicrographs of liver sections from (**a**) Control rats showing normal liver histology: clear centrioles with well fenestrated sinusoidal space and distinct hepatocytes, (**b**) rats treated with 250 mg/kg b.w DT and CCl_4 showing fatty accumulation although with clear centrioles and minimal hydropic degeneration, (**c**) rats treated with 500 mg/kg bw DT and CCl_4 showing less fatty accumulation and less hydropic degeneration of the hepatocytes although the centriole appears congested with neutrophilic infiltrates around it, (**d**) rats treated with 1000 mg/kg bw DT and CCl_4 showing mild fatty accumulation with congested centrioles amidst distinct hepatic nuclei, neutrophilic infiltrates are also present (**e**) rats treated with CCl_4 only showing fatty accumulation (macrovesicular steatosis) and severe hydropic degeneration of the hepatocytes besides multifocal distortion in liver histology. The liver sections were stained with H/E and observed with a 40X objective. DT stands for *Dennettia tripetala*

Several researchers have observed that the activities and expression of antioxidant enzymes such as SOD, catalase and glutathione peroxidase, drops drastically in the liver and kidney of animals exposed to an external source of free radicals compared to control animals [25, 27, 30, 32–34]. This may be explained by the fact that these radicals may inhibit (non-specifically) the synthesis as well as the activity of antioxidant enzymes in the liver and kidney at the DNA level and at the protein level respectively [31, 35–37].

On the other hand, some other researchers have observed that the activities and expression of antioxidant enzymes increases significantly in the liver and kidney of animals exposed to an external source of free radicals compared to control [38, 39]. This is not surprising because the cell may attempt to counteract the effects of radicals by activating genes responsible for encoding antioxidant enzymes [31]. It is also a known fact that certain reactive oxygen species can activate stress-induced transcription factors [31] and these transcription factors may include those that are critical to the expression of antioxidant enzymes. Put together, it stands to reason therefore that host response to oxidant-driven pathology may involve a two-phase process in which the host initially mobilizes its antioxidant defense system

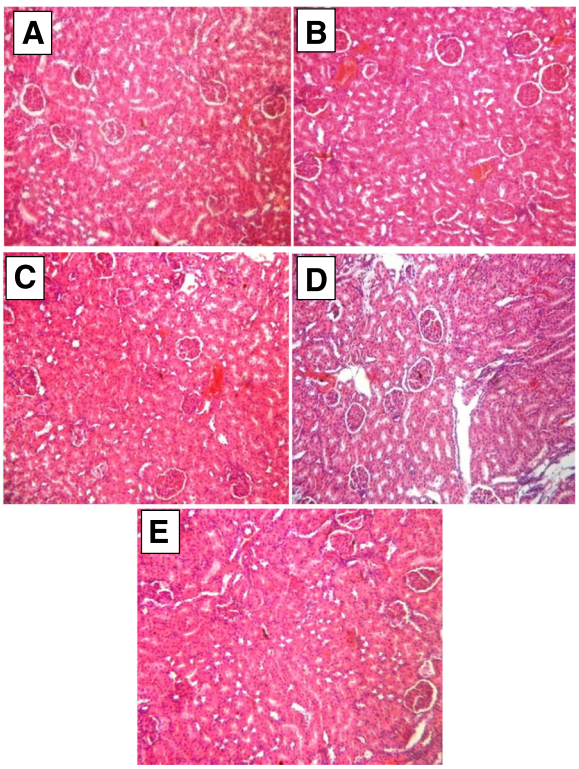

Fig. 3 Photomicrographs of kidney sections from (**a**) Control rats showing normal histological features. The section indicates a detailed cortical parenchyma and the renal corpuscles appear as dense rounded structures, (**b**) rats treated with 250 mg/kg b.w DT and CCl_4 showing some mild degree of distortion and disruption in microanatomy of the renal cortex, (**c**) rats treated with 500 mg/kg bw. DT and CCl_4 showing some distortion and disruption in microanatomy of the renal cortex, including mild queried edema, although with prominent renal corpuscles, (**d**) rats treated with 1000 mg/kg bw DT and CCl_4 showing some recovery in microanatomy of the renal cortex, (**e**) rats treated with CCl_4 only showing severe distortion and disruption in microanatomy of the renal cortex, including queried edema. The kidney sections were stained with H/E and observed with a 10X objective. DT stands for *Dennettia tripetala*

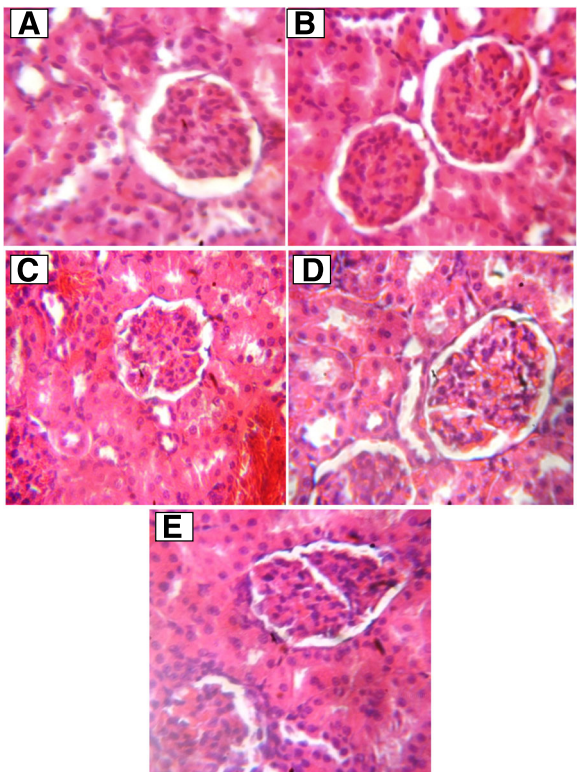

Fig. 4 Photomicrographs of kidney sections from (**a**) Control rats showing normal histological features. The section indicates a detailed cortical parenchyma and the renal corpuscles appear as dense rounded structures, (**b**) rats treated with 250 mg/kg b.w DT and CCl_4 showing some mild degree of distortion and disruption in microanatomy of the renal cortex, (**c**) rats treated with 500 mg/kg bw. DT and CCl_4 showing some distortion and disruption in microanatomy of the renal cortex, including mild queried edema, although with prominent renal corpuscles, (**d**) rats treated with 1000 mg/kg bw DT and CCl_4 showing some recovery in microanatomy of the renal cortex, it includes queried edema though, (**e**) rats treated with CCl_4 only showing severe distortion and disruption in microanatomy of the renal cortex, including queried edema. The kidney sections were stained with H/E and observed with a 40X objective. DT stands for *Dennettia tripetala*

until such a time, when such system becomes overwhelmed and begins to suffer a decline.

The mechanism by which carbon tetrachloride causes liver and kidney damage is free radical mediated. In this study, carbon tetrachloride decreased the activities of liver and kidney SOD and catalase. On the other hand, *Dennettia tripetala* caused an increase in liver and kidney SOD and catalase activities, indicating that one way in which this plant confers protection is by altering the activity of antioxidant enzymes. Further evidence in support of the antioxidant mechanism is seen in the reduction in malondialdehyde levels of the liver and kidney compared to that of the group administered with only carbon tetrachloride. Products of lipid peroxidation such as malondialdehyde, can inactivate many crucial cellular proteins [37]. Our results show that *Dennettia tripetala* extract was able to manage carbon tetrachloride-induced lipid peroxidation in the liver and kidney.

Conclusions

Under the conditions of this experiment, the aqueous extract of *Dennettia tripetala* fruits exhibits a profound ability to ameliorate liver and kidney damage caused by multiple exposures to carbon tetrachloride.

Authors' contribution

NEJO designed the experiments. SOI performed the experiments, analysed and interpreted the data. NEJO supervised the work. Both authors drafted and revised the manuscript. Both authors read and approved the final manuscript.

Competing interests
The authors declare that they have no competing interests.

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