HEPATOPROTECTIVE PROPERTIES OF AQUEOUS LEAF EXTRACT OF *PERSEA AMERICANA,* MILL (LAURACEAE) 'AVOCADO' AGAINST CCL4-INDUCED DAMAGE IN RATS

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Abstract

Background: Natural products from plants have received considerable attention in recent years due to their diverse pharmacological properties, including antioxidants and hepatoprotective activities. The protective effects of aqueous extract of *Persea americana* (AEPA) against carbon tetrachloride (CCl_4)-induced hepatotoxicity in male albino rats was investigated.

Materials and Methods: Liver damage was induced in rats by administering a 1:1 (v/v) mixture of CCl_4 and olive oil [3 ml/kg, subcutaneously (sc)] after pre-treatment for 7 days with AEPA. Hepatoprotective effects of AEPA was evaluated by estimating the activities of alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) and levels of total bilirubin (TBL). The effects of AEPA on biomarkers of oxidative damage (lipid peroxidation) and antioxidant enzymes namely, catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPx) and glutathione S-transferase (GST) were measured in liver post mitochondrial fraction.

Results: AEPA and Reducdyn® showed significant (p<0.05) hepatoprotective activity by decreasing the activities of ALT, AST, ALP and reducing the levels of TBL. The activities of antioxidant enzymes, levels of malondialdehyde and protein carbonyls were also decreased dose-dependently in the AEPA-treated rats. Pre-treatment with AEPA also decreased the serum levels of glutathione significantly.

Conclusion: These data revealed that AEPA possesses significant hepatoprotective effects against CCl_4-induced toxicity attributable to its constituent phytochemicals. The mechanism of hepatoprotection seems to be through modulation of antioxidant enzyme system.
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Key words: *Persea americana*; carbon tetrachloride; hepatotoxicity; lipid peroxidation; antioxidants enzymes.

Introduction

The experimental intoxication induced by carbon tetrachloride (CCl_4), is widely used for modeling liver injury in rats. Hepatotoxicity is associated with severe impairment of cell protection mechanisms with the liver, being mostly affected because it is the principal site for CCl_4 biotransformation. It is generally accepted that the hepatotoxicity of CCl_4 is the result of cytochrome P-450-dependent reductive dehalogenation to form a highly reactive trichloromethyl free radical, $CCl_3^{•}$ (McCay et al., 1984). CCl_4-induced damage is characterized by hepatocyte membrane damage, caused by lipid peroxidation, increased plasma levels of hepatic enzymes, fatty degeneration (steatosis i.e. accumulation of triglycerides in the liver), reduced ß-oxidation of fatty acids, and necrosis (Heimberg et al., 1962; Boll et al., 2001). Thus, quantitative measurements of plasma levels of liver enzymes, total cholesterol and hepatic triglyceride level, together with histopathological examination of hepatocytes provide a good assessment of the extent of liver damage or regeneration when challenged with CCl_4.

Plant derived natural products have received considerable attention in recent years due to their diverse pharmacological properties including antioxidants, and hepatoprotective activities (Banskota et al., 2000; Takeoka and Dao, 2003). Avocado is one of the plants that have been widely used in ethno-medicine. In Nigeria, the leaf has various local names such as *Ewé pia* (Yoruba), *Akwukwo Ube oyibo* (Igbo), and *Ganyen piya* (Hausa). The plant is usually 20m (60 ft), tall and the leaves are 12-25cm long in alternate arrangement. The flowers are greenish yellow and bear a pear-shaped fruit (Morton, 1987). Avocado fruits have culinary and nutritional values. The leaves when consumed raw are harmful to animals because of the constituent fatty acid derivative called persin (Oelrichs et al., 1995). Persin from the leaves have been shown to have anti-fungal properties and to be toxic to silkworms (Oelrichs et al., 1995). The leaves are chewed by man as a remedy for pyorrhea, and the aqueous extract of the leaves has a prolonged hypotensive effect. It has been shown to possess anti-inflammatory, anti-convulsant, anti-diabetic, and vasorelaxant activities (Adeyemi et al., 2002; Owolabi et al., 2005; Ojewole and Amabeoku 2006; Gondwe et al., 2008). However, there is paucity of information on hepatoprotective activity of *Persea americana*. Therefore, this study was designed to assess the hepatoprotective and anti-oxidative properties of aqueous extract of *Persea americana* against CCl_4-induced hepatotoxicity in rats.

Materials and methods
Preparation of plant extract

Fresh leaves of *P.americana* were collected from a cultivated plant in Lagos. The leaves were authenticated at the Department of Botany, University of Lagos, and a voucher specimen (LUH 4199), was deposited at the herbarium of the department. The leaves were air-dried and powdered. The aqueous extract was prepared by loading the powdered leaves into Soxhlet apparatus. The extraction lasted for 12hrs, and the extract solution was evaporated to dryness in a rotary evaporator at $40^{o}C$, and stored in clean vials until used.

Experimental Animals

Thirty male Wistar strain albino rats (130 – 160 g), were purchased from the Animal House of Nigerian Institute of Medical Research (NIMR), Lagos. The animals were acclimatized for 1wk., and maintained under standard conditions of temperature ($23 \pm 2^{o}C$), and (12hrs light/dark cycle). The rats were treated with human care, fed with a standard diet and water *ad libitum*.

Experimental Design

The procedure described by Wang et al. (2004), was used for induction of hepato-toxicity. The albino rats were randomly divided into five treatment groups of six rats per group as follows:

Group I (normal control): received distilled water (3ml/kg).
Group II received distilled water (3ml/kg).
Group III pre-treated with the standard drug Reducdyn at a dose of 100mg/kg/day.
Group IV pre-treated with AEPA at a dose of [100mg/kg/day]
Group V pre-treated with AEPA at a dose of [200mg/kg/day].
All the treatments were administered orally for 7 days.

On the seventh day, animals in groups II – V were injected with a fresh mixture of CCl_4 and olive oil (3ml/kg, 1:1, sc), half an hour after the administration of the last dose of the pre-treatment drug, or extract, or distilled water. Rats in group I, were administered olive oil (3ml/kg, sc). After 24hrs, the animals were sacrificed by cervical dislocation and blood samples were collected by cardiac puncture into plain sterile tubes and allowed to coagulate. Serum was separated by centrifugation at 3,000rpm for 10min., at 4°C. Rat livers were quickly excised and perfused with chilled 1.15 % (w/v), KCl solution in order to remove all traces of hemoglobin. The livers were blotted dry, weighed and a portion was used to prepare post mitochondrial fraction and stored at -80°C pending analysis while the remaining parts were preserved in 10 % formalin saline for histopathological analysis

Assay of liver marker enzymes and bilirubin

Liver enzymes- alanine, and aspartate aminotransferases (ALT and AST), and alkaline phosphatase (ALP), were assayed using standard kits supplied by Randox Laboratories (U. K.), and Teco Diagnostics (U. S. A). Total bilirubin in the serum was determined using commercial kit supplied by Human Diagnostics (Germany).
Hepatoprotective activity of the extract was calculated according to the formula of Singh et al. (1998).

$$\text{Hepatoprotective activity (\%)} = 1 - \frac{[PC - W]}{[C - W]} \times 100$$

where,
PC, C, and W are the measurable variables in rats treated with *P. americana* leaf extract plus CCl_4, CCl_4 and distilled water treated animals respectively.

Protein estimation

Protein concentration was determined by using the Bradford method based on the absorbance maximum of Coomassie Brilliant Blue G-250, at 595nm.

Preparation of liver post mitochondrial fraction

Liver homogenate (10% w/v), was prepared by homogenizing 1g of hepatic tissue sample with 10ml of ice-cold homogenizing buffer (8mM Na_2HPO_4, 12mM NaH_2PO_4, 1.15% KCl, pH 7.4). The homogenate was centrifuged at 12,000rpm for 20min., at 4 °C to obtain a supernatant called post mitochondrial fraction.

Antioxidant assays

The extent of lipid peroxidation was estimated in the post mitochondrial fraction by quantitative determination of thiobarbituric acid reactive substances, and protein carbonyls according to the methods described by Buege and Aust (1978), and Levine et al. (1990), respectively. The amount of glutathione (GSH), in the post mitochondrial fraction was determined by the method of Sedlak and Lindsay (1968), based on the ability of GSH to reduce 5,5'-Dithio-Bis [2-Nitrobenzoic Acid], (DTNB) forming a highly colored yellow anion that can be measured colorimetrically at 412nm. Catalase (CAT), activity was assayed by the *in vitro* spectrophotometric method of Aebi (1984), which monitors the breakdown of hydrogen peroxide (H_2O_2) at 240nm. Superoxide dismutase (SOD)], activity was estimated by the method of Misra and Fridovich (1972), based on the inhibitory effect of SOD in the initial rate of epinephrine auto-oxidation at elevated pH. Glutathione peroxidase (GPx), activity was determined by the method of Paglia, and Valentine (1962), using the RANSEL kit supplied by Randox Laboratories (U. K). Glutathione S-transferase (GST), activity was assayed by the method of Habig et al. (1974), which involves the catalysis of 1-chloro-2, 4-dinitrobenzene (CDNB), conjugation by GST to form 2, 4-dinitrobenzene-S-glutathione.

Histopathological examination

Livers of rats from different groups per-fused with 10% neutral formalin solution were dehydrated and embedded in paraffin. Paraffin sections were made and stained using haematoxylin-eosin (H & E). The stained sections were examined under a microscope for histopathological changes in liver architecture, and their photomicrographs were taken.

Statistical analysis

The data obtained were analyzed using one-way analysis of variance (ANOVA), and expressed as mean ± SEM. Statistical significance of the difference of the means was evaluated. A value of $p < 0.05$ was considered significant.

Results
Biomarkers of liver function

The effects of pre-treatment with AEPA on serum AST, ALT and ALP activities, and total bilirubin concentration in CCl_4-intoxicated rats are shown in Table 1. Intoxication of rats with CCl_4 caused hepato-cellular damage as shown by significant elevation ($p < 0.05$), in the activities of serum AST (83 %), ALT (586 %), and ALP (195 %), compared to control. However, pre-treatment of rats with AEPA for 7, days (at both 100 and 200mg kg^{-1} b. wt), protected the rats against CCl_4-induced hepato-toxicity as evidenced by reductions in the activities of the hepatic enzymes in the serum. Pre-treatment with 100mg kg^{-1} b. wt. of AEPA decreased AST, ALT and ALP activities were decreased by 43 %, 66 % and 28 %, respectively. Similarly, pre-treatment of rats with 200 mg kg^{-1} b. wt. of AEPA decreased these enzymes by 57 %, 63 % and 20 %, respectively

Table 1: Effects of pre-treatment with aqueous leaf extract of *P. americana* (AEPA) on CCl_4-induced liver damage in rats

Treatment (mg/kg)	AST (U/L)	ALT (U/L)	ALP (U/L)	TBL (µmol/L)
Control	64.2 ± 11.6	11.7 ± 3.3	27.6 ± 9.3	12.8 ± 6.2
CCl_4 + AEPA (100)	67.4 ± 15.0^a	27.7 ± 9.3^a	58.5 ± 8.0	31.1 ± 8.1^a
CCl_4 + AEPA (200)	50.1 ± 5.4^a	29.7 ± 5.2^a	65.6 ± 3.8	10.8 ± 1.5^b
CCl_4 + Reducdyn (100)	57.1 ± 1.5^a	24.9 ± 4.7^a	51.5 ± 8.6	21.1 ± 2.7^b
CCl_4 only	117.4 ± 20.7^b	80.5 ± 23.8^b	81.5 ± 21.2	48.5 ± 18.9^a

AEPA = Aqueous extract of *P. americana*
ALT = Alanine amino transferase
AST = Aspartate amino transferase
ALP = Alkaline phosphatase
TBL = Total bilirubin
Values are expressed as means $\pm$ SEM (n = 6).
Values not sharing a common superscript differ significantly at $p<0.05$.

Total serum bilirubin was significantly elevated ($p<0.05$), following intoxication of rats with CCl_4, however pre-treatment with 100 and 200 mg kg^{-1} b. wt of AEPA resulted in substantial decreases of 36 % and 78 % respectively. Reducdyn® also decreased activities of AST, ALT, ALP and total bilirubin levels by 51 %, 69 %, 37 % and 57 %, respectively. The calculated percentage protection shows that AEPA was hepatoprotective at both 100 and 200 mg kg^{-1} b. wt. (Table 2).

Table 2: Hepatoprotective activity of aqueous leaf extract of *P. americana* against CCl_4-induced hepatotoxicity in rats

Liver function Indicator	Pre-treatment		
	AEPA		Reducdyn
	(100 mg kg^{-1}) [% protection]	(200 mg kg^{-1}) [% protection]	(100 mg kg^{-1}) [% protection]
AST	94.0	126.5	113.4
ALT	76.7	73.9	80.9
ALP	42.7	29.6	55.8
Bilirubin	48.6	105.5	76.7

AEPA = Aqueous extract of *P. americana*
See Table 1 for other abbreviations
These results were comparable to the hepato-protective activity obtained by pre-treatment of rats with Reducdyn®.

Biomarkers of oxidative damage

Table 3 shows the effect of pre-treatment with AEPA on liver antioxidant enzymes system of CCl_4-intoxicated rats. CCl_4 administration resulted in significant elevations ($p < 0.05$), in the activities of CAT and SOD as compared to control rats. Pre-treatment with AEPA at 100mg and 200mg kg^{-1} b. wt. produced significant ($p<0.05$), reductions in the activities of CAT (55 % and 63%), and SOD (57 % and 56 %), respectively.

Liver GSHPx activity was slightly increased in CCl_4-intoxicated rats, but pre-treatment with AEPA at both concentrations lowered GSHPx activity non-significantly in the rats. On the contrary, liver GST was markedly decreased ($p < 0.05$) in CCl_4-intoxicated rats by 84 % compared to

control. Pre-treatment with 100 mg and 200 mg kg^{-1} b. wt. of AEPA caused an increase of 37 % and 13 %, respectively in GST activity while pre-treatment with Reducdyn® increased GST activity by 36 % as compared to CCl$_4$- treated animals. Serum GSH concentration was elevated significantly (p < 0.05), by CCl$_4$ intoxication but pre-treatment with 100 mg and 200 mg kg^{-1} b. wt. of AEPA reduced GSH concentration by 25 % and 42 % respectively. Pre-treatment with the standard drug Reducdyn® decreased GSH concentration by 45 % (Fig. 1). There was no significant difference (p>0.05) in liver GSH concentration across the groups (Figure 2).

Table 3: Effects of pre-treatment with aqueous leaf extract of *P. americana* on antioxidant enzymes in post mitochondrial fraction of CCl$_4$ -induced hepatotoxic rats.

Treatment (Dose, mg kg^{-1})	CAT (U mg^{-1} protein)	GSHPx (U mg^{-1} protein)	SOD (µM mg^{-1} protein)	GST (µM mg^{-1} protein)
Control	0.2 ± 0.01	147.6 ± 51.0	42.8 ± 1.3	26.2 ± 1.8
CCl$_4$ + AEPA (100)	0.3 ± 0.01[a]	135.8 ± 14.4[a]	46.4± 0.85[a]	19.5 ± 1.1[a]
CCl$_4$ + AEPA (200)	0.3± 0.1[a]	136.4 ± 13.8[a]	47.5 ± 1.4 [a]	16.0 ± 1.2[a]
CCl$_4$ + Reducdyn (100)	0.2 ± 0.01[a]	123.0± 34.4[b]	43.3 ± 1.5 [a]	19.3 ± 0.8[a]
CCl$_4$	0.7 ± 0.2[b]	157.0 ± 6.5[a]	108.9 ± 18.3[b]	14.2 ± 1.1[a]

AEPA = Aqueous extract of *P.americana*; CAT = Catalase; GSHPx = Glutathione peroxidase; SOD = Superoxide dismutase; GST = Glutathione S-transferase. Values are expressed as means ± SEM (n = 6). Values not sharing a common superscript differ significantly at p<0.05.
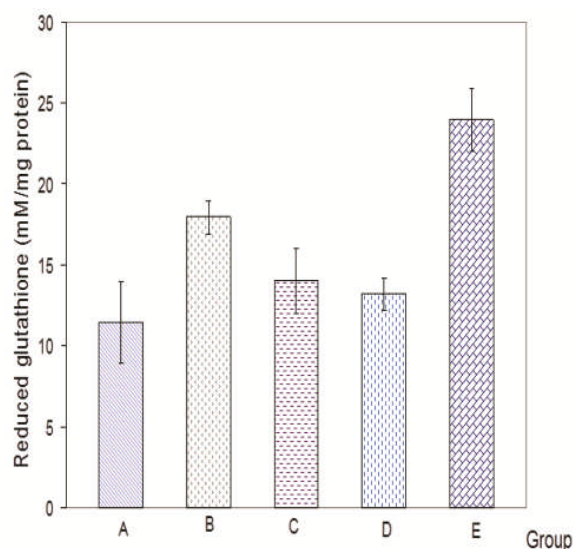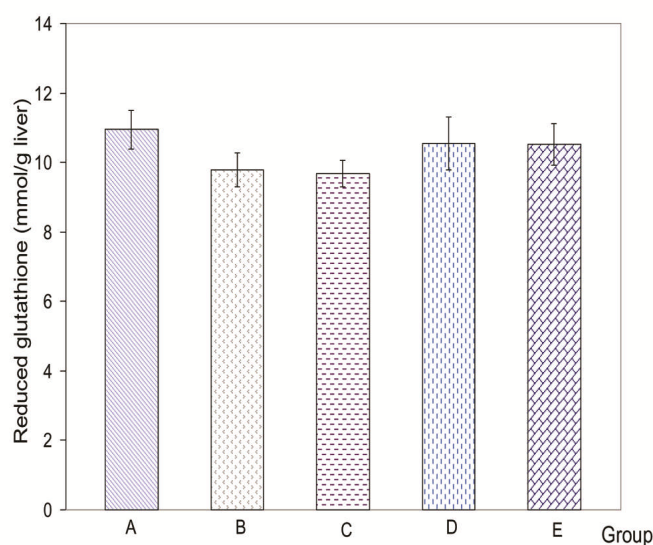

Figure 1: Effect of aqueous extract of *P. americana* on reduced glutathione content in the serum of CCl$_4$-treated rats.
Values are expressed as means ± SEM (n = 6). * Significantly different (p<0.05) from CCl$_4$ treated rats.
A = distilled water; **B** = CCl$_4$ + 100 mg kg^{-1} b.wt AEPA ; **C** = CCl$_4$ + 200 mg kg^{-1} b.wt AEPA*;* **D** = CCl$_4$ + 100 mg kg^{-1} b.wt Reducdyn®; **E** = CCl$_4$ only.

Figure 2: Effects of aqueous extract of *P. americana* on reduced glutathione content in the liver of CCl$_4$-treated rats.

Values are expressed as means ± SEM (n = 6).

A, standard rat chow; **B**, CCl$_4$ + 100 mg kg^{-1} b.wt AEPA ; **C**,CCl$_4$ + 200 mg kg^{-1} b.wt AEPA*;* **D**, CCl$_4$ + 100 mg kg^{-1} b.wt Reducdyn®; **E**, CCl$_4$ only.

The effects of pre-treatment with AEPA on the extent of lipid peroxidation in CCl$_4$-treated rats are presented in Table 4. The levels of MDA and protein carbonyl were used as indices for measuring the extent of lipid peroxidation. Liver MDA and protein carbonyl concentrations in CCl$_4$-intoxicated rats increased by 100% and 61% respectively, as compared to control rats. The increases in MDA and protein carbonyl resulting from CCl$_4$-intoxication were substantially reversed by pre-treatment with 100 mg/kg AEPA by 50 % and 67 %, while 200 mg/kg AEPA decreased these lipid peroxidation products by 65 % and 70 % respectively. Similarly, Reducdyn® decreased these by-products by 51 % and 75 %, respectively.

Table 4: Effect of pre-treatment with aqueous leaf extracts of *P. americana* on the extent of lipid peroxidation in CCl$_4$ -intoxicated rats

Treatment (Dose, mg/kg)	Liver MDA (µM/mg protein)	Liver Carbonyls (µM/mg protein)
Control	0.3 ± 0.1	5.0 ± 0.17
CCl$_4$ + AEPA (100)	0.3 ± 0.1[a]	3.2 ± 0.9[b]
CCl$_4$ + AEPA (200)	0.2 ± 0.01[a]	2.4 ± 0.1[b]
CCl$_4$ + Reducdyn (100)	0.3 ± 0.04[a]	2.0 ± 0.8[b]
CCl$_4$	0.6 ± 0.03[b]	8.0 ± 1.3[a]
AEPA = Aqueous extract of *P. americana*		

Values are expressed as means ± SEM (n = 6).

Values not sharing a common superscript differ significantly at p<0.05.

Histopathology

The photomicrographs obtained from the histo-pathological examination are depicted in Plate 1. Liver sections from normal control rats revealed hepatocytes with numerous portal tracts dividing them into lobules. Livers of CCl$_4$-treated rats showed marked widespread necrosis of hepatocytes with areas of fatty change, ballooning degeneration and diffuse mononuclear infiltration. Pre-treatment with 100 mg and 200 mg kg^{-1} b. wt. AEPA reduced the severity of hepatic damage as shown by the mild and few diffuse fatty changes and less periportal necrosis, respectively.
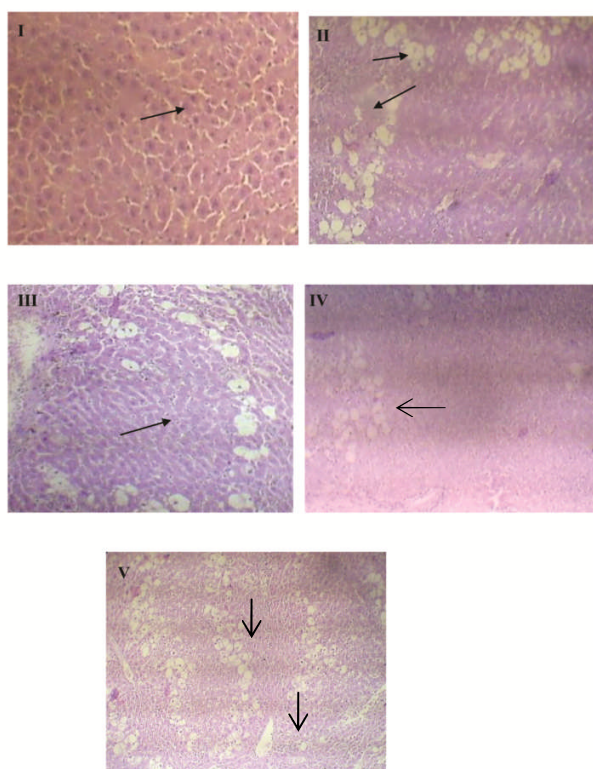

Plate 1: Photomicrographs of liver sections of rats H & E x 100. **I** (Control): Normal liver architecture and hepatocytes with numerous portal tracts dividing them into lobules; **II** (CCl$_4$ + 100 mg/kg AEPA): Mild, diffuse fatty change, periportal necrosis and mononuclear cell infiltration; **III** (CCl$_4$ + 200 mg/kg AEPA): Mild, diffuse fatty change with less periportal necrosis; **IV** (CCl$_4$ + Reducdyn®): Diffuse fatty change and mild mononuclear infiltration; **V** (CCl$_4$ only): Extensive necrosis of hepatocytes with areas of fatty change and diffuse mononuclear infiltration.

Discussion

In order to ascertain whether the aqueous leaf extract of *P. americana* (AEPA), would reduce hepatic lipid accumulation in fatty liver disease and ameliorate liver damage, rats were pre-treated with 100 and 200 mg kg^{-1} b. wt. AEPA for 7, days before challenging them with CCl$_4$ (3 ml/kg b. wt.). Administration of CCl$_4$ to rats markedly increased serum AST, ALT and total bilirubin levels. Increase in the levels of serum aminotransferases is known to reflect the severity of liver injury (Lin et al., 1996). The leakage of large quantities of enzymes into the blood stream is associated with massive centrilobular necrosis, ballooning degeneration and cellular infiltration of the liver. Serum ALP and bilirubin levels are related to the function of the hepatic cell and increase in serum level of ALP is due to increased synthesis of this enzyme (Moss and Butterworth, 1974). The increase in the transaminases and alkaline phosphatase is a clear indication of cellular leakage and loss of functional integrity of the membrane resulting from liver damage (Saraswat et al., 1993). This study demonstrated that pre-treatment of rats with AEPA caused substantial decreases in ALP and bilirubin levels and this decline was significant for bilirubin at extract concentration of 200 mgkg^{-1} b. wt. Effective controls of bilirubin level and ALP activity point towards an early improvement in the secretory mechanism of the hepatic cell (Gupta et al., 2004). The significant reduction in liver enzymes and bilirubin after pre-treatment with AEPA suggests that the extract is hepatoprotective (Sakr et al., 2011) Histopathological analysis and the decrease in the serum transaminases levels provided supportive evidence that pre-treatment with AEPA reduced the severity of toxic injuries caused by CCl$_4$ administration. The reduction in the severity of necrosis and fatty infiltration observed in molecular architecture also showed that AEPA has hepatoprotective activity against CCl$_4$-induced damage in these rats. The observed hepatoprotection by AEPA suggests that the extract tends to prevent liver damage and suppress the leakage of enzymes into the blood stream by preserving hepatocyte membranes. The calculated percentage hepatoprotection also showed that the administration of AEPA was substantially hepatoprotective, and was comparable to the standard drug Reducdyn® used in this study. The hepatoprotective activity of AEPA is similar to that exhibited by hepatoprotective activity against CCl$_4$ exhibited by *Bauhinia racemosa* (Gupta et al., 2004), *Bupleurum kaoi* (Wang et al., 2004), *Telfairia occidentalis, Amaranthus caudatus, Occimum graticimum* (Salawu and Akindahunsi, 2007), *Acalypha racemosa* (Iniaghe et al., 2008), *Vernonia amygdalina* (Adesonoye and Farombi, 2010) and *Rumex crispus* (Maksimovic et al., 2011).

The mechanism by which CCl$_4$ causes liver damage involves the biotransformation of CCl$_4$ by the cytochrome P-450 enzyme system to the toxic trichloromethyl free radical (CCl$_3$$^{\bullet}$), and then transforming this free radical into a more reactive trichloromethyl peroxyl radical (CCl$_3$O$_2$$^{\bullet}$), which causes lipid peroxidation, disrupts Ca^{2+} homeostasis, and eventually kills cells (McCay et al., 1984; Recknagel *et al*., 1989). Elevation in the levels of end products of lipid peroxidation in the liver of rat treated with CCl$_4$ was observed. The increases in MDA and protein carbonyl levels in these rat livers suggest occurrence of lipid peroxidation. This observation is similar to earlier reports that there is an elevation of MDA in liver of rats treated with CCl$_4$ which is attributed to enhanced lipid peroxidation, leading to tissue damage and failure of antioxidant defense mechanisms to prevent the formation of excessive free radicals (Shenoy et al., 2001; Wang et al., 2004). Pre-treatment with AEPA decreased MDA concentrations and significantly reduced protein carbonyl levels. Thus, suggesting that the mechanism of hepatoprotection of AEPA may be due in part to its antioxidant effect. It has been suggested that the protective effect of plant extracts against CCl$_4$-induced liver damage may be attributed to the presence of constituents including flavonoids, tannins, triterpenoids and alkaloids (Gilani and Janbaz, 1995; Tran et al., 2001; Gupta et al., 2004). Flavonoids are known to be antioxidants, free radical scavengers and anti-lipoperoxidants which cause hepato-protection (Yuting et al., 1990; Cook and Samman 1996; Khalid et al., 2002; Al-Qarawi, et al*.,* 2004; Mankani et al., 2005). The hepatoprotective effect of *P. americana* against CCl$_4$-induced liver damage could also be attributed in part to its antioxidant effect and free radical scavenging activity as shown in the decreased MDA and protein carbonyl levels in this study. Thus, eliminating deleterious effects of toxic metabolites from CCl$_4$ and inducing liver cell regeneration. It is possible that trichloromethyl radical or lipid peroxides generated by CCl$_4$ treatment may be scavenged by the extract resulting in depression of lipid peroxidation in the liver. The antioxidant and free radical scavenging activity of AEPA could be due to its constituent flavonoids and phenolic compounds (Arukwe et al., 2012). The elevation of serum GSH in CCl$_4$-intoxicated rats agrees with the findings of Harisch and Meyer, (1985). Increased GSH level is known to represent increased GSH synthesis due to transcriptional activation of the ɣ-glutamyl cysteinyl synthetase gene (Mari and Cederbaum, 2000). Up regulation of these antioxidant genes may reflect an adaptive mechanism to detoxify CYP2E1-derived oxidants. The elevation of serum GSH in this study may be due to the presence of free radical generated by CCl$_4$-intoxication. Pre-treatment with AEPA decreased GSH concentration that was elevated in response to the toxicant. However, it was observed that hepatic GSH was not depleted in the animals. GSH plays a pivotal defensive role against oxidative insult as an endogenous scavenger of free radicals and maintenance of liver GSH under conditions of increased lipoperoxidation has been suggested as a supportive and compensatory mechanism (Cooper and Kristal, 1997; Spolarics and Meyenhofer, 2000). Also, CCl$_4$ is known to cause lipid peroxidation but do not deplete GSH (Jaeschke et al., 2002). These observations and the free radical scavenging activity of the extract could explain the non-depletion of GSH in the liver of rats in this study. CCl$_4$-intoxication also caused significant elevation in SOD and CAT activities and an increase in GSHPx activity in the liver of CCl$_4$-intoxicated rats. It is known that under oxidative stress some endogenous antioxidant protective factors such as SOD and CAT are activated in the defense against oxidative injury (Kyle et al., 1987; John et al., 2001). The increase in enzymes activities in the liver observed in this study was probably in response to increased reactive oxygen species generation induced by CCl$_4$ administration. Similarly, CCl$_4$ may cause oxidative stress and the consequent up-regulation of antioxidant enzymes to render cells more resistant to subsequent oxidative damage (Halliwell, 2000). In this study, pre-treatment with AEPA decreased the activities of CAT, SOD and GSHPx that were raised by CCl$_4$-intoxication. The extract may have scavenged the free radicals generated thereby decreasing lipid peroxidation and oxidative stress in the animals. Hepatic GST is known to be released into the serum after treatment with CCl$_4$ (Aniya and Anders, 1985; Recknagel *et al*., 1989). The reduction in GST activity in this study could be due to the release of the enzyme into the serum following CCl$_4$-intoxication. Hepatic GST activity was however recovered by pre-treatment with AEPA.

These results showed that AEPA possesses significant protective effects against CCl$_4$-induced hepatotoxicity in rats and the hepato-protection appears to be dose dependent. The mechanism of the hepatoprotection seems to involve the modulation of the antioxidant enzyme systems. These beneficial effects may be attributed to the individual or combined action of the phytoconstituents present in the extract. Therefore, it is pertinent to further determine, isolate and purify the exact bioactive constituents with the potential hepatoprotective property.

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