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Aqueous Extract of *Dennettia Tripetala* (Pepperfruit) Protects the Liver and Kidney against Carbon Tetrachloride-Induced Damage in Rats

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Abstract
Evidence has shown that Carbon tetrachloride (CCl_4) is metabolized in the liver into a highly reactive trichloromethyl radical that can induce damage to the liver. Previous studies have shown that Dennettia tripetala possesses remarkable in vitro antioxidant properties. In this study, we tested the ability of aqueous extract of D. tripetala fruit to protect the liver and kidney of rats from CCl_4 -induced damage. Thirty female albino rats of the Wistar strain were used for the study. They were sorted into six groups of five rats each. Group A served as control and these rats were not given any treatment, whereas groups B to D were given D. tripetala at a daily oral dose of 250, 500 and 1000 mg/kg body weight respectively, for 14 days prior to CCl_4 administration. Animals in group E were given the highest dose (1000mg/kg body weight) of the extract for 14 days without CCl_4 while their group F counterparts received only CCl_4 on day 14. Where applicable, animals received 3ml/kg body weight of a 1:1 (CCl_4: olive oil) preparation. The results obtained showed that administration of CCl_4 resulted in a significant increase ($p<0.05$) in the activities of plasma ALT, ALP and GGT. Hepatic steatosis which manifested as markedly increased hepatic triglyceride levels, also accompanied carbon tetrachloride toxicity in this study. These hepatic lesions and the observed mild distortion in renal function were significantly prevented by pre-treatment with aqueous extract of D. tripetala. The study suggests that aqueous extract of D. tripetala fruits holds some promise in the prevention of carbon tetrachloride-induced liver and kidney damage.
Key words: *Dennettia tripetala*, Pepperfruit, CCl_4, antioxidant, liver, brain.

Introduction
Carbon tetrachloride (CCl_4) is widely used in research for the induction of experimental liver and kidney damage. The primary site for the biotransformation of CCl_4 is the liver. This is because the liver is very rich in Cytochrome P450, an enzyme responsible for its conversion to the highly reactive trichloromethyl radical (1). This agent provokes a free radical-mediated damage to lipids and other cellular constituents, via a chain of chemical processes that are now fairly well understood (1). Potential agents for the mitigation of CCl_4-induced liver damage should therefore be able to interfere with either the generation of the highly reactive CCl_3 radical or the quenching of free radicals once generated. Free radical 'quenchers' exert their biological effects by preventing or inhibiting the oxidation of critical biomolecules within the living cell, which would otherwise be targets for oxidative damage.
Historically, plants and plant-derived products have provided a veritable source of phytochemicals with enormous antioxidant properties. These phytochemicals include flavonoids, alkaloids, saponins, steroids and glycosides amongst others. Several earlier reports have provided significant insights into the medicinal value of plants and plant-derived products in a variety of disease conditions including diabetes (2), ulcer (3) and malaria (4).
Dennettia tripetala commonly known as Pepper fruit occurs widely in the rain forest zone of West Africa. It is widely valued for its spicy and peppery taste (5). It is also used traditionally as a remedy against fever, cough, toothache and other ailments (5). Phytochemical screenings have shown that *Dennettia tripetala* is rich in alkaloids, saponins, flavonoids, steroids, cardiac glycosides and terpenoids (6, 7).
Although there is paucity of information on the medicinal value of *D. tripetala*, there is proof that this plant has a lot of medicinal potential. Some earlier reports (8) have shown that the essential oil of *D. tripetala* possesses very powerful analgesic and antiinflamatory effects in rodents. Additional findings (9) suggest that the plant has anti-hyperglycemic potentials in rodents with experimentally-induced hyperglycemia. The seeds have also been shown to be effective in reducing the intraocular pressure of normotensive emmetropic humans (10). Other workers (11) reported that *D. tripetala* roots possess *in vitro* antioxidant activity comparable to those of ascorbic acid and vitamin E. There are interesting but somewhat inconclusive data to suggest that ethanolic extract of *D. tripetala* fruits could alter the secretion and composition of bile in normal rats (12).
Amongst many other aberrations, liver damage resulting in elevated plasma levels of the liver marker enzymes and excessive accumulation of fat in hepatocytes are considered cardinal features of experimental carbon tetrachloride

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toxicity. The present study was designed therefore to assess the hepatoprotective properties of the aqueous extract of the fruits of *D. tripetala* using the afore-mentioned biomarkers as indices.

Materials and Methods

Collection of plant materials

Mature fresh fruits of *Dennettia tripetala* (Pepperfruit) were purchased from New Benin market in Benin City, Nigeria. The fruits were washed with distilled water, sun-dried and ground into fine powder by using a blender.

Preparation of plant extract

The fine powder (500 g) was soaked in 2.5 L of distilled water for 24 hr with regular stirring. Thereafter, the extract was filtered using a clean cheese cloth. The filtrate obtained was concentrated by means of a freeze-drier. A stock solution of the resulting aqueous extract of *D. tripetala* (200 mg/ml) was prepared by dissolving 20 g of the freeze-dried sample in 100 ml of distilled water.

Preparation of CCl_4 stock solution

Carbon tetrachloride was dissolved in olive oil in a 1:1 (v/v) ratio. The CCl_4: olive oil preparation was administered to the rats, where applicable at a dose of 3ml/kg body weight.

Animal Experiment and Sample Preparation

Thirty female albino rats of the Wistar strain were purchased from the animal house of the Department of Anatomy, University of Benin, Benin City. The animals were subsequently maintained at the animal house of the Department of Biochemistry, University of Benin. Throughout the duration of the experiment, all animals had unhindered access to normal chow (product of Bendel Feeds and Flour Mill, Ewu, Edo State, Nigeria) and water except when it became necessary to withdraw food on the eve of animal sacrifice. The animals were weighed, allocated to six groups of five animals each and allowed to acclimatize for 14 days prior to the commencement of the experiment. The animal groupings are as defined below:

Group A (Control): The rats were given only feed and water.

Group B: The rats were treated orally with the aqueous extract of *Dennettia tripetala* fruit at a dose of 250 mg/kg body weight daily for 14 days prior to the administration of a single oral dose of CCl_4 (3 ml/kg body weight).

Group C: The rats were treated orally with the aqueous extract of *Dennettia tripetala* fruit at a dose of 500 mg/kg body weight daily for 14 days prior to the administration of a single oral dose of CCl_4 (3 ml/kg body weight).

Group D: The rats were treated orally with the aqueous extract of *Dennettia tripetala* fruit at a dose of 1000 mg/kg body weight daily for 14 days prior to the administration of a single oral dose of CCl_4 (3 ml/kg body weight).

Group E: The rats were treated orally with the aqueous extract of *Dennettia tripetala* fruit at a dose of 1000 mg/kg body weight daily for 14 days.

Group F: The rats received a single dose of CCl_4 (3 ml/kg body weight) on day 14 without prior treatment with *D. tripetala*.

All animals were sacrificed on day 15 under chloroform anaesthesia, and following an overnight fast. Blood samples were collected directly from the heart into heparinized bottles and centrifuged at 3000 rpm to obtain plasma. Similarly, the liver and kidney of each animal was carefully excised and a weighed portion homogenized in 5 ml of iced cold normal saline. This was followed by centrifugation at 3000 rpm and careful recovery of the resultant supernatant which was used for subsequent biochemical analyses. All animals were handled in strict accordance with the NIH guidelines for the care and use of laboratory animals.

Biochemical assays

All biochemical analyses (ALT, ALP, GGT, triglycerides, urea and creatine) were carried out using previously described standard assay procedures (13-18), and with the aid of commercially available test kits. With the exception of ALP which was assayed using test kits from Quimica Clinica Aplicada, Spain, all other assays were carried out using Randox test kits, products of Randox Laboratories, United Kingdom. In all instances, the manufacturer's instructions were strictly adhered to.

Statistical analysis

The results of the study are expressed as mean ± standard error of mean. The differences among means were analyzed using one-way ANOVA. Any differences in mean from the one-way ANOVA was confirmed using Tukey's test. Values were considered statistically significant at $p < 0.05$. GraphPad Prism 6 was used for this statistical analysis.

Results

The results obtained from this study are presented in tables 1-3. The effect of *Dennettia tripetala* on Plasma ALT, ALP and GGT activities in rats exposed to CCl_4 is shown in table 1. Administration of carbon tetrachloride resulted

in a significant increase ($p < 0.05$) in plasma ALT, ALP and Gamma glutamyl transferase (GGT) activities relative to control. Pre-treatment with *Dennettia tripetala* extract provided significant protection ($p < 0.05$) at different doses and to varying degrees.

Table 1: Effect *Dennettia tripetala* on ALT, ALP and GGT activities in rats exposed to CCl_4

Groups	Treatment	Plasma GGT (U/L)	Plasma ALP (U/L)	Plasma ALT (U/L)	Liver ALT (U/g wet tissue)
A	Control	1.74 ± 0.58^{a}	60.94 ± 2.4^{a}	60.7 ± 1.7^{a}	73.3 ± 1.3^{a}
B	250 mg DT + CCl_4	3.86 ± 0.39^{a}	104.9 ± 6.9^{ab}	91.7 ± 0.3^{b}	72.3 ± 5.4^{a}
C	500 mg DT + CCl_4	4.63 ± 0.01^{a}	85.57 ± 4.7^{a}	88.3 ± 0.7^{b}	77.0 ± 7.0^{a}
D	1000 mg DT + CCl_4	4.25 ± 0.39^{a}	79.10 ± 14.6^{a}	91.3 ± 2.3^{b}	70.0 ± 8.5^{a}
E	1000 mg DT	2.70 ± 0.39^{a}	68.7 ± 16.8^{a}	59.0 ± 1.0^{a}	66.0 ± 6.1^{a}
F	CCl_4	6.95 ± 0.47^{b}	173.8 ± 7.36^{b}	90.7 ± 2.7^{b}	72.3 ± 0.3^{a}

Values represent the mean ± SEM; n=5. Values with different superscripts are significantly different from each other. Statistical significance was set at $P<0.05$.

Table 2: Effect *Dennettia tripetala* on Triacylglycerol (TAG) Levels in rats exposed to CCl_4

Groups	Treatment	Liver TAG (mg/g wet tissue)	Plasma TAG (mg/dl)
A	Control	45.24 ± 4.98^{a}	159 ± 2.18^{a}
B	250 mg DT+ CCl_4	45.71 ± 1.61^{a}	160.0 ± 3.7^{a}
C	500 mg DT + CCl_4	53.28 ± 2.78^{a}	161.5 ± 0.8^{a}
D	1000 mg DT + CCl_4	58.18 ± 4.52^{a}	164.1 ± 0.42^{a}
E	1000 mg DT	62.72 ± 1.11^{a}	154.5 ± 0.66^{a}
F	CCl_4	79.77 ± 1.85^{b}	160.3 ± 2.00^{a}

Values represent the mean ± SEM; n=5. Values with different superscripts are significantly different from each other. Statistical significance was set at $P<0.05$.

Table 3: Effect *Dennettia tripetala* on Plasma Urea and Creatinine Levels in rats exposed to CCl_4

Groups	Treatment	Plasma Urea (mg/dl)	Plasma Creatinine (mg/dl)
A	Control	81.0 ± 2.46^{a}	2.00 ± 0.14^{a}
B	250 mg DT+ CCl_4	81.0 ± 2.56^{a}	1.99 ± 0.24^{a}
C	500 mg DT + CCl_4	83.0 ± 3.81^{a}	1.21 ± 0.45^{b}
D	1000 mg DT + CCl_4	76.0 ± 2.35^{a}	1.97 ± 0.38^{a}
E	1000 mg DT	79.0 ± 0.58^{a}	1.36 ± 0.10^{b}
F	CCl_4	118.0 ± 15.52^{b}	2.75 ± 0.26^{a}

Values represent the mean ± SEM; n=5. Values with different superscripts are significantly different from each other. Statistical significance was set at $P<0.05$.

The effect of *Dennettia tripetala* on the liver and plasma triacylglycerol levels of rats exposed to CCl_4 is shown in table 2. In the liver, there was a significant increase ($p<0.05$) in triacylglycerol levels following CCl_4 administration. There was however no significant difference in the plasma triacylglycerol level following CCl_4 administration when compared with control. Again the protection offered by *D. tripetala* extract is readily seen as the extract significantly prevented the carbon tetrachloride-induced hepatic steatosis.

The effect of *Dennettia tripetala* on urea and creatinine levels in the plasma of rats exposed to CCl_4 is shown in table 3. Treatment with CCl_4 significantly ($p<0.05$) elevated the level of plasma urea. On the other hand, treatment of CCl_4-exposed rats with the plant extract significantly ($p<0.05$) prevented this increase, with the highest dose being the most effective. There was however no significant difference in plasma creatinine levels of CCl_4-treated animals relative to control, although mild increases were observed.

Discussion

The liver is known to be the primary target of CCl_4 toxicity (1). The trichloromethyl peroxy radical that results from CCl_4 metabolism in the liver, causes oxidative damage to lipids, proteins and nucleic acids in the cells of the liver (1). Cell membranes tend to lose their integrity when the constituent lipids undergo peroxidation and this can result in the leakage of cellular enzymes into plasma. This leads to an increase in the plasma levels of such enzymes. It is pertinent to note that under normal conditions, the activities of these enzymes are kept fairly constant and maintained within a narrow limit. Therefore increases in their plasma activity may be used as an indication of leakage from their intracellular store occasioned by damage to the membrane (19).

GGT is a microsomal enzyme present in hepatocytes. It is also present in the cell membrane of tissues, including the kidneys, bile duct and pancreas. GGT is a sensitive indicator of hepatobiliary disease and its elevation excludes a bone source for elevated ALP in blood. An increase in serum GGT is a defense mechanism reflecting the induction of cellular GGT, when there is oxidative stress (20). Alkaline phosphatase is produced in the bile duct, kidney and bones. This enzyme is used to determine if the disease is as a result of damage to the bile duct or the liver. When the level of ALP is high, and the level of ALT and AST are fairly normal, problems associated with the bile duct may be concluded. ALP activity is related to the functioning of hepatocytes. Suppression of increased ALP activity suggests the prevention of biliary dysfunction in rat liver during chronic administration with CCl_4 (21). Aspartate transaminase (AST) and alanine transaminase (ALT) are the most commonly used biochemical markers of liver injuries (22). These enzymes are normally found in liver cells, but injury to hepatocytes could lead to their leakage into the blood. The ALT is thought to be a more specific indicator of liver inflammation as AST is also found in other organs such as the heart and skeletal muscle. In acute injury to the liver, the level of ALT and AST may be used as a general measure of the degree of liver inflammation or damage (19).

In this study, administration of CCl_4 to animals led to an increase in the plasma activity of GGT, ALP and ALT. The mechanism by which this occurred may be due to leakage of the enzymes from the liver cells as a result of a breach in the integrity of the hepatocellular membranes brought about by the peroxidation of membrane lipids by the free radical metabolite of CCl_4 (23). Other workers have reported similar results using CCl_4 (24, 25).

Administration of aqueous extract of *Dennettia tripetala* (DT) fruit extract may have prevented this increase by combating the oxidative stress associated with CCl_4 metabolism. DT is known to possess antioxidant properties similar to that of ascorbic acid (11, 26). This antioxidant property could be attributed to the high amount of flavonoids as well as vitamins A and C present in DT (6). The plant extract may also have direct effect on membrane integrity as it contains tannins (6) which has been shown to be capable of 'tarring' the outermost layer of the mucosa thereby increasing its resistance to chemical and mechanical stress and limiting its permeability (27). Tannins have also been reported to speed up the healing of inflamed mucous membranes and wounds (28).

A previous study (29) showed that CCl_4 triggers the post-translational degradation of microsomal triglyceride transfer protein (MTTP) which is responsible for the proper assembly and secretion of liver lipoproteins. This may explain at least in part, the result obtained in the present study where the group treated with only CCl_4 had a significantly higher ($p<0.05$) concentration of liver triglyceride when compared to the control. The pre-treatment with DT was found to suppress the increase in liver triacylglyceride content. The result is in conformity with previous findings where *Oren-gedoku-to* extract prevented the CCl_4-induced increase in liver triglycerides (30).

In consonance with results previously reported (31), there was no significant difference in plasma triglyceride levels between the control group, CCl_4 and DT treated groups. This may suggest that the failure in hepatic export of triglycerides was not sufficient to cause a significant drop in the plasma levels. This may be explained by the acute nature of the carbon tetrachloride challenge. It is probable that a more chronic course of CCl_4 toxicity would be sufficient to bring about a significant decrease in plasma triglyceride levels. One would think that mobilization of body fats or replenishment from diet may provide possible explanations as to why the observed failure of hepatic export of triglycerides did not manifest as a decrease in plasma triglyceride levels. However, neither of these lines of

thought seems to be credible enough to stand the test of reasonable argument. This is clearly so, because whether from the diet or body fat, the liver would still be required to play a critical role in the metabolism, processing, package and export of fat-based molecules.

Urea and creatinine clearance tests are commonly used to determine the glomerular filtration rate of the kidneys and consequently, renal function. An impaired ability to clear these metabolites from the blood into the urine gives rise to high plasma levels of urea and creatinine; Hence, abnormally high levels of urea and creatinine is diagnostic of impaired renal function (32).

Several earlier reports have shown that exposure to CCl_4 is capable of causing elevations in both plasma urea and creatinine (33, 34). The data obtained for these parameters in this study seem to suggest that aberrations in liver functions precedes alterations in renal function. Similarly, significant changes in plasma urea levels are suspected to be more of early events in CCl_4-treated animals relative to changes in plasma creatinine.

In conclusion, *Dennettia tripetala* has been confirmed by various researchers to contain components that elicit its antioxidant properties *in vitro*. This study attempted to take the current state of knowledge further by investigating the hepatoprotective and nephroprotective potentials of DT *in vivo*. Results from this study lay credence to the fact that the aqueous extract of *Dennettia tripetala* fruit significantly protects the liver and possibly the kidney from CCl_4-induced damage most probably in a free radical scavenging manner.

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