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Full Length Article

In vivo Antioxidant Effect of Methanolic Extract of *Afzelia africana* Seed on Carbon Tetrachloride-induced Acute and Chronic Oxidative Injury in Rats

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Abstract

The *in-vivo* antioxidant effect of the methanol extract of *Afzelia africana* SM seed on carbon tetrachloride (CCl_4)-induced oxidative stress in rats was investigated. In the acute liver injury experiment, rats were intraperitoneally pre-treated with the extract (5 mg/kg) for three days before CCl_4 intoxication at 0.6 mL/kg, while in the chronic liver injury experiment, rats were administered the extract (2.5 mg/kg) for ten consecutive days, with 72 h administration of CCl_4 (0.3 mL/kg body weight) following initial CCl_4 intoxication (0.6 mL/kg). Results showed a significantly higher levels ($p<0.05$) of packed cell volume (PCV), haemoglobin, superoxide dismutase (SOD) and catalase activities in the extract-treated rats compared to the CCl_4 control. Also, there was a significant reduction ($p<0.05$) in the levels of malondialdehyde (MDA) in the organs of *A. africana* seed extract – treated rats compared to CCl_4 control. There results indicate that the seeds of *A. africana* commonly consumed in eastern and central Nigeria possess antioxidant properties that could protect the kidney and liver from both acute and chronic injuries caused by oxidative stress. © 2014 Friends Science Publishers

Keywords: *Afzelia africana*; Antioxidant effect; Hepatoprotection; Disease chemoprevention; Oxidative stress

Introduction

Oxidative stress is among the major causative factors in the induction of many chronic and degenerative diseases including atherosclerosis, diabetes mellitus, cancer, Parkinson's disease, immune dysfunction and ageing (Souri *et al*., 2008). Antioxidants, can be effective in the prevention of free radical formation by scavenging or promotion of their decomposition and thus, suppress such disorders. Hence, there is growing interest in natural antioxidants from herbal and food plant sources (Atawodi, 2010; Atawodi and Onaolapo, 2010; Ghanbari *et al*., 2012; Zia-Ul-Haq *et al*., 2012a) as epidemiological and laboratory studies have strongly supported their therapeutic and protective efficacies in biological systems (Souri *et al*., 2008; Atawodi, 2011a, b). Besides, vegetables, spices and herbs are rich in natural antioxidants that protects against oxidative stress and thus play important roles in the therapy and chemoprevention of diseases that have their etiology and pathophysiology in reactive oxygen species (Atawodi *et al*., 2010a; b; Atawodi, 2011a; Manzoor *et al*., 2012).

Afzelia africana SM, (family *Leguminosae;* sub-family C*aesalpinaceae*), called "Ojawala" and "ukpehie" in the Igbo and Igala languages of Nigeria, respectively, is largely found wild in the fringing and the drier parts of the forest regions of Africa. It has a broad and rather open crown and massive branches with conspicuous hard blackish fruits, height of up to 30.5 m and seeds that are waxy orange with cup-like structure at their base. It is used in Nigeria and other African countries as a soup thickening ingredient (Ejikeme *et al.*, 2010). The foliage contains proteins, while the seeds contain about 27% crude proteins, with the 18% of the dry seed being oil (Eddy and Udoh, 2005). The phytochemical and nutritional constituents as well as the importance of different parts of the plants in human and animal nutrition have been reported (Akinpelu *et al*., 2008; Ojiako *et al*., 2010). In addition, the leaf is used in traditional medicine for management of pain, gastrointestinal disorders, gonorrhea, vomiting and for internal bleedings or haemorrhage (Akinpelu *et al*., 2008).

Despite these numerous nutritional and medicinal uses, information comparing the acute and chronic *in-vivo* antioxidant effect of the seeds of *A. africana* are hard to find in literature. Therefore, since it is used as food on a regular basis, it was thought necessary to evaluate its therapeutic and chemopreventive value in chronic and acute liver disease models.

Materials and Methods

Plant Collection and Authentication

A. africana SM seeds and plant parts were collected from Achalla village in Awka North Local Government of Anambra State Nigeria. It was authenticated Mallam Musa Muhammed at the Herbarum Unit of the Department of

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Biological Sciences, Ahmadu Bello University, Zaria, Nigeria where voucher number 900245 was assigned.

Sample Preparation and Extraction

A. africana seeds were dried at room temperature and pulverized using laboratory mortar and pestle. Fifty grams (50 g) of the sieved sample was weighed and extracted beginning with petroleum ether (for 9 h) to free the sample from lipid, followed by methanol (5 h × 3 times) using soxhlet extractor. The methanol extracts were combined and taken to dryness *in vacuo* at 45^{o}C using rotary evaporator (Büchi Labortechnik AG, Switzerland), weighed with Metler analytical balance and stored at 4^{o}C until required (Atawodi *et al*., 2012; Zubair *et al*., 2012).

Experimental Animals

Male albino rats (7-8 weeks old) and weighing between 120 and 150 g were purchased from the animal house of the National Research Institute for Chemical Technology (NARICT), Basawa Zaria, Kaduna State, Nigeria. They were preconditioned for two weeks prior to experimentation. Rats were maintained *ad-libitum* on tap water and growers mash (Vital feeds, Bukuru Jos, Plateau State, Nigeria) and weighed prior to commencement and at termination of the experiment.

***In vivo* Antioxidant Activity of Plant Extract**

In the chronic liver damage model, rats were divided into 7 groups, with five rats in each group. Group 1 was administered the plant extract only, group 2 was administered plant extract and carbon tetrachloride, group 3 was administered solvent only (corn oil), group 4 was administered solvent and carbon tetrachloride, group 5 was administered vitamin E only, group 6 was administered vitamin E and carbon tetrachloride, and group 7 was untreated control. Carbon tetrachloride was administered at a dose of 0.6 mL/kg body weight before the administration of the first extract or vitamin E, and subsequently at 0.3 mL/kg every 72 h for 10 days. After the first day, extract and vitamin E (10 mg/kg) were administered daily at a dose of 2.5 mg/kg for10 days (Atawodi, 2011c; Asuku *et al*., 2012).

In the acute liver injury model designed to study the protective effects of the methanol extract of the seed extract of *A. africana*, rats were divided into 7 groups as in the chronic experiment, with each group containing 5 rats**.** Animals were pre-treated with the extract (5 mg/kg) or vitamin E (50 mg/kg) for three days before intoxication with carbon tetrachloride (0.6 mL/kg), which was administered one hour after the extract or vitamin E treatment on the third day. After animals sacrifice, major organs and blood were collected and serum separated and and stored at -20^{0}C for assay of biochemical parameters (Atawodi, 2011c; Asuku *et al*., 2012).

Tissue Collection, Storage and Homogenization

Major organs of the rats were harvested after sacrifice, rinsed immediately in ice cold normal saline and stored in ice. The tissues were homogenized after collection with phosphate buffer (pH7.4) and centrifuged at 3000 x *g* for 10 min. The clear supernatants were collected in Eppendorf tubes for *in vivo* assay. At the point of sacrifice, blood from each rat was withdrawn from carotid artery at the neck and collected in previously labeled test tubes and allowed to stand for 4 h. Clear serum were collected from the blood in Eppendorf tubes and stored under -20^{o}C for biological assays (Atawodi, 2011a, b; Asuku *et al*., 2012).

Determination of Lipid Peroxidation Level

The level of lipid peroxidation was determined as MDA based on the principle that lipid peroxidation generates peroxide intermediates, which upon cleavage release MDA, a product which reacts with thiobarbituric acid to form a coloured complex that absorbs light at 535 nm. In the procedure, 1 mL of 14% trichloroacetic acid was measured into a test tube followed with 1 mL of thiobarbituric acid and 50 µL of the tissue homogenate. The mixture was incubated at 80^{o}C for 30 min in a water bath and allowed to cool rapidly under tap water before centrifugation at 3000×*g* for 10 min before the absorbance of the clear supernatant was read spectrophotometrically at 535 nm.

Antioxidant Activities

Catalase: Catalase (CAT) activity was measured using the procedure reported by Abei (1979). Briefly, the method is as follows: 10 µL of serum was added to test tube containing 2.8 mL of 50 mM phosphate buffer (pH 7.0). The reaction was initiated by adding 0.1 mL of fresh 30 mM H_2O_2 and the decomposition rate of H_2O_2 was measured at 240 nm for 5 min. on a spectrophotometer (Jenway 640 UV/Vis). A molar extinction coefficient of 0.0411 $mM^{-1}cm^{-1}$ was used to calculate catalase activity.

Superoxide Dismutase

Assay for superoxide dismutase (SOD) activity was performed according to the procedure described by Usha *et al.* (2007), based on the principle that there is an SOD-mediated decrease in the rate of auto-oxidation of hematoxylin in aqueous alkaline solution, which yields a chromophore with maximum absorbance at 560 nm, and hence can be measure spectrophotometrically. The procedure involved exactly 920 µL of phosphate buffer (pH 7.8), which was added into clean test tube containing 40 µL of sample, mixed and incubated for 2 min at 25^{o}C, before 40 µL of hematoxylin was added, mixed quickly and the absorbance was measured at 560 nm.

Determination of Packed Cell Volume and Hemoglobin Level

Packed cell volume (PVC) and haemoglobin concentration were determined by collecting whole blood into heparinized capillary tubes, filled up to two-thirds of the length during animal sacrifice, sealing with plasticine and centrifuging at 3000 × *g* for 10 min. Packed cell volume was determined using a hematocrit reader, and expressed as percentage erythrocytes content of the blood, while haemoglobin concentration was calculated by dividing the PCV concentration by three.

Statistical Analysis

The results obtained were statistically analyzed using Analysis of Variance (ANOVA) followed by Duncan's Multiple Range Test (DMRT) to separate means with significant difference. The significance level was set at $p<0.05$.

Results

Effect of *A. africana* Seed Methanolic Extract on Levels of Hemoglobin and PCV after Chronic and Acute Oxidative Stress

In the chronic liver injury model, the CCl_4 control group showed lower percentage level of PCV and haemoglobin concentration which was significantly ($p<0.05$) different from the untreated control group. However, administration of methanolic extract of *A. africana* seed elevated these parameters to the levels that there were no significant ($p>0.05$) difference with respect to packed cell volume and haemoglobin concentration among the groups treated with *A. africana* extract, vitamin E and normal control (Tables 1, 2).

Malondialdehyde in Rats Organs during Chronic or Acute Oxidative Stress

Assessment of the effect of concomitant administration of low doses of methanol extract of *A. africana* seed on chronic oxidative stress condition showed that while there was increase in the level of MDA in the liver, kidney and heart of control rats administered CCl_4 only, which was significantly ($p<0.05$) higher than all the other groups, administration of *A. africana* extract, brought the MDA content to a level that there were no significant difference ($p>0.05$) between the groups treated with *A. africana* extract and vitamin E for all the organs (Tables 3 and 4). However, MDA levels in these two groups were significantly lower ($p<0.05$) than the CCl_4 control group for both the chronic (Table 3) and acute (Table 4) experiments.

Antioxidant Enzymes in Rats during Oxidative Injuries

Evaluation of the antioxidant enzyme catalase, revealed a lower activity for CCl_4 control group that was significantly ($p<0.05$) different from the untreated control group with respect to the liver, kidney, heart and the serum. However, for these organs, there were no significant differences ($p>0.05$) between the groups treated with extract and vitamin E, although these groups were significantly

Table 1: Mean percentage haemoglobin concentration and packed cell volume (PCV) following daily intraperitoneal administration of the methanolic extract of *A. africana* seed extract (2.5mg/kg) with 72 h injection of carbon tetrachloride (0.3 mL/kg) for 10 days

Group	Treatment	Packed Cell Volume %	Hemoglobin Concentration
i.	*A. africana* only	51 ± 3^{b}	16.9 ± 0.85^{b}
ii.	*A. africana* + CCl_4	49 ± 2^{b}	16.3 ± 0.73^{b}
iii.	Solvent only	50 ± 3^{b}	16.77 ± 0.85^{b}
iv.	CCl_4 only	41 ± 2^{a}	13.73 ± 0.80^{a}
v.	Vit E only	51 ± 3^{b}	17.1 ± 1.02^{b}
vi.	Vit E + CCl_4	49 ± 2^{b}	16.37 ± 0.75^{b}
vii.	Normal control	50 ± 2^{b}	16.5 ± 0.75^{b}

Values are Mean ± SD; Values having different letters across the column are significantly different ($p<0.05$)

Table 2: Mean percentage haemoglobin concentration and percentage packed cell volume in rats intoxicated with carbon tetrachloride (0.6 mL/kg) following three days pre-treatment with the methanolic extract of *A. africana* seed extract (5.0 mg/kg)

Group	Treatment	Packed Cell Volume (%)	Hemoglobin concentration
i.	*A. africana* only	46 ± 4^{b}	15.4 ± 0.99^{b}
ii.	*A. africana* + CCl_4	47 ± 1^{b}	15.67 ± 0.39^{b}
iii.	Solvent only	45 ± 1^{ab}	14.9 ± 0.37^{ab}
iv.	CCl_4 only	47 ± 4^{b}	15.5 ± 1.22^{b}
v.	Vit E only	45 ± 3^{ab}	14.93 ± 0.99^{ab}
vi.	Vit E + CCl_4	44 ± 1^{ab}	14.67 ± 0.39^{ab}
vii.	Normal control	42 ± 4^{a}	14.03 ± 1.20^{a}

Values are Mean±SD; Values having different letters across the column are significantly different ($p<0.05$)

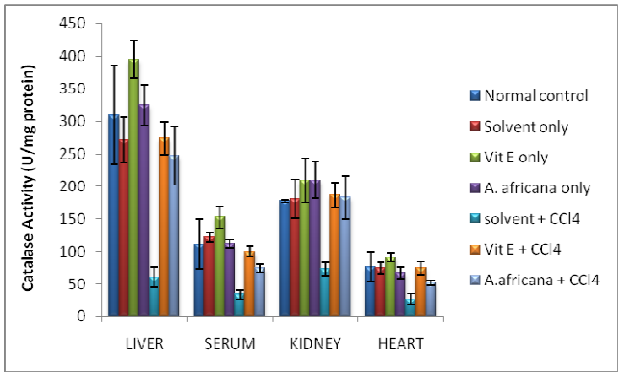

Fig. 1: Catalase activity in the liver, serum, kidney and heart following daily intraperitoneal administration of the methanolic extract of *A. africana* seed extract (2.5 mg/kg) with 72 h injection of carbon tetrachloride (0.3 mL/kg) for 10 days

Table 3: Mean MDA levels in the liver, kidney and heart homogenates following daily intraperitoneal administration of the methanolic extract of *A. africana* seed extract (2.5mg/kg) with 72 h injection of carbon tetrachloride (0.3 mL/kg) for 10 days

Group	Treatment	MDA levels (nmol/mg protein)		
		Liver	Kidney	Heart
i.	*A. africana* only	93±17^{a}	249±68^{a}	192±59^{a}
ii.	*A. africana* + CCl_4	189±9^{b}	282±75^{a}	311±15^{b}
iii.	Solvent only	204±18^{b}	237±42^{a}	372±91^{bc}
iv.	CCl_4 only	546±83^{c}	460±109^{b}	480±62^{d}
v.	Vit E only	125±40^{a}	197±60^{a}	165±30^{a}
vi.	Vit E + CCl_4	204±19^{b}	200±61^{a}	316±84^{b}
vii.	Normal	235±37^{b}	253±37^{a}	426±83^{cd}

Values are Mean±SD; Values having different letters across the column are significantly different (p<0.05)

Table 4: MDA levels in the liver of rats intoxicated with carbon tetrachloride (0.6 mL/kg) following three days pre-treatment with the methanolic extract of *A. africana* seed extract (5.0 mg/kg)

Group	Treatment	MDA Concentration (nmol/mg protein)		
		Liver	Kidney	Heart
i.	*A. africana* only	629±171^{a}	762±122^{b}	348±118^{a}
ii.	*A. africana* + CCl_4	621±133^{a}	765±74^{b}	375±59^{a}
iii.	Solvent only	707±230^{ab}	618±97^{a}	416±78^{ab}
iv.	CCl_4 only	998±124^{c}	1032±87^{c}	512±124^{ab}
v.	Vit E only	536±79^{a}	767±124^{b}	422±109^{ab}
vi.	Vit E + CCl_4	756±155^{ab}	774±71^{b}	509±166^{ab}
vii.	Normal control	891±186^{bc}	660±74^{ab}	553±147^{b}

Values are Mean ± SD; Values having different letters across the column are significantly different (p<0.05)

(p<0.05) higher than the CCl_4 control group in the chronic (Fig. 1) and acute (Fig. 2) models of liver injury. I addition, the group treated with vitamin E showed higher activities in the serum and heart, and these were significantly (p<0.05) different from the activities of the extract-treated group (Fig. 2).

Fig. 3 presents data on the effect of methanol extract of *A. africana* seed on superoxide dismutase in rats under chronic oxidative stress, while those in Fig. 4 shows the corresponding effect in the acute stress model. The CCl_4 control group showed a significantly (p<0.05) lower activities of SOD in the liver, kidney, heart and the serum compared with the normal control group, but there were no significant (p>0.05) differences between groups treated with extracts, vitamin E and untreated control. Also there was no significant difference (p>0.05) between the vitamin E only treated group and the untreated control in all the organs and serum Fig. 3. Although to a varying extent, similar results were obtained for the acute experiment Fig. 4.

Discussion

The significant (p<0.05) decrease in the packed cell volume (PCV) and haemoglobin concentration of the CCl_4 control group may be due to red blood cell destruction (Collen *et al*., 2003), which in this experiment, is connected with

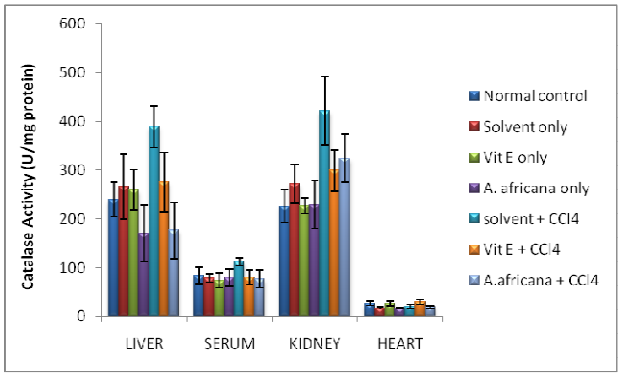

Fig. 2: Catalase activity in the liver, serum, kidney and heart of rats intoxicated with carbon tetrachloride (0.6 mL/kg) following three days pre-treatment with the methanolic extract of *A. africana* seed extract (5.0 mg/kg)

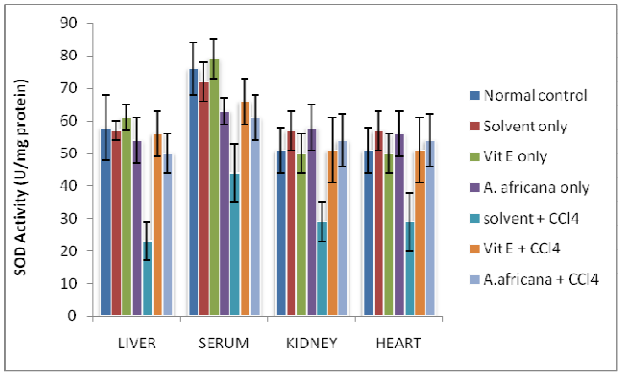

Fig. 3: Superoxide dismutase activity in the liver, serum, kidney and heart following daily intraperitoneal administration of the methanolic extract of *A. africana* seed extract (2.5 mg/kg) with 72 h injection of carbon tetrachloride (0.3 mL/kg) for 10 days

CCl_4 toxicity. Thus, low PCV might suggest oxidative stress-related red blood cell destruction in the CCl_4-control group, while the higher level of PCV in the vitamin E and *A. africana* extract-treated group in both the chronic and acute experiments (Table 1) suggest the potency of the extract to alleviate or prevent oxidative-stress related damage to red blood cell membrane (Atawodi, 2011c; Ahmad et al., 2012a, b; Asuku *et al*., 2012).

Carbon tetrachloride (CCl_4) intoxication leads to formation of lipid peroxides, which in turn produce MDA that cause damage to cell membranes (Siddhartha *et al*., 2011). That the groups of rats administered extract of *A. africana* showed significantly lower level of MDA in the liver, kidney and heart compared to the CCl_4 control group (Table 2) suggest that the lipid peroxidation of membranes caused by CCl_4 intoxication was prevented or ameliorated by administration of the methanolic extract of the seed of *A. africana* extract and thus implying that this seed contain antioxidant compounds that were capable of scavenging free radicals from the tissues of the treated rats (Atawodi, 2011c;

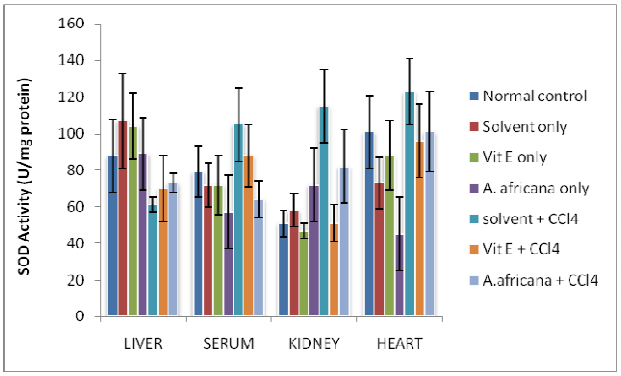

Fig. 4: Superoxide dismutase activity in the liver, serum, kidney and heart of rats intoxicated with carbon tetrachloride (0.6 mL/kg) following three days pre-treatment with the methanolic extract of *A. africana* seed extract (5.0 mg/kg)

Asuku *et al*., 2012).

Analysis of catalase, which is found mainly in peroxisomes of the kidney and the liver, and to a lesser extent in the cytosol and microsomal fractions of the cell, together with superoxide dismutase, which is abundant in the mitochondria, was based on the fact that this enzyme catalyzes the decomposition of hydrogen peroxide (H_2O_2) to water and oxygen and the removal of hydrogen peroxide from the cell by catalase is believed to provide protection against oxidative damage cells (Collen *et al*., 2003). Hence, the significant ($p<0.05$) elevation in the activities of superoxide dismutase and catalase in all the organs and serum of the extract-treated groups compared to the CCl_4 control group strongly suggest high anti-oxidative effect of the *A. africana* seed methanolic extract (Collen *et al*., 2003). These endogenous antioxidant enzymes are effective because conversion of superoxide anion to hydrogen peroxide and oxygen by SOD is the primary defense mechanism against oxidative stress since superoxide is such a strong initiator of chain reactions. Here then seems to lay the main function of the endogenous antioxidant enzymes, superoxide dismutase and catalase.

The ability of the methanolic extract of the seed of *A. africana* to exhibit significant antioxidant capacity is not surprising since earlier work has established that other African foods of Nigerian origin, including *Moringa oleifera* (Atawodi *et al.,* 2010a), Palm oil (Atawodi *et al.,* 2011c), *Canarium schweinfurthii* oil (Atawodi *et al.,* 2010), *Syzygium aromaticum* (Atawodi *et al.*, 2010b), *Hibiscus esculentum* (Atawodi *et al.*, 2009a) and *Dacryodes edulis* (Atawodi *et al.*, 2009c) have been demonstrated to contain appreciable levels of antioxidant polyphenols, especially, flavonoids, that have proven therapeutic potential (Ullah and Khan, 2008; Anwar and Przybylski, 2012). Therefore, it would be interesting to examine this seed for its contents of antioxidant compounds including polyphenols and antioxidant vitamins Bafeel and Ibrahim, 2008; Yao and Vieira, 2011).

In conclusion, the data on levels of MDA and the endogenous enzymes, namely, superoxide dismutase and catalase in the homogenates of major organs in animals of both chronic and acute oxidative stress experiments strongly suggest that regular consumption of *A. africana* seeds as is the case in eastern and north central Nigeria contributes significantly to the capacity of individuals in this population to protect their vital organs, especially the liver and the kidney against oxidative injury arising from exogenous or endogenous sources.

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