Prevention of Carbon Tetrachloride-induced Hepatic Steatosis and Cellular Damage by Aqueous Extract of *Dacryodes edulis* Seeds in Wistar Rats

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Abstract

The protective effect of the aqueous extract of *Dacryodes edulis* seeds on the liver of rats exposed to carbon tetrachloride was investigated. Thirty female albino rats of 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 (1000 mg/kg body weight) 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 seen in the significant ($p < 0.05$) reduction in the activities of the enzymes alanine transaminase, aspartate transaminase and alkaline phosphatase in the plasma of the animals treated with the plant extract when compared to the group administered CCl_4 only. The extract also inhibited cholesterol and triacylglycerol accumulation in the liver. The hepatoprotective properties of *Dacryodes edulis* observed in this study may be related to its high content of antioxidant compounds such as flavonoids and alkaloids previously reported. The study represents a novel attempt at exploring the medicinal potential of the seeds of *D. edulis* which are typically discarded after eating the fleshy pulp.

Key words: *Dacryodes edulis*, CCl_4, Triacylglycerols, Hepatotoxicity, Steatosis.

Introduction

Carbon tetrachloride (CCl_4) is commonly used for the experimental induction of liver damage. CCl_4-induced toxicity results in a variety of effects depending on dose and duration of exposure. These include: loss of Ca^{2+} homeostasis, lipid peroxidation, release of noxious or beneficial cytokines, fatty degeneration, fibrosis, cirrhosis, liver failure, respiratory failure and even cancer (Weber *et al*., 2003). Medicinal plants may be effective in managing these effects, due to their content of phytochemicals which are known to cure a wide range of ailments.

Dacryodes edulis (hereafter referred to as DE) is commonly known as African or bush pear. It is grown in West Africa. The fruits are edible and the bark, leaves, stem and roots have been employed for medicinal purposes (Waruhiu *et al*., 2004). Its content of phytochemicals such as: saponins, alkaloids, flavonoids and tannins have been used to explain its observed potential to treat a variety of skin diseases and inflammation (Okwu and Nnamdi, 2008). Antimicrobial, anti- sickle cell anaemia and antioxidant potentials have also been reported for DE extracts (Ajibesin, 2011). Other biochemical studies have been carried out on various parts of DE. In one study, the leaves of DE were shown to elicit a very high antioxidant activity due to its content of flavonoids (Agbor *et al*., 2007). The essential oil of DE resin has also been shown to possess antioxidant activity due to the presence of mono and sesquiterpenes (Obame *et al*., 2008). The resin oil of DE inhibits lipid peroxidation and may therefore help to prevent oxidative damage in humans during ageing, cancer, artherosclerosis, and diabetes (Kodou *et al*., 2004). The ethanolic extract of DE leaves has been shown to ameliorate oxidative stress induced by pretreatment with CCl_4 (Conrad and Uche, 2013).

The present study was designed to investigate the hepatoprotective effect of the aqueous extract of *Dacryodes edulis* seeds in Wistar rats subsequently treated with CCl_4. The results obtained showed that *Dacryodes edulis* plays a protective role in the liver of rats exposed to CCl_4.

Materials and Methods

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 to reveal the seeds. The seeds were chopped into tiny bits, sun-dried to constant weight and then ground into fine powder using an industrial grinder. The powdered seed (500 g) was soaked in 1.5L of distilled water 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 100 ml 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.

Animal Experiment and Sample Collection: Thirty female albino rats of 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 a two week 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 were 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 aqueous 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 administration of carbon tetrachloride. In addition to normal chow, animals in group E were given only *D. edulis* extract at a daily oral dose of 1,000 mg/kg body weight for two weeks. The animals in group F served as negative control. They were maintained on normal chow, no *D. edulis* treatment but received carbon tetrachloride on day 14 of the 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 plasma. The liver of each animal was carefully excised, blotted with filter paper and a known portion homogenized in 5ml of saline. The homogenate was subsequently centrifuged at 3000 rpm for 5 minutes and the clear supernatant carefully recovered for the biochemical assays which followed. Animals were handled in accordance with Guidelines for Care and Use of Laboratory Animals in Biomedical Research of the National Institutes of Health of the United States (NIH Publication, revised in 1985).

Biochemical assays: Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities were determined using the method described by Reitman and Frankel (1957). Alkaline phosphatase (ALP) assay was carried out based on the principles previously reported by Klein *et al.,* (1960). Total cholesterol and total triacylglycerols concentrations were determined according to the methods of Richard (1973) and Trinder (1969) respectively.

Statistical analysis: The results of the experiments were expressed as mean ± standard error of mean. The differences among means were analyzed using one-way analysis of variance (ANOVA). Differences gotten from the one-way ANOVA were confirmed using the Tukey's test. Values were considered statistically significant at $p < 0.05$. GraphPad Prism 6 was used for this statistical analysis.

Results and Discussion

Carbon tetrachloride (CCl_4) caused a significant increase ($p < 0.05$) in the plasma levels of ALT, AST and ALP when compared to rats in the control group (Table 1). This increase was prevented by all doses of the *D. edulis* aqueous seed extract.

Table 1: Effect of aqueous extract of *Dacryodes edulis* (DE) seeds on Plasma ALT, AST and ALP Activities

	ALT (U/L)	AST (U/L)	ALP (U/L)
A (Control)	19.83±2.67^a	84.09±2.57^a	71.15±8.63^a
B (250 mg DE + CCl_4)	41.95±1.35^b	100.23±7.73^b	86.38±10.30^b
C (500 mg DE + CCl_4)	45.42±0.78^b	102.33±6.69^b	94.42±5.57^b
D (1000 mg DE + CCl_4)	46.48±1.67^b	100.56±6.66^b	93.38±3.34^b
E (1000 mg DE)	16.40±0.48^a	73.50±5.36^a	88.74±7.64^b
F (CCl_4)	54.94±4.00^c	115.72±2.82^b	152.33±2.33^c

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

In the liver, there was a significant increase in triacylglycerols concentration in the rats exposed to CCl_4 compared to the control (Table 2). This increase was significantly prevented by the 250 and 1000 mg/kg bw doses of the plant extract. Plasma triacylglycerol concentration was found to decrease significantly ($p < 0.05$) in the rats exposed to CCl_4 compared to the control (Table 2). This decrease was significantly prevented by all doses of the plant extract. The concentration of cholesterol in the liver increased significantly ($p < 0.05$) in the rats exposed to CCl_4 only compared to the control and this increase was prevented by all doses of the plant extract. On the other hand, plasma cholesterol decreased significantly ($p < 0.05$) in the rats exposed to CCl_4 only compared to the control (Table 2). This decrease was significantly prevented by all doses of the plant extract.

Table 2: Effect of aqueous extract of *Dacryodes edulis* (DE) seeds on total cholesterol and triacylglycerols

Groups	Liver Triacylglycerols (mg/dl)	Plasma Triacylglycerols (mg/dl)	Liver Cholesterol (mg/dl)	Plasma Cholesterol (mg/dl)
A (Control)	66.74 ± 8.23^a	51.19 ± 1.97^a	37.07 ± 1.20^a	39.50 ± 1.61^a
B (250 mg DE + CCl_4)	78.03 ± 4.85^b	51.23 ± 9.21^a	42.21 ± 3.89^b	39.06 ± 1.27^a
C (500 mg DE + CCl_4)	89.19 ± 2.17^c	53.90 ± 3.08^a	38.17 ± 2.43^a	35.36 ± 1.93^b
D (1000 mg DE + CCl_4)	73.24 ± 4.54^b	47.52 ± 4.06^b	39.78 ± 4.68^b	37.70 ± 1.35^b
E (1000 mg + DE)	63.66 ± 9.79^a	55.11 ± 1.38^a	38.78 ± 2.67^a	41.39 ± 1.56^a
F (CCl_4)	86.25 ± 7.71^c	42.35 ± 2.20^c	48.52 ± 3.60^c	34.91± 1.23^c

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

In the present study, the administration of CCl_4 resulted in significant increases in plasma ALT, AST and ALP activities. This is similar to the results obtained from several earlier studies (Reyes-Gordillo *et al*. 2007; Mohamed *et al*. 2014; Adewale and Orhue, 2015). Prior treatment with aqueous extract of the seeds of *Dacryodes edulis* (DE) significantly ($p < 0.05$) prevented the CCl_4-induced increases in plasma ALT, AST and ALP. This may reflect possible protective effects of DE extract on liver integrity. CCl_4 is widely known for its adverse effects on the liver and other tissues (Weber *et al*., 2003). When CCl_4 is metabolized in the liver, the trichloromethyl peroxy radical that results causes damage to cellular macromolecules in a free-radical related mechanism (Weber *et al*., 2003). This damage includes peroxidation of membrane lipids with a resultant leakage of certain diagnostic enzymes or biomarkers that are specific to the tissue where the damage occurred (Visen *et al*., 1998). This therefore, leads to increases in plasma activities of such enzymes. When considering the liver for instance, the activity of the aminotransferases: ALT and AST are commonly estimated, as markers of liver function. Since these enzymes are usually located within the liver and are maintained at fairly constant levels in the plasma, a marked increase in the plasma activities of these enzymes is suggestive of a disruption in the integrity of the liver.

Triacylglycerols and cholesterol are majorly synthesized and exported by the liver. Therefore, the concentration of these lipids in the liver is a vital indicator of the wellbeing of the liver. In the present study, hepatic steatosis /(accumulation of lipids in the liver) was observed in the rats exposed to CCl_4. This suggests a problem with export of these lipids into the plasma. This result is similar to those of other investigators (Ohtar *et al*., 1997; Adewale and Orhue, 2015). It has been suggested that CCl_4-induced damage to MTTP (Microsomal triglyceride transfer protein) can lead to an accumulation of triacylglycerols in the liver (Pan *et al*., 2007). MTTP is thought to mediate the export of triacylglycerols from the liver into the plasma. Our results also showed that the aqueous extract of DE seeds was significantly able to prevent the CCl_4-induced hepatic steatosis. As previously mentioned, DE contains phytochemicals with antioxidant properties (Agbor *et al.,* 2007; Obame *et al*., 2008), and these phytochemicals may have been responsible for preventing oxidative damage to biomolecules that are considered critical to the proper functioning of liver cells.

Conclusion

Previous investigators have shown that CCl_4 exerts its effect through oxidative damage. *Dacryodes edulis* contains antioxidant phytochemicals that can combat free radicals and prevent oxidative damage. In this study, the aqueous extract of *Dacryodes edulis* seeds was effective in preventing liver damage in rats exposed to CCl_4.

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