Pre-Exposure to *Dennettia tripetala* Ethanolic Fruit Extract Prevents Biochemical Alterations in Rats Subsequently Exposed to a Single Dose of Carbon Tetrachloride

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Abstract. *Dennettia tripetala* (DT) is consumed in West Africa as a spice. It is also used in traditional medicine for treating cough, fever and other ailments. Its fruits have been shown to possess phytochemicals with proven antioxidant capabilities. DT roots elicit *in vitro* antioxidant capabilities similar to that of ascorbic acid. In this study, we evaluated the potency of the ethanol extract of DT fruits in preventing liver damage induced by a single oral administration of Carbon tetrachloride (CCl₄). Thirty female Wistar albino rats were randomized into six groups of five animals each: Group A served as control, Groups B-D were given increasing doses (250, 500 and 1000mg/kg b.w respectively) of DT extract for 14 days by gavage; on day 15, they were given CCl₄ (3ml/kg bw) by gavage. Group E was given the highest dose of DT for 14 days without CCl₄ while Group F was given only CCl₄ on day 15. Administration of CCl₄ resulted in liver and kidney injury detected as significant increases in plasma ALT, AST, ALP and GGT activities as well as plasma total protein, urea, creatinine, plasma and liver triglyceride and cholesterol as well as liver and kidney malondialdehyde concentrations. CCl₄ also caused a reduction in liver and kidney SOD and catalase activities as well as plasma albumin concentration. Pre-treatment with *Dennettia tripetala* however, significantly prevented damage to the liver and kidney. Under the conditions of this study, *Dennettia tripetala* shows potential in preventing liver and kidney damage and has no noticeable side effects.

Introduction

Carbon tetrachloride (CCl₄) was once used as a solvent and degreaser in homes and industries until it was found to be carcinogenic and hepatotoxic [1]. The molecular mechanism of its hepatotoxicity has been well documented. It is currently used to model liver damage. CCl₄ metabolism occurs primarily in the liver (and to a smaller extent in other organs that contain a high amount of cytochrome P450, such as the kidney). The trichloromethyl radical, which is formed from the metabolism of CCl₄ by the cytochrome P450 enzyme, reacts rapidly with molecular oxygen to produce the trichloromethyl peroxy radical which triggers oxidative damage to macromolecular structures in cells [1]. The trichloromethyl radical itself can also cause adduct formation and subsequent molecular damage. In the liver, molecular damage induced by CCl₄ can take the form of lipid peroxidation, steatosis, fibrosis, cirrhosis, hepatocellular carcinoma and liver failure in extreme cases [1, 2]. CCl₄ damages the kidney in a similar fashion as the liver, causing oxidative damage as well as histological alterations [3, 4].

Plants have been used for many centuries as medicine for the management of a number of diseases. This is majorly due to the presence of phytochemicals which elicit medicinal properties. *Dennettia tripetala* is one such plant. It grows in the rainforests of West Africa and is known in Nigeria by several names including: 'Pepperfruit', 'Ako', 'Mmimmi' and 'Ata Igberere'. *Dennettia tripetala* is mostly consumed for its spicy taste. It is also used in Nigeria for the treatment of cough, sore throat, toothache, diabetes, fever and nausea during pregnancy [5-7]. Its phytochemical constituents include: Saponins, tannins, flavonoids, cardiac glycosides, *Dennettia* essential oils,

phenolic acids as well as alkaloids [5-8]. *Dennettia tripetala* contains antioxidant vitamins and has been shown to possess *in vitro* antioxidant activities [8-10]. The essential oil of *Dennettia tripetala* possesses antimicrobial and insecticidal potential [6, 11-13] as well as analgesic and anti-inflammatory potential [14]. *Dennettia tripetala* also lowers blood glucose [15, 16].

Over the years, several studies have been conducted investigating the role of medicinal plants in experimentally induced liver and kidney damage. However, there is limited knowledge on the ability of *Dennettia tripetala* to prevent liver and kidney damage. In view of the phytochemical constituents of *Dennettia tripetala*, and the proven *in vitro* antioxidant activity of this plant, we carried out this study to investigate the potential of the ethanolic extract of this plant in preventing the liver and kidney of rats from undergoing free-radical damage caused by a single administration of CCl₄.

Material and Methods

Plant material and extract preparation

Fresh *Dennettia tripetala* fruits (Figure 1) were obtained from a local market in Benin City, Nigeria. The fruits were washed with distilled water, macerated and ground into coarse powder using a milling machine. Ethanol extract of the fruit was prepared by soaking about 500g of the coarse powder in 2.5L of ethanol for 24 hours with regular stirring. The mixture was filtered and the extract freeze dried. A stock solution of the extract (200mg/ml) was constituted using distilled water.

Figure 1. Mature *Dennettia tripetala* fruits.

Carbon tetrachloride dilution

Carbon tetrachloride solution purchased from Sigma-Aldrich (USA), was diluted with olive oil in a 1:1 (v/v) ratio. It was administered to the rats at a dose of 3ml/kg body weight.

Experimental animals and design

Thirty (30) female Wistar albino rats weighing between 120-150g were used for this study. The animals were kept in clean disinfected iron cages, in a well ventilated room and allowed free access to water and feed. Following a two-week acclimatization period, the animals were randomized into six groups of five animals each and treated as follows:

GROUP A: Control (feed only)

GROUP B: Given 250mg/kg body weight of extract for 14 days + CCl₄ on day 15.

GROUP C: Given 500mg/kg body weight of extract for 14 days + CCl₄ on day 15.

GROUP D: Given 1000mg/kg body weight of extract for 14 days + CCl₄ on day 15.

GROUP E: Given 1000mg/kg body weight of extract for 14 days

GROUP F: Given CCl₄ on day 15.

All animals were handled in strict accordance with the NIH guide for the care and use of laboratory animals.

Administration of the extract and CCl₄

The stock solution of plant extract was administered orally at varying doses, (as stated above) for a period of fourteen days. On day fifteen, a single dose of CCl₄ was also administered orally to the animals.

Sample collection

On day sixteen the animals were sacrificed by chloroform anaesthesia after an overnight fast. Blood samples were collected and the plasma obtained, was used for biochemical analyses. The liver of each rat was also collected, weighed and homogenized with normal saline (1:5 w/v). The tissue homogenates were centrifuged at 4000 rpm for 10 minutes and the supernatant obtained were used for biochemical analysis.

Assay methods

The following kits were purchased from Randox Laboratories (United Kingdom): Alanine aminotransferase (ALT), Aspartate transaminase (AST), Alkaline phosphatase (ALP), Gamma glutamyl transferase (GGT), urea, creatinine, total protein, albumin, cholesterol and triglyceride kits. The assays were carried out following the manufacturer's protocols. Superoxide dismutase activity was assayed for using the method of Misra and Fridovich [17]. Catalase activity was assayed for using the method of Goth [18]. Malondialdehyde concentration was assayed for using the method of Buege and Aust [19].

Statistical analysis

Data are represented as Mean $\pm$ S.E.M (n = 5). Significance of Difference was tested by one-way ANOVA, followed by Tukey test. GraphPad Prism Version 6 (GraphPad Software Inc. San Diego, California USA) was used for this analysis. Statistical Significance was set at $P < 0.05$.

Results

Table 1 shows that CCl₄ caused a significant ($P < 0.001$) increase in AST, ALT, ALP and GGT activities compared to control. Pre-administration of the plant extract significantly ($P < 0.05$) prevented this increase in a dose-dependent manner. The plant extract when administered alone did not alter these parameters significantly when compared to control.

Table 1. Effect of Ethanolic extract of *Dennettia tripetala* fruits on liver marker enzymes in plasma of rats exposed to carbon tetrachloride.

Groups	Treatment	AST (U/L)	ALT (U/L)	ALP (U/L)	GGT (U/L) ($\times 10^{-3}$)
A	Control	27.25 $\pm$ 1.11 ^a	28.00 $\pm$ 0.45 ^a	107.9 $\pm$ 2.74 ^a	1158 $\pm$ 0.01 ^a
B	ETDT 250 + CCl ₄	70.25 $\pm$ 0.25 ^b	76.33 $\pm$ 0.33 ^b	208.3 $\pm$ 0.46 ^b	8878 $\pm$ 386.0 ^b
C	ETDT 500 + CCl ₄	57.50 $\pm$ 0.29 ^c	76.67 $\pm$ 0.33 ^b	171.2 $\pm$ 1.06 ^c	9264 $\pm$ 668.6 ^b
D	ETDT 1000 + CCl ₄	40.67 $\pm$ 0.33 ^d	73.67 $\pm$ 1.33 ^b	153.6 $\pm$ 1.03 ^c	5018 $\pm$ 386.0 ^a
E	ETDT 1000	24.50 $\pm$ 0.29 ^a	29.33 $\pm$ 0.67 ^a	117.5 $\pm$ 2.56 ^a	3474 $\pm$ 0.01 ^a
F	CCl ₄	116.70 $\pm$ 4.41 ^c	97.00 $\pm$ 0.41 ^c	267.9 $\pm$ 12.53 ^d	10,422 $\pm$ 1215 ^b

Values are presented as mean $\pm$ SEM. Values with different superscripts in the same column, differ significantly from one another. Values with the same superscript in a column are not significantly different from one another. n=5 rats per group. ETDT means ethanolic extract of *Dennettia tripetala*.

Table 2 shows that CCl₄ caused a significant ($P < 0.001$) increase in the concentrations of cholesterol and triglyceride in the liver of the rats. Pre-administration of the plant extract significantly ($P < 0.001$) prevented this increase. The plant extract when administered alone did not alter these parameters significantly when compared to control.

Table 2. Effect of ethanolic extract of *Dennettia tripetala* fruits on liver lipid profile of rats exposed to carbon tetrachloride.

Groups	Treatment	CHOL (mg/dl)	TAG (mg/dl)
A	Control	154.8 ± 0.28 ^a	146.4 ± 1.01 ^a
B	ETDT 250 + CCl ₄	159.0 ± 0.63 ^b	152.3 ± 0.66 ^b
C	ETDT 500 + CCl ₄	158.4 ± 0.53 ^b	152.9 ± 0.71 ^c
D	ETDT 1000 + CCl ₄	155.7 ± 0.45 ^a	154.3 ± 1.48 ^c
E	ETDT 1000	153.1 ± 0.91 ^a	146.1 ± 0.32 ^a
F	CCl ₄	168.8 ± 0.29 ^c	254.8 ± 2.18 ^d

Values are presented as mean ± SEM. Values with different superscripts in the same column, differ significantly from one another. Values with the same superscript in a column are not significantly different from one another. n=5 rats per group. ETDT means ethanolic extract of *Dennettia tripetala*.

Table 3 shows that CCl₄ caused a significant (P<0.01) increase in plasma cholesterol and triglyceride concentration. Pre-administration of the plant extract significantly (P<0.05) prevented this increase. The plant extract when administered alone did not alter these parameters significantly when compared to control.

Table 4 shows that CCl₄ caused a significant (P<0.001) increase in plasma total protein and a significant (P<0.001) decrease in plasma albumin compared to control. Pre-administration of the plant extract significantly (P<0.05) prevented the increase in total protein and decrease in albumin. Administration of only the plant extract did not significantly alter the total protein levels but led to a reduction in plasma albumin.

Table 3. Effect of ethanolic extract of *Dennettia tripetala* fruits on plasma lipid profile of rats exposed to carbon tetrachloride.

Groups	Treatment	CHOL (mg/dl)	TAG (mg/dl)
A	Control	129.6 ± 1.41 ^a	151.10 ± 0.51 ^a
B	ETDT 250 + CCl ₄	127.8 ± 0.95 ^a	159.9 ± 3.19 ^{ab}
C	ETDT 500 + CCl ₄	128.8 ± 0.62 ^a	160.8 ± 1.37 ^{ab}
D	ETDT 1000 + CCl ₄	132.4 ± 0.54 ^a	157.7 ± 0.96 ^{ab}
E	ETDT 1000	125.6 ± 1.98 ^a	149.9 ± 0.98 ^a
F	CCl ₄	143.3 ± 0.52 ^b	168.7 ± 3.62 ^b

Values are presented as mean ± SEM. Values with different superscripts in the same column, differ significantly from one another. Values with the same superscript in a column are not significantly different from one another. n=5 rats per group. ETDT means ethanolic extract of *Dennettia tripetala*.

Table 4. Effect of ethanolic extract of *Dennettia tripetala* fruits on plasma protein profile of rats exposed to carbon tetrachloride.

Groups	Treatment	Total protein (g/dl)	Albumin (g/dl)
A	Control	5.71 ± 0.03 ^a	4.34 ± 0.13 ^a
B	ETDT 250 + CCl ₄	6.05 ± 0.01 ^b	3.54 ± 0.09 ^b
C	ETDT 500 + CCl ₄	5.93 ± 0.01 ^c	3.63 ± 0.17 ^b
D	ETDT 1000 + CCl ₄	5.85 ± 0.01 ^c	3.31 ± 0.10 ^{bc}
E	ETDT 1000	5.79 ± 0.04 ^a	3.53 ± 0.07 ^b
F	CCl ₄	6.17 ± 0.01 ^d	2.75 ± 0.10 ^c

Values are presented as mean ± SEM. Values with different superscripts in the same column, differ significantly from one another. Values with the same superscript in a column are not significantly different from one another. n=5 rats per group. ETDT means ethanolic extract of *Dennettia tripetala*.

Table 5 shows that in the liver of the rats, CCl₄ caused a significant (P<0.05) decrease in superoxide dismutase (SOD) and catalase activities as well as a significant increase (P<0.05) in MDA levels compared to control. Pre-administration with the plant extract significantly (P<0.05) prevented these alterations. Administration of only the plant extract did not significantly alter the activity of SOD but led to a decrease in Catalase activity and a decrease in malondialdehyde (negligible when compared to the CCl₄ only group).

Table 5. Effect of ethanolic extract of *Dennettia tripetala* fruits on the activity of antioxidant enzymes and lipid peroxidation status in liver of rats exposed to carbon tetrachloride.

Groups	Treatment	SOD (Units/g wet tissue)	CAT (Units/g wet tissue)	MDA (Units/g wet tissue) (x 10 ³)
A	Control	377.8 ± 9.98 ^a	6108 ± 42.55 ^a	0.19 ± 0.01 ^a
B	ETDT 250 + CCl ₄	516.7 ± 32.10 ^{ab}	5519 ± 78.11 ^b	0.23 ± 0.01 ^b
C	ETDT 500 + CCl ₄	327.2 ± 45.76 ^{ac}	5810 ± 101.9 ^a	0.23 ± 0.01 ^b
D	ETDT 1000 + CCl ₄	450.8 ± 33.45 ^{abc}	5580 ± 101.4 ^b	0.23 ± 0.01 ^b
E	ETDT 1000	378.9 ± 36.62 ^a	5530 ± 12.00 ^b	0.24 ± 0.02 ^b
F	CCl ₄	263.3 ± 21.31 ^{ad}	4830 ± 52.98 ^c	0.27 ± 0.02 ^c

Values are presented as mean ± SEM. Values with different superscripts in the same column, differ significantly from one another. Values with the same superscript in a column are not significantly different from one another. n=5 rats per group. ETDT means ethanolic extract of *Dennettia tripetala*.

Table 6 shows that CCl₄ caused a significant (P<0.01) increase in urea and creatinine levels compared to control. The plant extract significantly (P<0.05) prevented this increase. Administration of only the plant extract did not significantly alter these parameters.

Table 7 shows that in the kidney of the rats, CCl₄ caused a significant (P<0.05) decrease in SOD and catalase activities as well as a significant increase (P<0.05) in MDA levels compared to control. Pre-administration with the plant extract significantly (P<0.05) prevented these alterations. Administration of only the plant extract did not significantly alter these parameters when compared to control.

Table 6. Effect of ethanolic extract of *Dennettia tripetala* fruits on urea and creatinine concentrations in plasma of rats exposed to carbon tetrachloride.

Groups	Treatment	Urea (mg/dl)	Creatinine (mg/dl)
A	Control	84.81 ± 0.18 ^a	1.86 ± 0.07 ^a
B	ETDT 250 + CCl ₄	90.15 ± 0.72 ^a	2.00 ± 0.03 ^a
C	ETDT 500 + CCl ₄	91.70 ± 2.06 ^a	1.61 ± 0.33 ^a
D	ETDT 1000 + CCl ₄	86.79 ± 3.99 ^a	2.23 ± 0.29 ^a
E	ETDT 1000	92.01 ± 1.08 ^a	1.01 ± 0.09 ^b
F	CCl ₄	120.20 ± 8.06 ^b	3.22 ± 0.13 ^c

Values are presented as mean ± SEM. Values with different superscripts in the same column, differ significantly from one another. Values with the same superscript in a column are not significantly different from one another. n=5 rats per group. ETDT means ethanolic extract of *Dennettia tripetala*.

Table 7. Effect of ethanolic extract of *Dennettia tripetala* fruits on the activity of antioxidant enzymes and lipid peroxidation status in kidney of rats exposed to carbon tetrachloride.

Groups	Treatment	SOD (Units/g wet tissue)	CAT (Units/g wet tissue)	MDA (Units/g wet tissue) (x 10 ³)
A	Control	108.0 ± 3.34 ^a	4005 ± 156.5 ^a	0.09 ± 0.01 ^a
B	ETDT 250 + CCl ₄	85.58 ± 7.65 ^b	3624 ± 46.86 ^{ab}	0.10 ± 0.01 ^b
C	ETDT 500 + CCl ₄	100.20 ± 6.00 ^a	3744 ± 49.57 ^a	0.10 ± 0.02 ^b
D	ETDT 1000 + CCl ₄	77.53 ± 3.39 ^b	4125 ± 77.13 ^{ac}	0.09 ± 0.02 ^a
E	ETDT 1000	85.21 ± 6.23 ^b	3606 ± 26.15 ^{ab}	0.09 ± 0.01 ^a
F	CCl ₄	59.36 ± 0.68 ^c	2016 ± 78.63 ^d	0.15 ± 0.01 ^c

Values are presented as mean ± SEM. Values with different superscripts in the same column, differ significantly from one another. Values with the same superscript in a column are not significantly different from one another. n=5 rats per group. ETDT means ethanolic extract of *Dennettia tripetala*.

Discussion

The liver is an important organ in the body with multiple functions including the detoxification of xenobiotics, which predisposes it to drug-induced damage. Markers of liver damage such as ALT, AST, ALP and GGT are often used to assess liver function. The level of these enzymes in the blood is usually under tight regulation therefore an increase in the plasma level of these enzymes may indicate a breach in liver integrity [20]. In this study, the well-known hepatotoxicant, carbon tetrachloride (CCl₄) was used to test the effectiveness of the ethanolic extract of *Dennettia tripetala* fruits in protecting the liver of rats from molecular damage.

The results of this study showed that CCl₄ caused an increase in the plasma levels of ALT, AST, ALP and GGT. The increase in the plasma level of these enzymes is most likely due to free radical damage to the membrane of the hepatocytes; as studies have shown that trichloromethyl peroxy radical (a product of CCl₄ metabolism) is capable of causing oxidative damage to subcellular structures including the cell membrane [1]. In the present study, administration of *Dennettia tripetala* for 14 days prior to a single administration of carbon tetrachloride significantly protected the liver from this damage. This may be due to the intrinsic antioxidant content of the plant. *Dennettia tripetala* has been shown to possess antioxidant vitamins A and C as well as phytochemicals with proven antioxidant actions such as flavonoids [21]. *Dennettia tripetala* may have helped to manage or quench the free radicals generated by the metabolism of CCl₄.

Lipid homeostasis is tightly regulated in the liver. Liver injury can take the form of steatosis which is the accumulation of lipids, chiefly triglycerides in the liver [20]. CCl₄ can cause steatosis by increasing the synthesis of triglycerides and cholesterol in the liver [22], and by inhibition of beta-oxidation of fatty acids and decreasing the secretion of lipids [23]. Suppression of lysosomal acid triglyceride lipase activity also leads to accumulation of triglycerides in hepatocytes of rats exposed to CCl₄ [24, 25]. Results of the present study showed that CCl₄ caused triglyceride and cholesterol to accumulate in the liver. Pre-administration of *Dennettia tripetala* ethanolic extract protected the liver from steatosis.

Elevation of cholesterol in the serum may be due to the inability of the liver to remove cholesterol from the blood [26]. On the other hand, elevation in serum triglyceride levels may be as a result of a reduction in the activity of peripheral lipase [27]. In the present study, CCl₄ administration resulted in significant elevations in the plasma levels of triglyceride and cholesterol, indicating alterations to lipid metabolism. *Dennettia tripetala* ethanolic extract was able to prevent this disturbance in lipid homeostasis.

Albumin is synthesized by the liver and is the major protein found in blood. It is therefore used to determine the synthetic capacity of the liver [20]. The results from the present study showed that CCl₄ caused a reduction in plasma albumin. This is probably a result of decreased synthesis of albumin, indicating damage to the liver. The plant extract was able to prevent this decrease. On the other hand, the total protein content of the plasma increased. This may be due to the enzymes which leaked out of the liver into the plasma due to the porous membranes of the hepatocytes as previously discussed.

Superoxide dismutase and Catalase are enzymes that normally function to combat free radicals and a decrease in their activity may indicate free radical damage. The present study showed that CCl₄ administration caused significant decreases in the activities of SOD and Catalase in the liver. CCl₄ also caused an increase in malondialdehyde levels which indicates lipid peroxidation in the liver. *Dennettia tripetala* ethanolic extract was able to prevent the decline in SOD and Catalase activities as well as significantly inhibit lipid peroxidation.

Carbon tetrachloride is also known to cause damage to kidneys. It is distributed at high concentrations in the kidney [28]. It has a high affinity for the cortex of the kidney due to the high content of cytochrome P450 enzymes [4, 29]. The mechanism by which it exerts damage to the kidneys is similar to that of the liver (free radical damage from the trichloromethyl and its peroxyradicals).

Urea and creatinine are usually filtered from the blood by the kidney, therefore an increase in the levels of these metabolites in the blood shows that there is damage to the kidney. In the present study, CCl₄ caused significant elevations in plasma levels of urea and creatinine. Pre-administration of the *Dennettia tripetala* ethanolic extract significantly prevented this increase. CCl₄ also caused a reduction in SOD and catalase activities in the kidney as well as an increase in malondialdehyde levels in the kidney, indicating lipid peroxidation and oxidative stress. *Dennettia tripetala* ethanolic extract prevented these alterations.

Conclusion

Data from the present study show that the ethanolic extract of *Dennettia tripetala* fruits has the potential to protect the liver and kidneys of rats from damage when it is administered daily for two weeks prior to the administration of a single dose of CCl₄. The mechanism by which this protection occurs is most likely an inhibition of free radical damage, as the data in this paper shows that the plant extract prevented the decline in antioxidant enzymes in the liver and kidney of the rats; and also prevented lipid peroxidation. Molecular experiments designed to study the details of the mechanism by which *Dennettia tripetala* elicits this protective activity need to be carried out. It will also be interesting to find out if *Dennettia tripetala* will be effective in preventing liver and kidney damage caused by multiple exposures to carbon tetrachloride.

List of abbreviations. CCl₄ = Carbon tetrachloride, ETDT = Ethanolic extract of *Dennettia tripetala*, SOD = Superoxide dismutase, CAT = Catalase, CHOL = Cholesterol, TAG = Triacyl glycerol, SEM = Standard error of mean.

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