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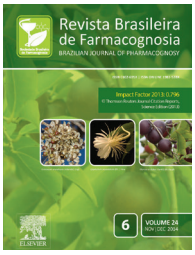

Original article

Phenolic extract of *Parkia biglobosa* fruit pulp stalls aflatoxin B1 – mediated oxidative rout in the liver of male rats

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ABSTRACT

The effect of phenolic extract of *Parkia biglobosa* (Jacq.) R. Br. ex G. Don, Fabaceae, pulp on aflatoxin B_1 induced oxidative imbalance in rat liver was evaluated. Thirty-five male rats were randomized into seven groups of five animals each. Rats in group A served as control and received vehicle for drug administration (0.5% DMSO) once daily at 24 h intervals for six weeks. Rats in groups B, D, E, F and G, received aflatoxin B_1 (167 μg/kg body weight) in 0.5% DMSO for three weeks, starting from the third week of the experimental period. Rats in Group C received 400 mg/kg bodyweight of the extract for six weeks, while groups D, E and F rats were treated with 100, 200 and 400 mg/kg bodyweight of the extract for six weeks respectively. Group G rats received 100 mg/kg body weight of vitamin C. Aflatoxin B_1-mediated decrease in the activities of superoxide dismutase, catalase, glutathione peroxidase, glutathione reductase and glucose-6-phosphate dehydrogenase were significantly attenuated. Aflatoxin B_1 mediated the elevation in malondialdehyde, conjugated dienes, lipid hydroperoxides, protein carbonyl, and significantly lowered DNA fragmentation percentage. Overall, the phenolic extract of *P. biglobosa* pulp stalls aflatoxin B_1-mediated oxidative rout by enhancing antioxidant enzyme activities leading to decreased lipid peroxidation, protein oxidation and DNA fragmentation.

Introduction

Exposure to food-borne mycotoxin aflatoxin B_1 (AFB_1), has been linked to the high incidence of liver cancer, growth retardation, decreased immunity and vaccination failure in Africa (Bankole et al., 2006). Oxidative modification of 8,9-vinyl bond in AFB_1 by cytochrome 2PE1 to metabolize the biologically active AFB_1-8,9-epoxide occurs in the liver (Scholl et al., 1997). AFB_1-8,9-

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epoxide is the ultimate hepatocarcinogen, having been found to produce DNA and protein adducts both *in vitro* and *in vivo* studies (Scholl et al., 1997; Wang and Groopman, 1999). The formation of AFB_1-guanine adducts in hepatic DNA is critical for the carcinogenic effects of AFB_1 in animals which result in mutations of key genes (Kensler et al., 1986).

In addition to the genotoxic and mutagenic actions, accumulated evidence have shown that oxidative damage plays a crtical role in aflatoxin B_1-induced cytotoxicity and carcinogenicity (Towner et al., 2003; Lee et al., 2005). The oxidative damage results from enhanced accumulation of ROS (such as O_2-, H_2O_2 and lipid hydroperoxides) during metabolic processing of AFB_1 by liver cytochrome P450 (Shen et al., 1996; 1995).

Consumption of both/either natural or synthetic antioxidants prevents/limits deleterious effects of oxidative damage when cells are overwhelmed by generated ROS (Ajiboye et al., 2010). Antioxidants such as silymarin (Rastogi et al., 2000; 2001), crocetin (a natural carotenoid) (Wang et al., 1991), green tea (Qin et al., 1997; Muto et al., 2001), and butylated hydroxyanisole (Choi et al., 1991) have been reported to stall oxidative damage associated with AFB_1 hepatocarcinogenesis. One fruit that is widely used for the treatment of liver-related diseases in Nigeria folklore is *Parkia biglobosa*.

Parkia biglobosa (Jacq.) R. Br. ex G. Don is a leguminous tree belonging to the Fabaceae family, which grows in the West Africa savannah (Sosulski et al., 1982). It is locally known as "*dawadawa*" (Hausa), "*iru*" and "*igba*" (Yoruba) (Campbell-Platt, 1980). *Parkia* tree recycles nutrients from deep soils, by holding the soil particles with the aid of roots to prevent soil erosion (Sosulski et al., 1982). The pulp of the fruit pods is rich in sucrose, and the seeds are rich in carbohydrates, proteins and lipids, thus constituting an important source of energy (Djakpo, 2005). The roots, bark, leaves, stems, flowers, fruits and seeds are all used medicinally to treat a range of ailments including diarrhoea, ulcers, pneumonia, burns, coughs and jaundice. The pulp contains phenolics, proanthocyanidins and flavonoids (Compaoré et al., 2011). Despite the folk use of the pulp for treating liver disorders such as jaundice, no study has validated this claim. Thus, this study investigates the effect of the phenolic extract of *P. biglobosa* pulp on AFB_1 mediated oxidative rout towards validating its use in treating liver disorders.

Materials and methods

Experimental animals

Healthy, two month old male albino rats (*Rattus norvegicus*) of Wistar strain (150 ± 2.54 g, n = 35) were obtained from the Animal House of the Department of Veterinary Physiology, Biochemistry and Pharmacology, University of Ibadan, Nigeria. They were kept in clean plastic cages in well-ventilated house conditions with free access to food (Ace Feeds Ltd., Osogbo, Nigeria) and tap water.

Plant materials

Fresh and ripe *Parkia biglobosa* (Jacq.) R. Br. ex G. Don, Fabaceae, fruits was collected at Ido-Osun, Osogbo, Nigeria in November 2011. A plant sample was authenticated and deposited in the Herbarium unit of Forestry Research Institute of Nigeria, Ibadan, Oyo state with Voucher No: FHI. 109508.

Chemicals and assay kits

Aflatoxin B_1 (AFB_1) and dimethyl sulfoxide (DMSO) were purchased from Sigma Chemical Co., St. Loius, MO. Diphenylamine, 5,5′-dithio-bis (2-nitrobenzoic acid), guanidine hydrochloride, *N*-ethylmaleimide (NEM), and salicylic acid 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 obtained from Randox Laboratories Ltd., Co. Antrim, United Kingdom. All other reagents used were supplied by Sigma-Aldrich Inc., St. Louis, USA.

Preparation of Phenolic Extracts

The edible pulp of *Parkia biglobosa* fruit was removed and separated from the seed. The pulp was grounded (162 g) and extracted with 1620 ml of acetone for 24 h with continuous shaking at room temperature to obtain the phenolics. The extracts was concentrated using a rotary evaporator to give 13.83 g and stored at -4°C.

Quantitative determination of total phenolics and flavonoids

Total phenolics

The concentration of phenolic compounds in *P. biglobosa* pulp was determined using the method described by Spanos and Wrostald (1991). Briefly, 2.5 ml of 10% Folin-Ciocalteu reagent and 2 ml of Na_2CO_3 (2% w/v) were added to 0.5 ml each of the extracts solution (1 mg/ml). The resulting mixture was incubated at 45°C under constant shaking for 15 min. The absorbance of the samples was read at 765 nm. This was done in triplicate. The total phenolic content in the extracts was expressed as mg of epicatechin (0-0.5 mg/ml) dissolved in distilled water.

Total flavonoids

The concentration of flavonoids in *P. biglobosa* pulp was determined using the method described by Zhishen et al. (1999). Briefly, the extract (1 ml) was mixed with 3 ml of methanol, 0.2 ml of 10% aluminum chloride, 0.2 ml of 1 M potassium acetate and 5.6 ml of distilled water. The mixture was allowed to stand at room temperature for 30 min. The absorbance of the reaction mixtures was read at 420 nm. The concentration of flavonoids in mg/ml was obtained from the calibration curve of epicatechin solution (0-0.8 mg/ml) in distilled water.

Experimental design

Thirty-five male rats were randomized into seven groups (A-G) of five animals each. The rats in group A served as the control and received the vehicle (0.5% DMSO). Groups B, D, E, F and G, received 167 µg/kg body weight of AFB_1 (20 µg/day) in 0.5% DMSO, a dose capable of inducing hepatocarcinogenesis,

through gavage for three weeks starting from the third week of the experimental period (Yates et al., 2006). Rats in Group C received 400 mg/kg bodyweight of *P. biglobosa* pulp extracts for six weeks, while group D, E and F rats were treated with 100, 200 and 400 mg/kg bodyweight respectively of phenolic extract of *P. biglobosa* pulp for six weeks. Group G rats received 100 mg/kg body weight of vitamin C. This study was approved by Al-Hikmah University Ethical Comittee on the use of laboratory animals (HUI/ECULA14/03/01), which is in accordance to the Guidelines of National Research Council Guide for the Care and Use of Laboratory Animals (NRC, 2011) and principles of Good Laboratory Procedure (WHO, 1998).

Tissue and serum preparation

Serum and liver homogenates were prepared using the procedure described by Yakubu et al. (2009) and Ajiboye et al. (2014), respectively.

Biochemical assays

Hepatocellular enzymes

Alkaline phosphatase (ALP) activity was assayed according to the method described by Wright et al. (1972). The methods described by Bermeyer et al. (1986a, b) were employed for the assay of alanine aminotransferase (ALT) and aspartate aminotransferase (AST).

Superoxide dismutase

The activity of superoxide dimustase (SOD) was determined as described by Misra and Fridovich (1972). Briefly, 0.2 ml of tissue homogenate was added to 2.5 ml of 0.05M carbonate buffer (pH 10.2) to equilibrate and the reaction was started by adding 0.3 ml of freshly prepared 0.3 mM epinephrine. An increase in absorbance was recorded at 480 nm every 30 s for 150 s. One unit of enzyme activity is 50% inhibition of the rate of autoxidation of pyrogallol as determined by changes in absorbance/min at 420 nm.

Catalase

Catalase activity was determined using the described Aebi's method (Aebi, 1984). Fifty microliters of the homogenate was added to a cuvette containing 2 ml of phosphate buffer (pH 7.0) and 1 ml of 30 mM H_2O_2. Catalase activity is measured at 240 nm for 1 min using a spectrophotometer. The molar extinction coefficient of H_2O_2, 43.6 M cm^{-1} was used to determine the catalase activity.

Glutathione peroxidase and glutathione reductase

The activities of glutathione peroxidase and glutathione reductase were determined using the procedure outlined in commercial kits (Randox Laboratories Ltd., Antrim, UK).

Glutathione reduced (GSH) and glutathione disulfide (GSSG)

The level of GSH in the tissue homogenate was determined using the procedure described by Ellman (1959). Briefly, 1.0 ml of tissue homogenate was added to 0.1 ml of 25% trichloroacetic acid (TCA) and the precipitate was removed by centrifugation at 5,000 × *g* for 10 min. Supernatant (0.1 ml) was added to 2 ml of 0.6 mM DTNB prepared in 0.2 M sodium phosphate buffer (pH 8.0). The absorbance was read at 412 nm.

GSSG level was determined using the procedure described by Hissin and Hilf (1976). A total of 50 µl of sample was mixed with 20 µl of 0.04 M *N*-ethylmaleimide (NEM) to prevent oxidation of GSH to GSSG. The mixture was incubated at room temperature for 30 min and 1.68 ml of 0.3 M Na_2HPO_4 solution was added, followed by 250 µl of DTNB reagent. The absorbance of the sample was measured at 412 nm.

Lipid peroxidation products and protein carbonyl

The levels of conjugated dienes, lipid hydroperoxides and malondialdehyde were assayed according to Reilly and Aust (2001). Protein carbonyl concentration was determined according to the procedure described by Levine et al. (1990).

Fragmented DNA

The quantity of fragmented DNA in the liver homogenates was determined using the procedure described by Burton (1956). Briefly, liver homogenate was centrifuged at 15,000 × *g*, for 15 min at 4°C. The supernatant was separated from the pellet and treated with trichloroacetic acid (1.50 ml, 10%). The pellet was treated with trichloroacetic acid (0.65 ml, 5%) as well. The reaction mixtures were allowed to precipitate overnight (≥ 4 h) in a refrigerator (4°C), centrifuged at 2500 × *g* for 10 min. The reaction mixtures were boiled at 100°C for 15 min, cooled to room temperature and further centrifuged at 2500 × *g* for 5 min. The supernatants (0.5 ml) were treated with diphenylamine reagent (1 ml) and incubated at 37°C for 4 h. Absorbance was read at 600 nm using a spectrophotometer. The fragmented DNA was calculated using the following expression:

$$\text{Fragmented DNA (\%)} = \frac{\text{Absorbance of the supernatant}}{\text{Absorbance of the supernatant + Absorbance of pellet}}$$

Statistical analysis

Results were expressed as the mean of five determinations ± SD. An Analysis of variance (ANOVA) followed by Tukey–Kramer test for differences between means were used to detect any significant differences ($p < 0.05$) between the treatment groups. Stat Plus, 2011 Software was used for statistical analysis (Analyst Soft Inc., Alexandria, VA, USA).

Results

Total phenolics and total flavonoids of P. Biglobosa pulp extracts

The phenolics and flavonoid contents of the extract of *P. biglobosa* pulp are 517.11 ± 2.19 mg/ml and 159.13 ± 0.54 mg/ml, respectively.

Cellular enzymes

The administration of AFB_1 (167 µg/kg body weight) alone brought about 74.46, 75.44 and 53.80% decrease in ALP, ALT and AST activities in rat liver respectively compared to controls ($p < 0.05$). This was also accompanied with significant ($p < 0.05$)

increase in the enzymatic activities in the serum (Table. 1). This trend was dose-dependently reversed in the rats pre-treated with *P. biglobosa* pulp extract, as the activity of the liver and serum enzymes compared significantly ($p < 0.05$) to the control. The highest dose of phenolic extract of *P. bliglobosa* (400 mg/kg bodyweight) prevented AFB_1-mediated decrease in the activities of ALP, ALT and AST by 73.12, 71.73 and 50.16%, respectively.

Oxidative stress biomarkers

Antioxidant enzymes

AFB_1 administration significantly ($p < 0.05$) decrease the activities of SOD, catalase, GSH-Px, GSH-red and Glc 6-PD by 69.16, 81.74, 73.48, 63.64 and 69.74%, respectively, when compared to the control. These enzymes were significantly ($p < 0.05$) induced by *P. biglobosa* pulp extract in a dose dependent manner. The inductions significantly ($p < 0.05$) attenuated AFB_1-mediated decrease in these activities (Figs. 1-3); with the highest dose of the extract, 400 mg/kg bodyweight, producing 67.11, 79.70, 72.77, 59.58 and 67.89% reversal of AFB_1-mediated decrease in SOD, catalase, GSH-Px, GSH-red and Glc 6-PD respectively.

Non-enzymatic antioxidant

The level of GSH reduced by 54.61% in the liver of AFB_1-intoxicated rats when compared to controls ($p < 0.05$). Conversely, the level of GSSG increased significantly ($p < 0.05$) when compared to the control. This trend was significantly reversed in rats pre-treated with phenolic extracts of *P. biglobosa* pulp in a dose-dependent manner. The highest dose used in this study produced a 53.35% reversal of AFB_1–mediated reduction in GSH. All the extract treated groups compared favourably ($p < 0.05$) with vitamin C (Table 2).

Lipid peroxidation products, protein carbonyl and DNA fragmentation

AFB_1-mediated elevation in the concentrations of oxidative stress biomarkers; malondialdehyde, conjugated dienes, lipid hydroperoxides, protein carbonyl and percentage DNA fragmentation were significantly ($p < 0.05$) lowered by phenolic extracts of *P. biglobosa* pulp (Tables 3 and 4).

Table 1
Specific activities of hepatic marker enzymes following six weeks of administration of phenolic extract of *Parkia bliglobosa* pulp to aflatoxin B_1-treated rats.

Treatment/tissues	Alkaline phosphatase		Alanine aminotransferase		Aspartate aminotransferase	
	Liver	Serum	Liver	Serum	Liver	Serum
0.5% DMSO (control)	7.40 ± 0.35^a	0.043 ± 0.002^a	62.53 ± 4.81^a	3.92 ± 0.21^a	93.12 ± 0.23^a	9.82 ± 0.16^a
AFB_1 treated	1.89 ± 0.02^b	0.452 ± 0.001^b	15.36 ± 1.67^b	8.03 ± 0.33^b	43.02 ± 1.14^b	20.32 ± 1.05^b
400 mg/kg body weight of extract	7.74 ± 0.23^a	0.050 ± 0.001^a	63.10 ± 0.26^a	3.61 ± 0.40^a	90.20 ± 0.23^a	8.50 ± 0.81^a
AFB_1 + 100 mg/kg body weight of extract	3.92 ± 0.15^c	0.332 ± 0.001^c	29.26 ± 0.34^c	6.12 ± 0.22^c	60.26 ± 0.24^c	16.03 ± 0.20^c
AFB_1 + 200 mg/kg body weight of extract	5.86 ± 0.31^a	0.100 ± 0.001^c	38.08 ± 2.48^d	4.35 ± 0.46^a	73.28 ± 1.24^c	13.60 ± 1.11^c
AFB_1 + 400 mg/kg body weight of extract	7.03 ± 0.51^a	0.050 ± 0.001^a	54.33 ± 0.12^a	3.64 ± 0.12^a	82.02 ± 5.12^b	11.15 ± 0.15^a
AFB_1 + 100 mg/kg body weight of Vitamin C	6.90 ± 0.24^a	0.054 ± 0.002^a	54.17 ± 0.22^a	3.91 ± 0.28^a	86.31 ± 0.60^a	11.44 ± 0.32^a

Data are expressed as mean of five determinations ± SD. Specific enzymatic activities are expressed as nmol/min/mgprotein. Values with superscripts different for the liver and serum of each enzyme are significantly different ($p < 0.05$).

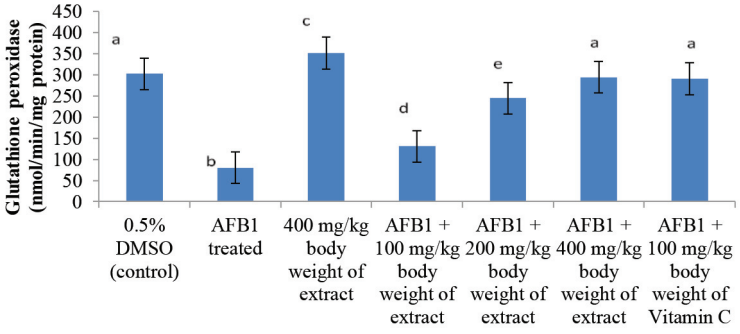

Figure 1 – Specific activity of glutathione peroxidase following six weeks of administration of phenolic extract of *Parkia bliglobosa* pulp to aflatoxin B_1-treated rats. Values are mean ± standard deviation (SD) of five determinations. [abcde]Bars with different superscripts for each parameter are significantly different ($p < 0.05$).

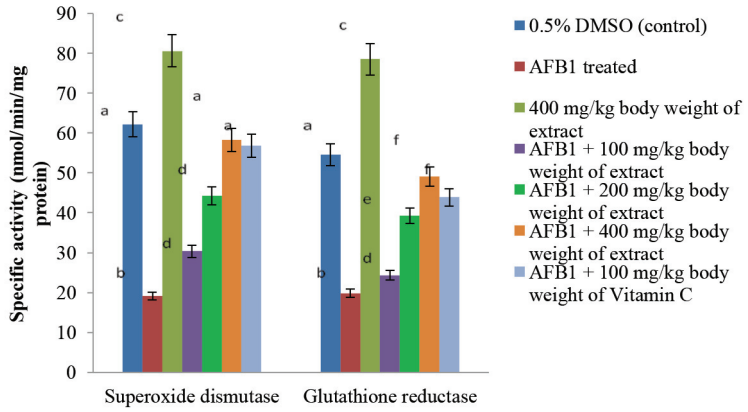

Figure 2 – Specific activities of SOD and Glutathione reductase following six weeks administration of phenolic extract of *Parkia bliglobosa* pulp to aflatoxin B_1-treated rats. Values are mean ± standard deviation (SD) of five determinations. [abcde]Bars with different superscripts for each parameter are significantly different ($p < 0.05$).

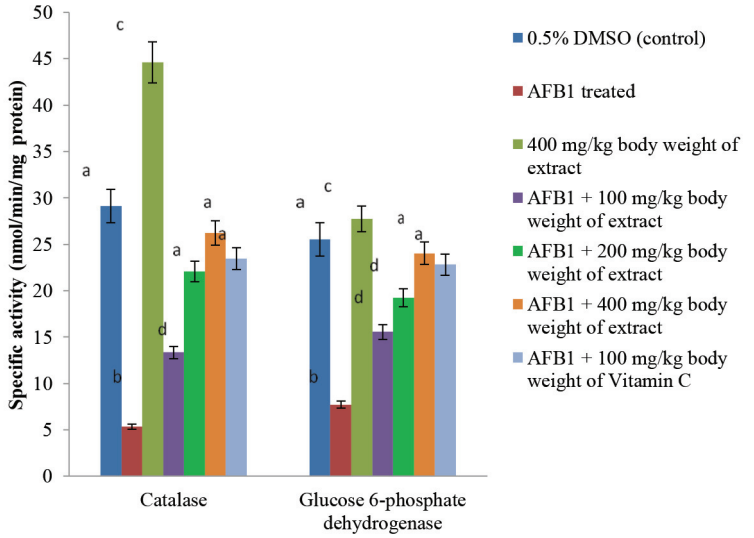

Figure 3 – Specific activities of catalase and glucose-6-phosphate dehydrogenase following six weeks administration of phenolic extract of *Parkia bliglobosa* pulp to aflatoxin B_1-treated rats. [abcde]Bars with different superscripts for each parameter are significantly different ($p < 0.05$).

Table 2
Levels of non-enzymatic antioxidants following six weeks of administration of phenolic extract of *Parkia bliglobosa* pulp to aflatoxin B_1-treated rats.

Treatments	Reduced glutathione (nmol/mgprotein)	Peroxidized glutathione (nmol/mgprotein)	GSH: GSSG ratio
0.5% DMSO (control)	62.52 ± 0.15^{a}	6.54 ± 0.11^{a}	9.56 ± 0.18^{a}
AFB_1 treated	28.38 ± 0.32^{b}	22.62 ± 1.10^{b}	1.25 ± 0.18^{b}
400 mg/kg body weight of extract	91.31 ± 0.43^{c}	5.50 ± 0.11^{a}	16.60 ± 0.11^{c}
AFB_1 + 100 mg/kg body weight of extract	37.06 ± 1.24^{d}	16.48 ± 0.27^{c}	2.25 ± 0.01^{d}
AFB_1 + 200 mg/kg body weight of extract	56.66 ± 3.62^{a}	10.89 ± 0.32^{d}	5.20 ± 0.15^{e}
AFB_1 + 400 mg/kg body weight of extract	60.83 ± 2.25^{e}	6.96 ± 0.57^{a}	8.74 ± 0.09^{a}
AFB_1 + 100 mg/kg body weight of Vitamin C	64.23 ± 1.18^{a}	6.40 ± 0.30^{e}	10.04 ± 0.12^{a}

Data are expressed as mean of five determinations ± SD. Specific enzymatic activities are expressed as nmol/min/mgprotein. Values with superscripts different for the liver and serum of each enzyme are significantly different ($p < 0.05$).

Table 3
Levels of lipid peroxidised products following six weeks of administration of phenolic extract of *Parkia bliglobosa* pulp to aflatoxin B_1-treated rats.

Treatments	Conjugated dienes (nmol/mgprotein)	Lipid hydroperoxide (nmol/mgprotein)	Malondialdehyde (nmol/mgprotein)
0.5% DMSO (control)	35.23 ± 0.20[a]	23.35 ± 0.91[a]	6.26 ± 0.23[a]
AFB_1 treated	92.03 ± 1.53[b]	117.26 ± 0.27[b]	20.13 ± 0.62[b]
400 mg/kg body weight of extract	25.12 ± 0.73[a]	17.84 ± 0.18[a]	5.01 ± 0.17[a]
AFB_1 + 100 mg/kg body weight of extract	71.12 ± 1.21[c]	84.71 ± 0.58[c]	15.23 ± 0.31[c]
AFB_1 + 200 mg/kg body weight of extract	57.31 ± 0.44[d]	55.70 ± 1.72[c]	10.07 ± 0.36[d]
AFB_1 + 400 mg/kg body weight of extract	40.92 ± 4.96[e]	38.18 ± 0.82[a]	6.43 ± 0.36[e]
AFB_1 + 100 mg/kg body weight of Vitamin C	43.62 ± 1.08[e]	33.10 ± 0.14[d]	6.22 ± 0.46[a]

Data are expressed as mean of five determinations ± SD. Specific enzymatic activities are expressed as nmol/min/mgprotein. Values with superscripts different for the liver and serum of each enzyme are significantly different ($p < 0.05$).

Table 4
Levels of protein carbonyl and fragmented DNA following six weeks administration of phenolic extract of *Parkia bliglobosa* pulp to aflatoxin B_1-treated rats.

Treatments	Protein carbonyl (nmol/mgprotein)	Fragmented DNA (%)
0.5% DMSO (control)	3.04 ± 0.02[a]	4.93 ± 0.31[a]
AFB_1 treated	11.84 ± 0.13[b]	90.34 ± 0.35[b]
400 mg/kg body weight of extract	2.02 ± 0.01[c]	5.04 ± 0.02[a]
AFB_1 + 100 mg/kg body weight of extract	8.14 ± 0.25[b]	55.72 ± 1.44[c]
AFB_1 + 200 mg/kg body weight of extract	5.02 ± 0.24[c]	34.62 ± 0.23[d]
AFB_1 + 400 mg/kg body weight of extract	3.72 ± 0.20[a]	10.49 ± 0.16[a]
AFB_1 + 100 mg/kg body weight of Vitamin C	3.55 ± 0.15[a]	13.81 ± 0.32[e]

Data are expressed as mean of five determinations ± SD. Specific enzymatic activities are expressed as nmol/min/mgprotein. Values with superscripts different for the liver and serum of each enzyme are significantly different ($p < 0.05$).

Discussion

Investigations into the phytochemical constituents of fruits, medicinal plants and vegetables are important for nutraceutics and phytomedicine development (Oloyede et al., 2013). This study investigated the capability of the phenolic extract of *Parkia biglobosa* (Jacq.) R. Br. ex G. Don, Fabaceae, pulp to stall oxidative rout mediated by AFB_1.

Hepatocellular marker enzymes

Alterations in the activities of cellular enzymes in both tissue and serum provide a valuable tool for evaluating cellular toxicity. The significant decrease in specific activity of ALP in the liver of AFB_1-treated rats with a corresponding increase in the serum indicates loss of plasma membrane integrity. Studies have reported disruption of ordered bilayer of membrane in AFB_1 toxicity (Ajiboye et al., 2013; Adeleye et al., 2014), which could be due to peroxidation of polyunsaturated fatty acid components of the membranes by ROS ($O_2^{\bullet}$-, $^{\bullet}OH$ and H_2O_2) generated during AFB_1 metabolism (Towner et al., 2003). The prevention of AFB_1-mediated alteration in ALP could be attributed to the inherent antioxidants present in the extract such as phenolics and flavonoids, capable of scavenging free radicals and ROS (Compaoré et al., 2011).

The activity of ALT (cytosolic) and AST (cytosolic and mitochondrial) is useful to confirm the integrity of the plasma membrane. The reduction in specific activities of ALT and AST in the liver of AFB_1-treated rats corroborates the indication of compromised plasma membrane as revealed by the alteration in ALP. This is so because damage to plasma membrane results to leakage of cytosolic content of cell to the external milieu. The reversal of ALT and AST in both liver and serum by *P. biglobosa* pulp extract further substantiate protection of the membrane by the phenolic and flavonoid constituent of the extract.

Reactive oxygen species detoxifying enzymes

The decrease in specific activities of antioxidant enzymes (SOD, catalase, GSH-Px, GSH-red and Glc 6-PD) could be due to excessive mobilization of antioxidant enzymes towards detoxification of ROS ($O_2^{\bullet}$-, $^{\bullet}OH$, $RO_2^{\bullet}$ and H_2O_2) during AFB_1 metabolism (Towner

et al., 2003). This can lead to uncontrolled oxidative attack on cellular macromolecules (lipid, protein, DNA etc.) and eventually cell death (Ajiboye et al., 2010). Similar reduction in activity of SOD, CAT, GSH-Px and GSH-Red (ROS detoxifying enzymes) were reported to be due to excessive generation of ROS during AFB_1 hepatocarcinogenesis (Ramakrishnan et al., 2006; Yadav and Bhatnagar, 2007; Sivaramakrishnan et al., 2008; Ajiboye et al., 2013). Thus, significant attenuation of AFB_1-mediated reduction in specific activities of ROS detoxifying enzymes by *P. biglobosa* pulp extract indicates a protective activity. This could be explained by the capability of the extract to scavenge ROS or enhance ROS detoxifying enzymes.

Non-enzymatic antioxidants

The significant ($p < 0.05$) reduction in GSH levels, a non-enzymatic antioxidant playing a complementary role in preventing ROS-induced oxidative damage, might have resulted from the depletion of GSH-Px and GSH-Red, as they have direct relationship with GSH (Kozer et al., 2003). Conversely, AFB_1-mediated increase in the level of GSSG might have resulted from the oxidation of GSH or mobilization of GSH towards production of GSH-Px. The reduction in GSH:GSSG following administration of AFB_1 suggests oxidative attack on liver cells. Thus, the preservation of GSH, high GSH:GSSG and low GSSG levels in the liver of AFB_1-treated rats following administration of *P. biglobosa* pulp extract indicates possible antioxidant activity.

Lipid peroxidation

AFB_1 has been reported to induce lipid peroxidation both *in vitro* and *in vivo* models of AFB_1-induced hepatotoxicity (Shen et al., 1994; Towner et al., 2003; Theumer et al., 2010; Ravinayagam et al., 2012; Ajiboye et al., 2013). Thus, the significant increase in the levels of lipid peroxidation products (conjugated dienes, lipid hydroperoxides and malondialdehydes) shows indiscriminate oxidative assaults on cellular lipids. This can lead to disturbances of the membrane organization, functional loss and modification of proteins and DNA bases (Niki, 2009). The increase in conjugated dienes could result in mutation (Das et al., 2010). The reduction of AFB_1-mediated increases in conjugated dienes, lipid hydroperoxide and malondialdehyde by phenolic extract of *P. biglobosa* shows protection of the membrane lipids. This might have also resulted from the capability of the extract to promote detoxification (through the induction of antioxidant enzymes) of ROS, which could cause the peroxidation of polyunsaturated fatty acids of the plasma membrane.

Protein oxidation

The significant increase in protein carbonyl, a marker of protein oxidation in the AFB_1-treated rat could have resulted from the protein oxidation by the free radicals and generated ROS during AFB_1 metabolism. This increase indicates irreversible oxidative damage to cellular proteins (Dalle-Donne et al., 2003). The attenuation of AFB_1-mediated increase in the levels of protein carbonyl by phenolic extract of *P. biglobosa* pulp further shows possible capability to promote the detoxification of ROS via the induction of antioxidant enzymes. Similar attenuation of AFB_1-mediated increase in protein carbonyl level following the administration of *Tridham* has been reported (Ravinayagam et al., 2012).

DNA fragmentation

Oxidative stress and accumulation of calcium ions have been reported to mediate DNA fragmentation (Amin and Hamza, 2005). This damage, which usually results from OH- 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 et al., 2006). Thus, the significant increase in the levels of fragmented DNA in the liver of AFB_1-treated rats proves genotoxic activity. It also denotes possible initiation of carcinogenesis. Golli-Bennour et al. (2010) reported a similar increase in fragmented DNA in AFB_1-treated rats. The reduction in the levels of fragmented DNA in the liver of AFB_1-treated rat by phenolic extract of *P. biglobosa* pulp shows antioxidant and antigenotoxic activities. The extract could have acted as an antigenotoxic complex, which enhances the DNA repair system or DNA synthesis (Brahmi et al., 2011).

Conclusion

It is evident from this study that the phenolic extract of *P. biglobosa* pulp enhanced the detoxification of AFB_1, possibly by enhancing the activities of reactive oxygen species detoxifying enzymes, thus preventing the oxidation and fragmentation of cellular macromolecules such as DNA, lipids and proteins as well as AFB_1 induced redox imbalance. Hence, the use of the phenolic extract of *P. biglobosa* pulp shows promising potentials as a dietary supplement/ functional food due to its prophylactic role.

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Authors' contributions

TOA participated in the design of the research, supervised the experiment and drafted the manuscript. AOA, OBO, NSA and SS collected the sample, identified, performed the study and analyzed the data. AKS contributed in the supervision and critical reading of the manuscript. TOS participated in the critical reading of the manuscript, supervised and provided mentorship support. All the authors have read the final manuscript and approved the submission.

Conflicts Interests

The authors declare no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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