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Anthocyanin extract of *Lannea microcarpa* fruits stall oxidative rout associated with aflatoxin B₁ hepatocarcinogenesis

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ABSTRACT

The capability of anthocyanins extract of *Lannea microcarpa* fruits to scavenge reactive oxygen species and stall aflatoxin B₁-mediated oxidative rout on cellular proteins, lipids and DNA was investigated. Reactive oxygen species (ROS) scavenging potentials of the anthocyanins extract of *Lannea microcarpa* fruits (0.2–1.0 mg/mL) was investigated using 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical, superoxide ion, hydrogen peroxide, hydroxyl radical and ferric ion reducing system. Anthocyanins extract at 1.0 mg/mL scavenged DPPH, superoxide ion, hydrogen peroxide, and hydroxyl radical by 91%, 88%, 90% and 83%, respectively, it also reduced ferric ion significantly. The specific activities of reactive oxygen species detoxifying enzymes (superoxide dismutase, catalase, glutathione peroxidase, glutathione reductase and glucose 6-phosphate dehydrogenase) were significantly ($P < 0.05$) induced by *Lannea microcarpa* fruits anthocyanins. These inductions significantly ($P < 0.05$) attenuated the aflatoxin B₁-mediated decrease in reactive oxygen species detoxifying enzymes and compared favorably with Vitamin C. Aflatoxin B₁-mediated elevation in the concentrations of conjugated dienes, lipid hydroperoxides, malondialdehyde, protein carbonyl and fragmented DNA were significantly ($P < 0.05$) lowered by *Lannea microcarpa* fruits anthocyanins. Overall, the results of this study show that *Lannea microcarpa* fruits anthocyanins possesses antioxidant properties and halted aflatoxin B₁-mediated oxidative rout on cellular proteins, lipids and DNA.

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1. Introduction

Dietary exposure to food-borne mycotoxins, aflatoxin B₁ (AFB₁), is responsible for the high incidence of liver cancer, growth retardation, decreased immunity and vaccination failure in Africa (Bankole, Schollenberger, & Drochner, 2006).

In the liver, cytochrome P2E1 catalyzed the oxidation of 8,9-vinyl bond in AFB₁ to biologically active AFB₁-8,9-epoxide (Scholl, Musser, & Groopman, 1997). The epoxide has been found to produce DNA and protein adducts in both *in vitro* and *in vivo* (Wang & Groopman, 1999). In addition to the genotoxic action, AFB₁ produces genetic damage by

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enhancing reactive oxygen species (ROS) accumulation (Shen & Ong, 1996). This thus shows involvement of the enhanced ROS production in the oxidative damage associated with AFB₁ mutagenicity, carcinogenicity and hepatotoxicity.

Studies have proven that dietary antioxidants/free radicals and ROS scavengers such as silymarin (Rastogi, Srivastava, & Rastogi, 2001; Rastogi, Srivastava, & Rastogi, 2000), crocetin (a natural carotenoid) (Wang, Hsu, & Lin, 1991), green tea (Muto, Fujita, Yamazaki, & Kamataki, 2001; Qin, Gopalan-kriczky, Su, Ning, & Lotlikar, 1997), and butylated hydroxyanisole (Choi, Park, Park, & Cha, 1991) could halt oxidative damage associated with AFB₁ hepatocarcinogenesis. These dietary antioxidants are widely distributed in cereals, fruits, vegetables and medicinal plants (Krishnaswamy & Polasa, 2001). One of the fruit that is widely used in treatment of liver related disease in Nigeria folklore is *Lannea microcarpa* fruit.

Lannea microcarpa Engl & K. Krauss (Anacardiaceae) is a wild fruit tree found in the Sudano-Sahelian regions of Africa. The tree grows up to 16 m. The bark is gray white with a spiral twist; it is smooth when the tree is young, becoming splintery with age. The slash is red. The leaves are comprised of 1–3 pairs of asymmetrical leaflets, plus the terminal one. The leaflets are ovate-lanceolate, obtuse and unequal at the base; they are 5.5–13 cm long and 2.7–4.5 cm wide. The new growth has short, close and simple hairs. The flowers are small, green yellowish with glabrous sepals. The flowers crowd at the end of the branches.

In Nigeria, leaves, bark, roots and fruits are applied to treat mouth blisters, rheumatism, sore throat, dysentery, as a cathartic and as a dressing on boils. Fruits of *L. microcarpa* are commonly consumed (and used in the production of local drinks) and are also utilized in traditional medicine to treat scurvy, rickets and cough. It contains 1300 mg/100 g of anthocyanins in the dry epicarp. Palé, Nacro, and Kouda-Bonafas (1998) reported the presence of cyanidin 3-O-(2-O-β-D-xylopyranosyl) β-D-galactopyranoside and cyanidin 3-O-β-D-galactopyranoside.

Despite the folkloric claim of *Lannea microcarpa* in the management of liver diseases and the presence of antioxidants, little or no study exist in literature to support or refute this claim. This study thus investigates the antioxidant capability of *Lannea microcarpa* fruits' anthocyanin extract in the oxidative stress associated with AFB₁ hepatocarcinogenesis.

2. Materials and methods

2.1. Materials

2.1.1. Plant materials

Lannea microcarpa fruits was obtained from Gaa Akanbi area in Ilorin, Kwara state Nigeria. The plant sample was authenticated and deposited at the Herbarium unit of Forestry Research Institute of Nigeria, Ibadan, Oyo state with voucher no: FHI. 109507.

2.1.2. Chemicals and assay kits

Diphenylamine 5,5'-Dithio-bis(2-nitrobenzoic acid), guanidine hydrochloride, and N-ethyl-maleimide (NEM) were procured from Research Organics, Cleveland, Ohio, USA. Superoxide

dismutase (SOD), glutathione peroxidase (GSH-Px), glutathione reductase (GSH-Red) and glucose 6-phosphate dehydrogenase (Glc-6-PD) were products of Randox Laboratories Ltd., Co. Antrim, United Kingdom. All other reagents used were supplied by Sigma-Aldrich Inc., St. Louis, USA.

2.1.3. Experimental animals

Healthy, 2 months, male albino rats (*Rattus norvegicus*) of Wistar strain, weighing 195.28 ± 4.2 g, were obtained from Animal House of the Department of Veterinary Physiology, Biochemistry and Pharmacology, University of Ibadan, Nigeria. They were kept in clean plastic cages contained in well-ventilated house conditions with free access to feeds (Capfeed Ltd., Osogbo, Nigeria) and tap water. The animals were used according to the Guidelines of National Research Council Guide for the Care and Use of Laboratory Animals (National Research Council, 2011) and in accordance with the principles of Good Laboratory Procedure (GLP) (WHO, 1998).

2.2. Methods

2.2.1. Preparation of anthocyanin extract of *Lannea microcarpa* fruits

The anthocyanin extract of *Lannea microcarpa* fruits was prepared using the procedure described by Ajiboye et al. (2011). The anthocyanin extract was stored in dark bottled and kept in a refrigerator until used.

2.2.2. Determination of in vitro antioxidant activity of anthocyanin extract of *Lannea microcarpa* fruits

2.2.2.1. DPPH radical scavenging assay. The DPPH radical scavenging activity of anthocyanin extract of *Lannea microcarpa* fruits was determined by measuring the capacity to bleach a purple-coloured ethanol solution of 2,2-diphenyl-1-picrylhydrazyl (DPPH), as described by Turkoglu, Duru, Mercan, Kivrak, and Gezer (2007). Briefly, 2 mL of various concentrations (200–1000 µg/mL) of the samples in ethanol were added to 2 mL of 0.2 mmol/L sample of DPPH in ethanol. After 30-minute incubation period at room temperature, the absorbance was read against blank at 517 nm. Inhibition rate (I%) on the DPPH radical was calculated using the following expression:

$$\text{Percentage Inhibition (I\%)} = \{(A_{\text{blank}} - A_{\text{sample}}) / A_{\text{blank}}\} \times 100$$

where A_{blank} is the absorbance of the blank, A_{sample} is the absorbance of the sample.

2.2.2.2. Superoxide anion radical (O_2^-) scavenging assay. The scavenging effect of anthocyanin extract of *Lannea microcarpa* fruits on superoxide anion radical was determined by the spectrophotometric measurement of the product formed on reduction of nitroblue tetrazolium (Yen & Chen, 1995). Briefly, superoxide anion radical was generated in a non-enzymic system. The reaction mixture contained 1 mL of the anthocyanin extract (0.2–1.0 mg/mL) in distilled water, 1 mL of 60 µM of phenazine methosulfate (PMS) in phosphate buffer (0.1 M, pH 7.4), 1 mL of 468 µM of NADH in phosphate buffer, and 1 mL of 150 µM of nitroblue tetrazolium (NBT) in phosphate buffer and was incubated at ambient temperature for

5 min, and the absorbance was read at 560 nm against blank samples.

2.2.2.3. Hydrogen peroxide (H_2O_2) scavenging assay. H_2O_2 scavenging activity of anthocyanin extract of *Lannea microcarpa* fruits was determined according to the procedure described by [Ruch, Cheng, and Klaunig \(1989\)](#). Briefly, 3.4 mL of varying concentrations (200–1000 $\mu\text{g/mL}$) of the anthocyanin extract in phosphate buffered saline (pH 7.4) were mixed with 0.6 mL of 40 mM H_2O_2 . The absorbance was read at 230 nm after 10 min of incubation at room temperature. The percentage H_2O_2 scavenging activity of anthocyanin extract of *L. microcarpa* fruits was calculated using the following expression:

$$\text{Percentage } H_2O_2 \text{ scavenged (\% } H_2O_2) = \frac{A_{\text{control}} - (A_{\text{sample}} - A_{\text{extract}})}{A_{\text{control}}} \times 100$$

where A_{control} is the absorbance of the mixture without extract, A_{sample} is the absorbance of the mixture with the extract and A_{extract} is the absorbance of the extract alone.

2.2.2.4. Hydroxyl radical ($OH^{\bullet}$) scavenging assay. $OH^{\bullet}$ scavenging activity of anthocyanin extract of *Lannea microcarpa* fruits was measured as described by [Smirnoff and Cumbes \(1989\)](#). Briefly, 2 mL of anthocyanin extract at concentrations ranging from 200 to 1000 $\mu\text{g/mL}$, 0.6 mL of 8 mM ferrous sulfate, 0.5 mL of 20 mM H_2O_2 and 2 mL of 3 mM salicylic acid were mixed and incubated at 37 °C for 30 min. Then, 0.9 mL of distilled water was added to each vial. The final solution was centrifuged at 4472g for 10 min after which the absorbance was read at 510 nm. The percentage $OH^{\bullet}$ scavenging activity of anthocyanin extract of *Lannea microcarpa* fruits was calculated using the following expression:

$$\text{Percentage } OH^{\bullet} \text{ scavenged (\% } OH^{\bullet}) = \frac{A_{\text{control}} - (A_{\text{sample}} - A_{\text{extract}})}{A_{\text{control}}} \times 100$$

where A_{control} is the absorbance of the mixture without the extract, A_{sample} is the absorbance of the mixture with the extract and A_{extract} is the absorbance of the extract alone.

2.2.2.5. Reducing power. The reducing power of anthocyanin extract of *Lannea microcarpa* fruits was evaluated by adopting the procedure described by [Oyaizu \(1986\)](#). Varying amounts (0.2–1.0 mg/mL) of anthocyanin extract were suspended in 1 mL of distilled water and mixed with 2.5 μL of 0.2 M phosphate buffer (pH 6.6) and 2.5 mL of 1% potassium hexacyanoferrate [$K_3Fe(CN)_6$]. The mixture was incubated at 50 °C for 20 min, after which 2.5 μL of trichloroacetic acid was added to the mixture. This was centrifuged at 402g for 10 min after which 2.5 μL of the supernatant was mixed with an equal amount of distilled water and 0.5 mL of 0.1% $FeCl_3$. The absorbance of the resulting solution was read at 700 nm.

2.2.3. Modulation of redox perturbation in AFB₁ hepatocarcinogenesis study

Thirty-five male rats were randomly grouped into 7 of 5 rats each. The rats in group A served as the control and received the vehicle for drug administration (0.5% DMSO). Group B rats were gavaged with AFB₁ (20 $\mu\text{g/day}$) in 0.5% DMSO starting from the third week to the end of the experimental period.

Group C, D, E and F received 400, 100, 200 and 400 of anthocyanin extract of *L. microcarpa* fruits for 6 weeks respectively. In addition, groups D, E and F rats were treated with AFB₁ (20 $\mu\text{g/day}$) starting from the third week of the experimental period. Rats in group G were treated like groups D, E and F, but received 100 mg/kg body weight of vitamin C instead of anthocyanin extract of *L. microcarpa* fruits. The rats were sacrificed 24 h after the last treatment, and livers from individual animals were used for isolation of subcellular fractions.

2.2.4. Preparation of serum and tissue homogenates

The procedure described by [Yakubu, Oladiji, and Akanji \(2009\)](#) was employed for the preparation of serum and tissue supernatants.

2.2.5. Biochemical assay

The activities of alkaline phosphatase (ALP), alanine and aspartate aminotransferases (ALT and AST) were determined as described by [Wright, Leathwood, and Plummer \(1972\)](#) and ([Bergmeyer, Hørdler, & Rej, 1986a, 1986b](#)) respectively. SOD, Catalase, GSH-Px, GSH-red and Glc 6-PD activities were assayed according to the procedures described by [Misra and Fridovich \(1972\)](#), [Beers and Sizer \(1952\)](#), [Rotruck et al. \(1973\)](#), [Mavis and Stellwagen \(1968\)](#) and [Kornberg and Horecker \(1955\)](#) respectively. Levels of glutathione reduced and oxidized were assay as described by respectively. The concentration of protein carbonyl in the liver homogenates was determined according to the procedure described by [Levine, Garland, Oliver, Amici, and Clement \(1990\)](#). The concentrations of conjugated dienes, lipid hydroperoxides and malondialdehyde were assessed according to the procedure described by [Bus, Costa, Hodgson, Lawrence, and Reed \(2001\)](#). The quantity of fragmented DNA was quantified according to the procedure described by [Burton \(1956\)](#).

2.2.6. Statistical analysis

Free radical and ROS scavenging activities were expressed in percentage. Results from *in vivo* study were expressed as the mean of five determinations $\pm$ SD. Analysis of variance (ANOVA) followed by Tukey–Kramer test for differences between means was used to detect any significant differences ($P < 0.05$) between the treatment groups in this study using StatPlus, 2011.

3. Results

3.1. In vitro reactive oxygen species scavenging effects of anthocyanin extract of *Lannea microcarpa* fruits

The anthocyanin extract of *Lannea microcarpa* fruits concentration dependently scavenged DPPH, $O_2^{\bullet}$, H_2O_2 and $OH^{\bullet}$, with the highest concentration (1 mg/mL) in each case producing 90.78, 88.03, 90.20 and 82.99% scavenging effects respectively ([Figs. 1–4](#)). All these were similar to that produced by vitamin C, a reference antioxidant ([Figs. 1–4](#)) used in this study. Furthermore, the reducing effect of the anthocyanin extract of *Lannea microcarpa* fruits on ferric ion was similar to that produced by ascorbic acid ([Fig. 5](#)).

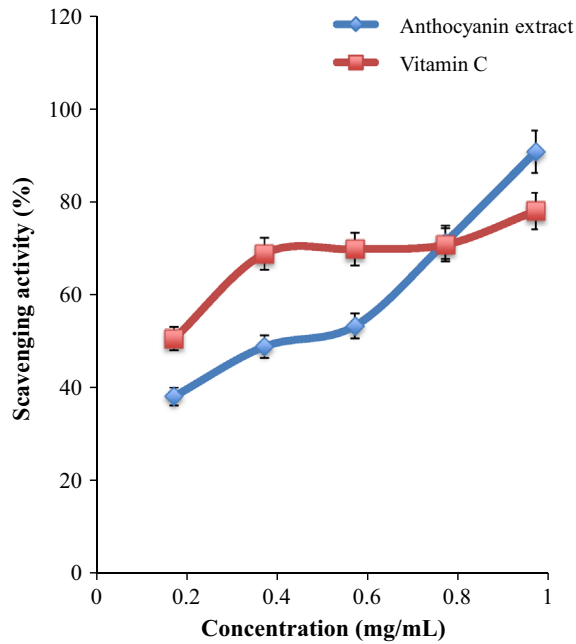


Fig. 1 – DPPH scavenging activity of anthocyanin extract of *Lannea microcarpa* fruits.

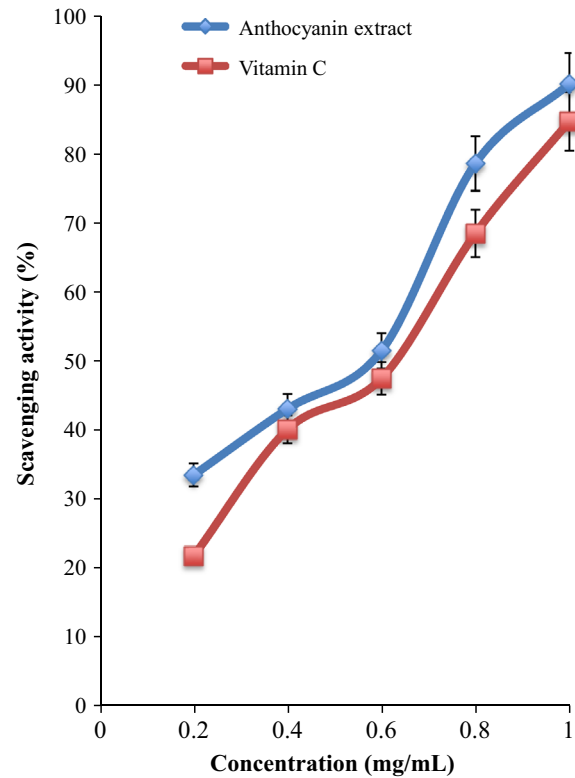


Fig. 3 – Hydrogen peroxide scavenging activity of anthocyanin extract of *Lannea microcarpa* fruits.

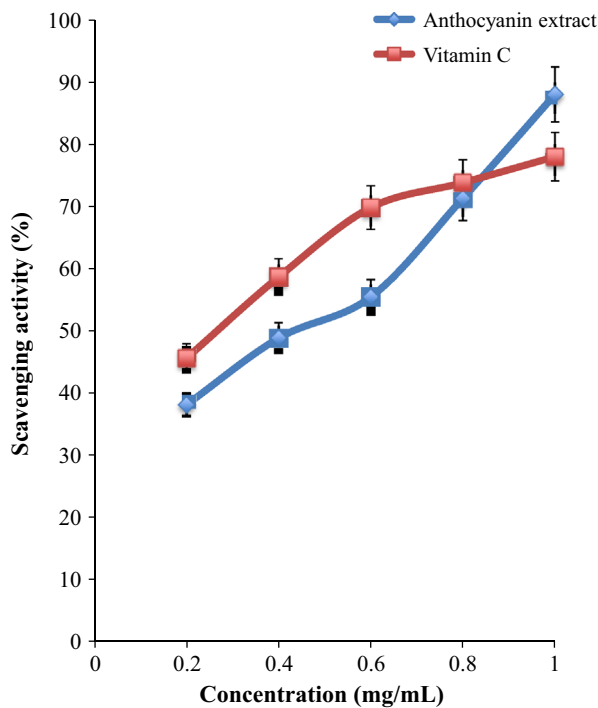


Fig. 2 – Superoxide ion scavenging activity of anthocyanin extract of *Lannea microcarpa* fruits.

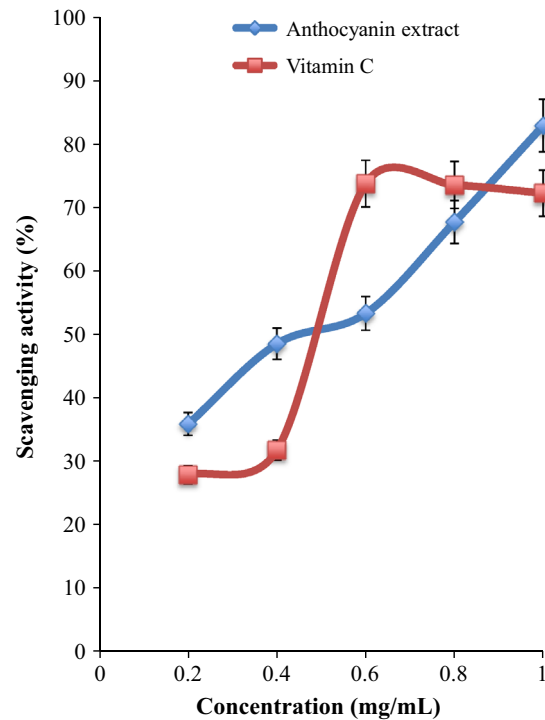


Fig. 4 – Hydroxyl radical scavenging activity of anthocyanin extract of *Lannea microcarpa* fruits.

3.2. Modulation of redox perturbation in AFB₁ hepatocarcinogenesis

3.2.1. Hepatocellular enzymes

There was significant decrease ($P < 0.05$) in the specific activities of ALP, ALT and AST in the liver of AFB₁-treated rats. These decrease resulted to corresponding increase in the activities of these enzymes in the serum (Table 1). Treatment

of rats with anthocyanin extract of *Lannea microcarpa* fruits at the doses investigated significantly ($P < 0.05$) reversed this trend as the activity of the liver and serum enzymes

compared favorably ($P > 0.05$) with that of the distilled water treated group. Similarly, the activities of the liver and serum enzymes (ALP, ALT and AST) of the animals simultaneously administered with Vitamin C and AFB₁ compared well with the control (Table 1).

3.2.2. Reactive oxygen detoxifying proteins

Reactive oxygen species detoxifying enzymes (SOD, CAT, GSH-Px, GSH-Red and Glc 6-PD) were significantly ($P < 0.05$) reduced in AFB₁-treated rats (Table 2). In addition to the increase in the activities of ROS detoxifying enzymes following the administration of anthocyanin extract of *Lannea microcarpa* fruits alone, the extract completely attenuated AFB₁-mediated decrease in these enzymes (Table 2).

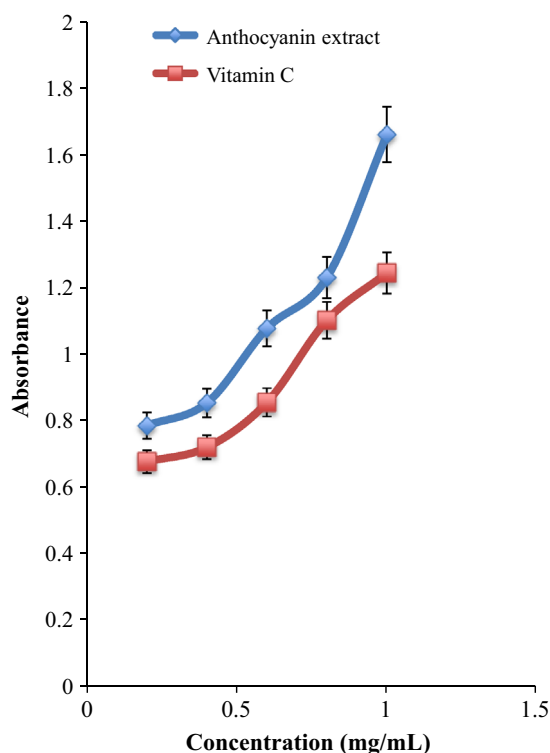


Fig. 5 – Reducing activity of scavenging activity of anthocyanin extract of *Lannea microcarpa* fruits.

AFB₁ treatment resulted to significant reduction in the level of the non-enzymatic antioxidants glutathione reduced (GSH) in the liver of rats when compared to the control. Conversely, the concentration of peroxidised glutathione (GSSG) in the liver increased significantly ($P < 0.05$). Furthermore, GSH:GSSG ratio decreased significantly following the administration of AFB₁ (Table 3). Administration of anthocyanin extract of *Lannea microcarpa* fruits significantly ($P < 0.05$) reversed the AFB₁-mediated alterations in the level of these non-enzymatic proteins (Table 3).

3.2.3. Lipid peroxidation

The levels of lipid peroxidation products (conjugated dienes, lipid hydroperoxides and malondialdehyde) were significantly raised in the liver of AFB₁-treated rats (Table 4). The AFB₁-mediated increase in the levels lipid peroxidation products were significantly ($P < 0.05$) reduced in the liver of rats by the anthocyanin extract of *Lannea microcarpa* fruits.

3.2.4. Protein oxidation

Oxidation of protein as indicated by the level of protein carbonyl increased significantly ($P < 0.05$) in the liver of AFB₁-treated rats. Although, treatment of rats with anthocyanin extract of *Lannea microcarpa* fruits produced no change in the level of protein carbonyl, anthocyanin extract of *L. microcarpa* fruits significantly ($P < 0.05$) reduced the protein carbonyl level in AFB₁-treated rats (Table 5).

3.2.5. DNA fragmentation

In a similar vein, there was a significant ($P < 0.05$) increase in the fragmented DNA in the liver of AFB₁-treated rats. Anthocyanin extract of *Lannea microcarpa* fruits significantly ($P < 0.05$) reversed the AFB₁-mediated increase in fragmented DNA (Table 5).

4. Discussion

Wise use of fruits, medicinal plants, and vegetables requires investigations into the phytochemicals and antioxidants as well as possible medicinal properties and prospective products, such as nutraceuticals and phytomedicines (Oloyede, Ajiboye, Komolafe, & Salau, 2013).

Table 1 – Specific activities of hepatic marker enzymes following the administration of anthocyanin extract of *Lannea microcarpa* fruits to aflatoxin B₁-treated rats.

Treatment/tissues	Alkaline phosphatase		Alanine aminotransferase		Aspartate aminotransferase	
	Liver	Serum	Liver	Serum	Liver	Serum
0.5% DMSO (control)	6.83 ± 0.35 ^a	0.049 ± 0.001 ^a	60.25 ± 2.04 ^a	4.52 ± 0.11 ^a	90.73 ± 2.43 ^a	12.98 ± 0.13 ^a
AFB ₁ treated	1.44 ± 0.01 ^b	0.532 ± 0.002 ^b	19.25 ± 1.14 ^b	12.23 ± 0.69 ^b	47.27 ± 1.23 ^b	24.31 ± 0.99 ^b
400 mg/kg body weight of extract	6.53 ± 0.12 ^a	0.031 ± 0.001 ^a	61.42 ± 0.22 ^a	4.70 ± 0.08 ^a	91.17 ± 2.13 ^a	11.74 ± 0.18 ^a
AFB ₁ +100 mg/kg body weight of extract	3.99 ± 0.03 ^c	0.420 ± 0.001 ^c	27.32 ± 1.32 ^c	10.23 ± 0.39 ^c	57.32 ± 2.26 ^c	19.01 ± 0.05 ^c
AFB ₁ +200 mg/kg body weight of extract	4.72 ± 0.31 ^a	0.173 ± 0.002 ^c	40.79 ± 0.21 ^d	7.50 ± 0.56 ^c	69.47 ± 1.65 ^c	15.09 ± 0.01 ^c
AFB ₁ +400 mg/kg body weight of extract	6.39 ± 0.15 ^a	0.061 ± 0.001 ^a	57.57 ± 0.80 ^a	5.05 ± 0.21 ^a	87.91 ± 2.37 ^b	11.89 ± 0.13 ^a
AFB ₁ + 100 mg/kg body weight of Vitamin C	6.10 ± 0.01 ^a	0.056 ± 0.001 ^a	54.70 ± 2.30 ^a	5.91 ± 0.21 ^a	87.07 ± 2.61 ^a	12.92 ± 0.01 ^a

Data are mean of five determinations ± SD. Specific enzyme activities are expressed as nmol min⁻¹ mg protein⁻¹. Values carrying superscripts different for the liver and serum of each enzyme are significantly different ($P < 0.05$).

Table 2 – Specific activities of antioxidant enzymes following the administration of anthocyanin extract of *Lannea microcarpa* fruits to aflatoxin B₁-treated rats.

Treatments	Superoxide dismutase (nmol min ⁻¹ mg protein ⁻¹)	Catalase (nmol min ⁻¹ mg protein ⁻¹)	Glutathione peroxidase (nmol min ⁻¹ mg protein ⁻¹)	Glutathione reductase (nmol min ⁻¹ mg protein ⁻¹)	Glucose 6-phosphate dehydrogenase (nmol min ⁻¹ mg protein ⁻¹)
0.5% DMSO (control)	63.24 ± 2.15 ^a	31.33 ± 1.32 ^a	303.34 ± 1.07 ^a	50.32 ± 1.26 ^a	27.84 ± 1.30 ^a
AFB ₁ treated	24.66 ± 0.14 ^b	5.86 ± 2.58 ^b	81.26 ± 1.23 ^b	18.45 ± 0.11 ^b	8.23 ± 0.14 ^b
400 mg/kg body weight of extract	80.25 ± 2.36 ^c	50.42 ± 0.18 ^c	593.21 ± 1.70 ^c	81.23 ± 1.76 ^c	26.25 ± 1.21 ^c
AFB ₁ +100 mg/kg body weight of extract	33.30 ± 1.60 ^d	17.16 ± 1.52 ^d	201.31 ± 1.26 ^d	29.42 ± 0.80 ^d	14.41 ± 0.31 ^d
AFB ₁ +200 mg/kg body weight of extract	44.12 ± 0.46 ^e	26.61 ± 0.21 ^e	275.11 ± 1.32 ^e	41.78 ± 1.14 ^e	21.15 ± 0.22 ^d
AFB ₁ +400 mg/kg body weight of extract	59.50 ± 2.80 ^a	33.09 ± 1.13 ^a	324.32 ± 0.21 ^a	47.02 ± 2.11 ^f	24.28 ± 1.26 ^a
AFB ₁ + 100 mg/kg body weight of Vitamin C	60.30 ± 2.12 ^a	28.92 ± 1.82 ^a	316.30 ± 1.22 ^a	43.21 ± 2.20 ^f	21.03 ± 1.51 ^a

Data are mean of five determinations ± SD. Values carrying superscripts different for each parameter are significantly different (*p* < 0.05).

4.1. Free radical and reactive oxygen species

Scavenging effect on DPPH radical is one of the models for testing for antiradical properties of chemical compounds including plant extract. Since free radical-mediated chain reaction (initiation, propagation, and termination) is a process in lipid peroxidation (Gago-Dominguez, Castela, Pike, Sevanian, & Haile, 2005). Thus, the DPPH radical scavenging activity produced by the anthocyanin extract of *Lannea microcarpa* fruits is an indication that it can terminate lipid peroxidation by donating hydrogen.

The ROS (•OH, O₂^{•-} and H₂O₂) scavenging activities of the anthocyanin extract of *Lannea microcarpa* fruits in this study shows its possible potentials to complement the ROS detoxifying enzymes *in vivo*, if effectively absorbed. It could also help terminate lipid peroxidation that could arise from O₂^{•-}, and the stronger ROS (O[•], •OH and H₂O₂) (Yen & Duh, 1994). Also, the capability of *L. microcarpa* fruits' anthocyanin extract to significantly reduce K₃Fe(CN)₆, shows its effectiveness to halt the oxidation of cellular macromolecules by oxidizing molecules that could arise from the metabolism of either drugs or toxins.

4.2. Hepatocellular marker enzymes

The significant decrease in specific activities of ALP, a marker enzyme for the plasma membrane, in the liver of AFB₁-treated rats with a corresponding increase in the serum shows compromise of the integrity of the plasma membrane (Akanji, Olagoke, & Oloyede, 1993). This could be due to loss of membrane component through peroxidation of the polyunsaturated fatty acids of plasma membrane by ROS (O₂^{•-}, •OH and H₂O₂) generated during AFB₁ metabolism (Towner, Qian, Kadiiska, & Mason, 2003). The capability of anthocyanin extract of *Lannea microcarpa* fruits to prevent the AFB₁-mediated alteration in ALP could be due to the free radical and ROS scavenging capabilities of anthocyanins extract of *Lannea microcarpa* fruits.

The reduction in the specific activities of ALT (cytosolic) and AST (cytosolic and mitochondrial) in the liver of AFB₁-treated rats is not surprising. This is because the pattern of alteration on ALP revealed that the integrity of the plasma membrane had been compromised, thus resulting to leakage of cytosolic content of the cell to the external milieu. The reversal this trend in a manner similar to vitamin C suggests antioxidant potential of the extract. This amelioration may be due to the ability of the extract to scavenge ROS (O₂^{•-}, •OH and H₂O₂) generated during AFB₁ metabolism (Towner et al., 2003).

4.3. Reactive oxygen species detoxifying enzymes

ROS detoxifying enzymes (SOD, CAT, GSH-Px, GSH-Red and Glc 6-PD) counteract redox imbalance-mediated oxidative onslaught of cellular macromolecules such as lipid, protein, DNA etc. (Ajiboye et al., 2010). The reduction in specific activities of these ROS detoxifying enzymes in the liver of AFB₁-treated rats could be due to excessive mobilization of the antioxidant enzymes toward the detoxification of ROS (O₂^{•-}, •OH, RO₂[•] and H₂O₂) during AFB₁ carcinogenesis

Table 3 – Levels of non-enzymatic antioxidants following the administration of anthocyanin extract of *Lannea microcarpa* fruits to aflatoxin B₁-treated rats.

Treatments	Glutathione (reduced) (nmol mg protein ⁻¹)	Glutathione (oxidized) (nmol mg protein ⁻¹)	GSH:GSSG ratio
0.5% DMSO (control)	54.62 ± 0.25 ^a	3.06 ± 0.11 ^a	17.85 ± 0.34 ^a
AFB ₁ treated	16.25 ± 0.62 ^b	20.31 ± 0.18 ^b	0.80 ± 0.09 ^b
400 mg/kg body weight of extract	75.34 ± 0.18 ^c	3.12 ± 0.32 ^a	24.15 ± 0.56 ^c
AFB ₁ +100 mg/kg body weight of extract	44.48 ± 0.61 ^d	14.24 ± 0.16 ^c	3.12 ± 0.14 ^d
AFB ₁ +200 mg/kg body weight of extract	50.72 ± 0.82 ^a	8.08 ± 0.25 ^d	6.28 ± 0.11 ^e
AFB ₁ +400 mg/kg body weight of extract	58.03 ± 1.38 ^e	3.84 ± 0.02 ^a	15.11 ± 0.39 ^a
AFB ₁ + 100 mg/kg body weight of Vitamin C	50.63 ± 0.22 ^a	4.90 ± 0.01 ^e	10.33 ± 0.22 ^f

Data are mean of five determinations ± SD. Values carrying superscripts different for each parameter are significantly different ($P < 0.05$).

Table 4 – Levels of lipid peroxidation products following the administration of anthocyanin extract of *Lannea microcarpa* fruits to aflatoxin B₁-treated rats.

Treatments	Conjugated dienes (nmol mg protein ⁻¹)	Lipid hydroperoxide (nmol mg protein ⁻¹)	Malondialdehyde (nmol mg protein ⁻¹)
0.5% DMSO (control)	50.16 ± 0.46 ^a	44.18 ± 0.90 ^a	6.95 ± 0.68 ^a
AFB ₁ treated	87.18 ± 1.02 ^b	103.29 ± 1.56 ^b	17.57 ± 0.64 ^b
400 mg/kg body weight of extract	50.68 ± 2.52 ^a	40.64 ± 0.12 ^a	6.26 ± 0.23 ^a
AFB ₁ +100 mg/kg body weight of extract	80.46 ± 2.68 ^c	87.66 ± 0.30 ^c	13.54 ± 0.12 ^c
AFB ₁ +200 mg/kg body weight of extract	67.64 ± 0.36 ^d	71.12 ± 0.42 ^c	9.94 ± 0.06 ^d
AFB ₁ +400 mg/kg body weight of extract	52.16 ± 0.37 ^e	47.24 ± 0.82 ^a	6.27 ± 0.32 ^e
AFB ₁ + 100 mg/kg body weight of Vitamin C	56.16 ± 0.68 ^e	49.10 ± 0.23 ^d	7.09 ± 0.16 ^a

Data are mean of five determinations ± SD. Values carrying superscripts different for each parameter are significantly different ($P < 0.05$).

Table 5 – Level of protein carbonyl and fragmented DNA following the administration of anthocyanin extract of *Lannea microcarpa* fruits to aflatoxin B₁-treated rats.

Treatments	Protein carbonyl (nmol mg protein ⁻¹)	Fragmented DNA (%)
0.5% DMSO (control)	3.12 ± 0.21 ^a	2.14 ± 0.08 ^a
AFB ₁ treated	9.57 ± 0.38 ^b	88.18 ± 2.50 ^b
400 mg/kg body weight of extract	3.01 ± 0.01 ^a	3.00 ± 0.31 ^a
AFB ₁ +100 mg/kg body weight of extract	8.32 ± 0.21 ^b	51.32 ± 1.62 ^c
AFB ₁ +200 mg/kg body weight of extract	6.01 ± 0.11 ^c	35.23 ± 0.24 ^d
AFB ₁ +400 mg/kg body weight of extract	3.41 ± 0.25 ^a	19.70 ± 0.23 ^e
AFB ₁ + 100 mg/kg body weight of Vitamin C	4.03 ± 0.02 ^c	20.15 ± 0.34 ^e

Data are mean of five determinations ± SD. Values carrying superscripts different for each parameter are significantly different ($P < 0.05$).

(Towner et al., 2003). These reductions could lead to uncontrolled oxidative attack on the cellular macromolecules resulting to oxidative damage and cell death. Similar reductions in the activities of these enzymes (SOD, CAT, GSH-Px and GSH-Red) were reported to be due to the excessive generation of ROS during AFB₁ hepatocarcinogenesis

(Ramakrishnan, Raghavendran, Vinodhkumar, & Devaki, 2006; Sivaramakrishnan, Shilpa, Praveen Kumar, & Niranjali Devaraj, 2008; Yadav & Bhatnagar, 2007). Thus, significant attenuation of AFB₁-mediated reduction in the specific activities of ROS detoxifying enzymes (SOD, CAT, GSH-Px, GSH-Red and Glc 6-PD) by anthocyanin extract of *L. microcarpa*

fruits might have resulted from the capability of anthocyanin extract to scavenge the ROS generated during AFB₁ metabolism. It might also have resulted from the capability of *L. microcarpa* fruits anthocyanin to enhance ROS detoxifying enzymes. Reports have shown elevation of antioxidant enzymes as well as attenuation of AFB₁-mediated decrease in the levels of antioxidant enzymes by medicinal plants and plant components (Brahmi et al., 2011; Sharma, Sharma, & Gupta, 2011).

4.4. Non-enzymatic antioxidants

The significant ($P < 0.05$) reduction in the level of GSH, a non-enzymatic antioxidant playing complementary role in the prevention of oxidative damage resulting from ROS generated during AFB₁ metabolism might have resulted from the depletion of GSH-Px and GSH-Red, as they have direct relationship with GSH (Kozer et al., 2003). Conversely, the AFB₁-mediated increase in the level of GSSG might have resulted from oxidation of GSH or mobilization of GSH toward production of GSH-Px. The reduction in GSH:GSSG ratio following administration of AFB₁ is an indication that the liver cell is prone to oxidative attack. Thus, preservation of GSH, high GSH:GSSG and low GSSG levels in the liver of AFB₁-treated rats following the administration of anthocyanin extract of *L. microcarpa* fruits indicates the possible antioxidant activity.

4.5. Lipid peroxidation

Lipid peroxidation induces disturbance of membrane organization and functional loss and modification of proteins and DNA bases (Niki, 2009). AFB₁ has been reported to induce lipid peroxidation in numerous *in vitro* and *in vivo* methods of carcinogenesis (Lee, Hye, Lee, & Sook, 2005; Ravinayagam, Jaganathan, Panchanadham, & Palanivelu, 2012; Shen, Shi, Lee, & Ong, 1994; Theumer et al., 2010; Towner, Mason, & Reinke, 2002). The significant increased levels of lipid peroxidation products (conjugated dienes, lipid hydroperoxides and malondialdehydes) in this study show indiscriminate oxidative assaults on cellular lipids. These increases (most especially conjugated dienes) could result to mutation (Das et al., 2010). The capability of the anthocyanin extract to reverse the AFB₁-mediated increases in conjugated dienes, lipid hydroperoxide and malondialdehyde might have resulted from ROS scavenging activity of the extract. It might have also resulted from capability of the extract to promote the detoxification (through the induction of antioxidant enzymes) ROS, which could cause the peroxidation of polyunsaturated fatty acids of plasma membrane.

4.6. Protein oxidation

Protein carbonyl is an indicator of irreversible oxidative damage to cellular proteins and may have lasting detrimental effects on cells and tissues (Dalle-donne, Rossi, Giustarini, Milzani, & Colombo, 2003). Thus, the significant increase in protein carbonyl, a marker of protein oxidation in AFB₁-treated rat could have resulted from the oxidation of protein by free radicals and ROS generated during AFB₁ metabolism.

Attenuation of AFB₁-mediated increase in the level of protein carbonyl by anthocyanin extract of *L. microcarpa* fruits further shows the possible ROS scavenging and its capability to promote detoxification of ROS via induction of antioxidant enzymes. Similar attenuation of AFB₁-mediated increase in protein carbonyl level following the administration of Tridham has been reported (Ravinayagam et al., 2012).

4.7. DNA fragmentation

Oxidative stress and accumulation of calcium ion have been reported to mediate DNA fragmentation (Amin & Hamza, 2005). This damage, which usually results from OH[•] and can lead to either the arrest or induction of transcription, induction of signal transduction pathways, replication errors and genomic instability, all of which are associated with carcinogenesis (Cooke, Evans, Dizdaroglu, & Lunec, 2003). Thus, the significant increase in the level of fragmented DNA in the liver of AFB₁-treated rat shows the genotoxic activity. It also denotes possible initiation of carcinogenesis. Golli-Bennour et al. (2010) reported similar increase in fragmented DNA in AFB₁-treated rats. The reduction in level of fragmented DNA in the liver of AFB₁-treated rat by the anthocyanin extract of *L. microcarpa* fruits shows the antioxidants and antigenotoxic activity. The anthocyanin extract could have acted as an antigenotoxic complex, which enhances the DNA repair system or DNA synthesis (Brahmi et al., 2011).

5. Conclusion

It is evident from the data obtained from this study that the anthocyanin extract of *Lannea microcarpa* fruits enhanced the detoxification of AFB₁ possibly by acting as free radicals and reactive oxygen species scavenger or by enhancing the activities of the reactive oxygen species detoxifying enzymes, thus preventing the oxidation and fragmentation of cellular macromolecules such as DNA, lipids and proteins as well as AFB₁ hepatocarcinogenesis. Hence, anthocyanin extract of *Lannea microcarpa* fruits show promising potentials of being used as a dietary supplements/functional foods because of its prophylactic role.

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