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Ethanol Extract of *Dacryodes Edulis* Seeds Suppresses Carbon Tetrachloride-Induced Liver Damage in Wistar Albino Rats

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Abstract
The protective effect of the ethanol extract of Dacryodes edulis seeds on the liver of rats exposed to Carbon tetrachloride (CCl_4) was investigated. Thirty female albino rats of the Wistar strain were randomly allocated to six groups consisting of five rats each. Group A served as control. Groups B - D were given increasing oral doses (250, 500 and 1000 mg/kg body weight, respectively) of Dacryodes edulis extract daily for two weeks prior to the administration of a single dose of CCl_4 (3 ml/kg body weight) on the fourteenth day. Group E was given only Dacryodes edulis extract daily for two weeks, while group F received only a single dose of CCl_4 on day 14. The extract was found to possess hepatoprotective properties as observed in the significant ($p < 0.05$) reduction in the plasma activities of the liver function enzymes alanine transaminase, aspartate transaminase and alkaline phosphatase of the animals pre-treated with the plant extract relative to the animals exposed to CCl_4 only (group F). The plant extract also inhibited lipid peroxidation and triglyceride accumulation in the liver of rats administered with the extract, prior to CCl_4 administration. The hepatoprotective properties of Dacryodes edulis may be related to its previously reported high content of antioxidant compounds such as flavonoids and alkaloids.

Keywords: CCl_4, hepatotoxicity, *Dacryodes edulis*, antioxidants

Introduction
Plants have been used by man for medicinal purposes since ancient times. Humans who consume plant parts specifically for its medicinal effects believe that they contain bioactive compounds that may elicit actions against different ailments. Scientific investigations have revealed the presence of phytochemicals such as alkaloids, flavonoids and steroids in a myriad number of plants, and most of the medicinal effects of such plants have been linked to these phytochemicals (1).

Dacryodes edulis (hereafter referred to as DE) is commonly known as "African pear" or "bush pear". It is a fruit tree native to West Africa. The fruits are edible and the bark, leaves, stem and roots have been employed for medicinal purposes (1). Saponins, alkaloids, flavonoids and tannins have been identified in DE with implications in the treatment of a variety of skin diseases and inflammation (2). Antimicrobial, anti-sickle cell anaemia and antioxidant potentials have also been reported for DE extracts (3).

In vitro studies have shown that the leaves of DE possess a very high antioxidant activity (4). This antioxidant activity has been attributed to the presence of flavonoids. Other workers (5) have also reported that the essential oil of DE resin possesses antioxidant activity attributable to the presence of mono and sesquiterpenes. According to Koudou et al, (6), the resin oil of DE inhibits lipid peroxidation and may be useful in the prevention of oxidative damage in humans during ageing, cancer, artherosclerosis, and diabetes. More recently, the ethanol extract of the leaves of *D. edulis* was found to possess *in vivo* antioxidant activities, ameliorating oxidative stress induced by carbon tetrachloride (7).

In the light of these findings, the present study was designed to investigate the possible hepatoprotective effect of the ethanol extract of the seeds of *Dacryodes edulis* in Wistar albino rats subsequently treated with CCl_4.

Materials and Method
Collection of plant materials and preparation of plant extract
Fresh and fully ripe fruits of *Dacryodes edulis* (African pear) were harvested from a farm in the suburb of Benin City, Nigeria. The fruits were washed with distilled water and opened up to reveal the seeds. The seeds were chopped into tiny bits, sun-dried and then ground into fine powder. The powdered seed (500 g) was soaked in 1.5L of 99.7% ethanol for 48 hours with regular stirring. Thereafter, the extract was filtered using a clean cheese cloth. The filtrate obtained was freeze-dried. To prepare the stock solution, 20 g of the freeze-dried sample was dissolved in 100ml of distilled water, giving a stock solution of 200 mg/ml.

Preparation of CCl_4 stock solution
CCl_4 was dissolved in olive oil in a 1:1 (v/v) ratio. This was administered to the rats at a dose of 3 ml/kg body weight.

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Animal experiment and sample collection

Thirty female albino rats of the Wistar strain were purchased from the animal house of the Department of Anatomy, University of Benin, Benin City, Nigeria. They were subsequently housed in the Animal house of the Department of Biochemistry, University of Benin where all the studies were carried out. All animals were allowed 2 weeks acclimatization prior to the commencement of the study. They were also allowed unlimited access to food and drinking water except on the eve of animal sacrifice when feeds had to be withdrawn in order for animals to undergo an overnight fast.

Animals were randomly allocated to six groups of five animals each. Animals in group A served as controls while those in groups B to D in addition to normal chow, received ethanol extract of *D. edulis* at a daily oral dose of 250, 500 and 1,000 mg/ kg body weight, respectively for 14 days prior to the administration of carbon tetrachloride. In addition to normal chow, animals in group E were given only *D. edulis* at a daily oral dose of 1,000 mg/ kg body weight but without carbon tetrachloride. The animals in group F served as negative control; they were maintained on normal chow, but received carbon tetrachloride on day 14 of experiment. In all instances, carbon tetrachloride was administered as a 1:1 (carbon tetrachloride: olive oil) preparation and at a single oral dose of 3 ml/ kg body weight.

After a period of 14 days, the rats were deprived of food overnight, and were sacrificed under chloroform anaesthesia on day 15. Blood was collected directly from the heart into heparinized bottles and centrifuged at 3000 rpm for 5 minutes to obtain the plasma. The liver of each animal was carefully excised, blotted with filter paper and a known portion homogenized in 5ml of physiological saline. The homogenate was subsequently centrifuged at 3000 rpm for 5 minutes and the clear supernatant carefully recovered for the biochemical assays which followed.

Biochemical assays

Alanine aminotranseferase (ALT) and aspartate aminotransferase (AST) activities were determined using the method described by Reitman and Frankel (8). Alkaline phosphatase (ALP) assay was carried out based on the principles previously reported by Klein *et al*. (9), gamma glutamyl transferase (GGT) as described by Tietz (10) while total protein was carried out using the method earlier reported by Doumas *et al*. (11). Estimation of albumin was determined using the method of Doumas *et al*. (12) while total cholesterol and triglycerides were determined based on the principles described by Richard (13) and Trinder (14) respectively. 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 above, the manufacturer's instructions were strictly adhered to. Estimation of malondialdehyde was according to the method of Buege and Aust (15).

Statistical analysis

The results of the study were expressed as mean ± standard error of mean. The differences among means were analyzed using one-way ANOVA and post hoc test were carried out using the Tukey's test. Values were considered statistically significant at $p < 0.05$ (95% confidence level). GraphPad Prism 6 was employed for this statistical analysis.

Results

The results obtained from this study are presented in tables 1 to 3.

Table 1 shows the effect of ethanol extract of *Dacryodes edulis* (DE) seeds on plasma AST, ALT, ALP and GGT activities in rats exposed to CCl_4.

Table 1: Effect of ethanol extract of *Dacryodes edulis* (DE) seeds on Plasma AST, ALT, ALP and GGT activities in rats exposed to CCl_4

Groups	Treatment	AST (U/L)	ALT (U/L)	ALP (U/L)	GGT (U/L)
A	Control	28.49 ± 6.49^{a}	4.96 ± 0.80^{a}	181.10 ± 16.92^{a}	2.90 ± 0.33^{a}
B	250 mg DE+ CCl_4	107.72 ± 6.30^{c}	42.46 ± 1.99^{b}	171.44 ± 30.62^{a}	5.40 ± 0.39^{a}
C	500 mg DE + CCl_4	47.32 ± 6.43^{a}	24.83 ± 1.78^{b}	152.28 ± 14.25^{a}	8.11 ± 0.67^{b}
D	1000 mg DE + CCl_4	70.28 ± 3.94^{b}	36.38 ± 0.43^{b}	164.81 ± 9.37^{a}	2.60 ± 0.73^{a}
E	1000 mg DE	15.88 ± 3.23^{a}	7.31 ± 0.97^{a}	106.37 ± 4.79^{b}	5.33 ± 0.28^{c}
F	CCl_4	102.02 ± 5.95^{c}	47.93 ± 1.36^{c}	242.57 ± 19.94^{c}	4.17 ± 0.59^{c}

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

The result reveals that administration of a single oral dose of carbon tetrachloride to rats was accompanied with a significant increase ($p < 0.05$) in the activities of liver function enzymes: ALT, AST, ALP and GGT. Whereas, pre-treatment of the animals with *D. edulis* for 14 days before administration of CCl_4 resulted in significant reduction ($p < 0.05$) in the aforementioned enzymes activities.

Table 2: Effect of ethanol extract of *Dacryodes edulis* (DE) seeds on plasma total protein, albumin, total cholesterol and total triglyceride levels in rats exposed to CCl_4.

Groups	Treatment	Total protein (g/dl)	Albumin (g/dl)	Total cholesterol (mg/dl)	Total triglyceride (mg/dl)
A	**Control**	**77.19±1.68^a**	**3.82±0.27^a**	**116.90±7.88^a**	**127.74±6.50^a**
B	**250 mg DE+ CCl_4**	**77.49±1.78^a**	**3.95±0.10^a**	**119.65±9.76^a**	**111.32±9.40^a**
C	**500 mg DE + CCl_4**	**73.98±0.88^a**	**3.79±0.04^a**	**97.20±3.10^a**	**83.30±6.67^b**
D	**1000 mg DE + CCl_4**	**74.50±0.61^a**	**3.91±0.08^a**	**111.06±4.76^a**	**93.51±9.07^b**
E	**1000 mg DE**	**74.23±1.80^a**	**3.80±0.14^a**	**115.00±8.26^a**	**98.47±8.54^b**
F	**CCl_4**	**74.20±0.78^a**	**3.42±0.45^a**	**114.58±2.30^a**	**81.51±3.15^b**

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

Results in table 2 represent the effect of ethanol extract of *Dacryodes edulis* (DE) seeds on plasma total protein, albumin, total cholesterol and total triglyceride levels in rats exposed to CCl_4. Results in table 3 represent the effect of ethanol extract of *Dacryodes edulis* (DE) seeds on liver triglyceride, cholesterol and malondialdehyde levels in rats exposed to CCl_4.

Table 3: Effect of ethanol extract of *Dacryodes edulis* (DE) seeds on liver triglyceride, cholesterol and malondialdehyde levels in rats exposed to CCl_4.

Groups	Treatment	Total cholesterol (mg/dl)	Total triglyceride (mg/dl)	MDA (units/mg wet tissue) x 10^{-6}
A	**Control**	**87.34±3.12 ^{a}**	**125.45±7.67 ^{a}**	**19.12±2.05^a**
B	**250 mg DE+ CCl_4**	**86.26±7.50 ^{a}**	**126.77±14.82^a**	**18.01±1.55^a**
C	**500 mg DE + CCl_4**	**82.00±4.03 ^{a}**	**140.50±11.06^a**	**17.02±1.25^a**
D	**1000 mg DE + CCl_4**	**56.98±7.51^b**	**136.70±22.73^a**	**13.80±1.50^a**
E	**1000 mg DE**	**65.01±2.49 ^{b}**	**149.28±10.83^a**	**18.86±2.46^a**
F	**CCl_4**	**54.79±1.93 ^{b}**	**196.04±10.59^b**	**28.67±2.90^b**

Values represent the mean ± SEM; n=5. Values with different subscripts are significantly different from each other. Values with similar subscript are not significantly different from each other. Statistical significance is

Although there was no significant change in plasma total cholesterol, there was a significant decrease in the level of this parameter in the liver of carbon tetrachloride-treated animals relative to the control. The pattern of changes in triglycerides shows that there was no significant change in the plasma but a significant build up in the liver of the CCl_4-treated group when compared with the control. Both this and the carbon tetrachloride-induced

elations in MDA levels were significantly prevented by the 14 day pre-treatment with the ethanol extract of *D. edulis.*

Discussion

Carbon tetrachloride is commonly used in the laboratory to induce hepatotoxicity. The mechanism by which it induces its hepatotoxic effect has long been elucidated. When administered orally, the liver is the primary target organ for CCl_4 toxicity as it contains a high amount of cytochrome P450 enzymes (16). CCl_4 is metabolized by cytochrome P450 enzymes into a trichloromethyl peroxy radical which is capable of causing oxidative damage to macromolecular constituents of the cell including proteins, lipids and nucleic acids (17). The membrane of the cell tends to lose its integrity when the constituent lipids undergo peroxidation and this can result in the leakage of cellular enzymes. For this reason, the leakage of cytosolic enzymes into plasma is routinely used to assess CCl_4 hepatotoxicity (18).

In this study, CCl_4 when administered alone to experimental rats caused a significant increase in plasma ALT, AST and ALP activities when compared with the control animals. This suggests that CCl_4 induced hepatoxicity in these rats. Elevations in the plasma levels of these enzymes may have resulted from their leakage from their intracellular stores into plasma occasioned by the peroxidation of membrane lipids by the free radical metabolite of CCl_4 (19). This result is in agreement with previous findings that the activities of ALT, AST and ALP in plasma are significantly increased ($p < 0.05$) in rats following CCl_4 administration (20-22).

Pre-treatment of the rats with the ethanol extract of *Dacryodes edulis* (DE) seeds for 14 days prior to the administration of CCl_4 significantly decreased the plasma activities of ALT, AST and ALP when compared to the group given only CCl_4. This decrease is in consonance with earlier reports (23). This suggests that the ethanol extract of *D. edulis* has potentials for preventing damage to liver cells and subsequent leakage of intracellular enzymes. Previous reports indicate that *Dacryodes edulis* is reasonably rich in tannins (24-26). Tannin indeed has been found to 'tar' the outermost layer of the mucosa thereby reducing its permeability and increasing its resistance to chemical and mechanical injury as well as irritation (27). Tannins have also been reported to speed up the healing of inflamed mucous membranes and wounds (28). This may provide some insight into the possible protective effect of the extract aside from its well established antioxidant properties.

There was no significant difference in the concentration of total protein and albumin among the groups studied. Plasma albumin is the protein synthesized most abundantly by the liver. It therefore serves as a reflection of the extent of functioning liver cell mass. Albumin has a fairly long half life of 20 days and is therefore not a very reliable indicator of acute liver diseases (29). However, values of albumin and total protein obtained in this study provide information that rules out the possibility of drawing a biased conclusion as a result of chronic disease and infectious hepatitis present in the experimental animals.

There was a significant decrease in the liver total cholesterol in the group treated with only CCl_4 compared to the control group. This may have been due to an inhibition in the synthesis of the enzymes required for cholesterol synthesis and esterification. Pre-treatment with *D. edulis* prevented this decrease in total cholesterol concentration. These findings are consistent with some earlier documented reports (30, 31), which observed that the decrease in total cholesterol in the CCl_4-treated animals was significantly reversed by the extract of *T. decandra* to levels comparable to those of the control group.

Also, another study has shown 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 (32). This may explain the result from the present study where the group treated with only CCl_4 had a significantly higher ($P<0.05$) concentration of liver triglyceride compared to the control. Whereas, the pre-treatment of animals with *D. edulis* before subsequent administration of CCl_4 was found to suppress the increase in liver triglyceride content. This result is in conformity with that previously reported by Nasir et al. in which the leaf extract of *Andrographis paniculata* prevented the increase in liver triglycerides following CCl_4 administration (33). In the plasma, there was a decrease in triglyceride concentration in the CCl_4-treated group when compared to the control group. The observations for plasma triglyceride concentration could also be explained in terms of the MTTP mechanism (32). Reduced export of triglycerides from the liver may provide one possible explanation for the reduced plasma triglyceride levels in CCl_4-treated animals.

Malondialdehyde (MDA) is a decomposition product of lipid hydroperoxides (34). It is widely used as a marker of lipid peroxidation (35). An elevated level of MDA could indicate a high degree of peroxidative damage in hepatocytes (36). In this study, hepatic MDA levels in CCl_4 treated rats were significantly elevated compared to the control. Pre-treatment of the rats with *D. edulis* significantly ($P<0.05$) suppressed the elevation of hepatic MDA concentration triggered by CCl_4.

The ability of the extract to reduce the CCl_4-induced lipid peroxidation is a pointer to the antioxidant potentials of the plant. Several other workers have provided evidence for the *in vitro* free radical scavenging activity of other plant parts of *Dacryodes edulis* (4-6). The reason for this property of *Dacryodes edulis* has been attributed to the high content of phenols, flavonoids and terpenoids. Antioxidant properties have been shown to have a

direct relationship with phytochemical constituents of plants. Phenolics and flavonoids for instance are commonly known for their free radical scavenging activity and hence, antioxidant activity. They modify responses to allergens, viruses and carcinogens and exhibit anti-allergic, anti-inflammatory, anti-microbial and anti-cancer activity (37).

In conclusion, the results from this study indicate that the ethanol extract of *Dacryodes edulis* seeds has a potential hepatoprotective activity against CCl_4-induced liver damage in rats. Pre-treatment of rats with the extract for 14 days prior to CCl_4 administration resulted in inhibition of CCl_4-induced lipid peroxidation, inhibition of triglyceride accumulation in liver, and enhancement of liver function as adjudged by the activities of plasma ALT, AST, ALT and GGT. The mechanism proposed for this activity is clearly related to the antioxidant activity of the plant extract which may be attributed to the high content of flavonoids, phenolic compounds and terpenoids present in the seed extract of *D. edulis*

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