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African locust bean (*Parkia biglobosa,* Jacq Benth) leaf extract affects mitochondrial redox chemistry and inhibits angiotensin-converting enzyme in vitro

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Abstract

Background: *Parkia biglobosa* leaf has popular ethnomedicinal use in tropical Africa. However, little is known about its molecular biological effects. This study sought to investigate the in vitro antioxidant activity, angiotensin-I-converting enzyme (ACE) inhibition and effects of aqueous-methanolic extract of *P. biglobosa* leaf (PBE) on mitochondrial membrane potential and reactive oxygen species (ROS) generation.

Methods: Antioxidant activity was determined by extract's $DPPH^{\cdot}$ (1,1-diphenyl-2-picrylhydrazyl radical), $ABTS^{\cdot+}$ [2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt radical cation] scavenging ability, reducing property and propensity to inhibit lipid peroxidation induced by prooxidants ($FeSO_4$/sodium nitroprusside, SNP) in isolated rat tissue preparations. Determination of angiotensin-converting enzyme (ACE) inhibition was based on the hydrolysis of N-hippuryl-His-Leu hydrate (HHL) by the enzyme. Subsequently, the effects of PBE on toxicant-induced mitochondrial ROS formation and basal membrane potential (ΔΨm) were determined by 2′, 7′-dichlorodihydrofluorescin (DCFH) oxidation and safranine fluorescence respectively.

Results: PBE significantly reduced ferric ions ($P < 0.001$), scavenged DPPH (EC_{50} = 98.33 ± 1.0 μg/mL) and ABTS (EC_{50} = 45.30 ± 0.1) radicals, with moderate Fe^{2+}- chelating effect (40%). In rat liver and brain homogenates respectively, PBE prevented membrane peroxidation induced by $FeSO_4$ (EC_{50}: 75.87 ± 2.1 μg/mL and 89.34 ± 2.5 μg/mL) and SNP (EC_{50}: 28.10 ± 1.6 μg/mL and 17.25 ± 0.78 μg/mL). The extract's inhibition of ACE (IC_{50} = 51.30 ± 5.1 μg/mL) and mild depolarization of isolated liver mitochondria membrane potential were concentration-dependent. Finally, PBE was more effective than catechin in attenuating calcium and SNP-induced surge in mitochondrial ROS generation.

Conclusion: *Parkia biglobosa* leaf exhibits considerable ACE inhibitory effect, antioxidant activity and affects mitochondrial redox chemistry. These present findings also justify the ethnobotanical applications of the plant in the indigenous system of medicine.

Keywords: *Parkia biglobosa*, Angiotensin, Antioxidant, Mitochondria, Membrane potential

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Background

There is increasing awareness of potential health benefits of naturally occurring phytochemicals from plants. Herbs have long been used for a large range of nutritional and medicinal purposes. In fact, herbs were the basis for nearly all medicinal therapy until synthetic drugs were developed in the nineteenth century [1]. Bioactive molecules from natural sources could have fewer side effects than synthetic ones. The established inverse relationship between intake of plant-derived foods and mortality from age-related degenerative diseases such, as cancer, diabetes, emphysema, cardiovascular diseases and brain dysfunction [2] has been attributed to their antioxidant property. Thus, antioxidant principles from natural sources can provide a multifaceted approach to modulate oxidative imbalance found in human degenerative diseases. Electron leakage from the mitochondrial electron transport chain (ETC) during cellular respiration accounts for about 2% of oxygen consumed being converted to superoxide anion ($O_2^{\cdot-}$) and this is thought to be the major source of ROS generation in somatic cells [3] with the rate of $O_2^{\cdot-}$ production being dependent on mitochondrial potential [4]. Mitochondrial ROS production under certain conditions is capable of overwhelming the endogenous antioxidant defence mechanisms, resulting in oxidative stress, with a grave implication in numerous pathological conditions and contributes to retrograde redox signaling from the organelle to the cytosol and nucleus. Early ROS production in the mitochondria could be detected using dichlorodihydrofluorescein (DCFH) derivatives which localize to the mitochondrial matrix [5] and is sensitive to H_2O_2 which is immediately generated on spontaneous or catalyzed dismutation of superoxide anion by mitochondrial SOD. The resulting H_2O_2 is capable of diffusing out of the mitochondria into the cytoplasm.

Of particular therapeutic significance, polyphenolics appear to play a significant role as antioxidants in the protective effect of medicinal plants [6] and have become the focus of current nutritional and therapeutic interest. Generally, antioxidant compounds like phenolic acids, polyphenols and flavonoids scavenge free radicals such as peroxide, hydroperoxide of lipid hydroxyl and thus inhibit the oxidative mechanisms involved in the progression of degenerative diseases [7]. These natural antioxidants have been shown to have access to metabolic processes and are capable of interrupting free radical-mediated reactions by donating hydrogen from the phenolic hydroxyl groups to free radicals [8]. They also have the aptitude to scavenge oxygen-nitrogen derived free radicals by donating hydrogen atom or an electron, chelating metal catalysts and activating antioxidant enzymes [8, 9]. Plant phytochemicals have a multifunctional nature. It is, therefore, necessary that in vitro antioxidant investigations combine radical scavenging and lipid peroxidation inhibitory effects in order to arrive at a solid conclusion on the total antioxidant potential of phytochemicals and plant products.

Parkia biglobosa (Jacq.) Benth., commonly known as 'African locust bean', is a plant used extensively in West Africa for timber, food and medicine. It was largely prescribed in traditional medicine for its multiple medicinal virtues in tropical Africa. A decoction of the bark, root and leaves is used in treating toothaches, leprosy, hypertension and fevers [10]. The phenolic constituents [11] and hypotensive effect of the leaf extract was earlier reported [12]. However, the mechanism underlying the hypotensive effect is still a subject of investigation. Also, there is a paucity of information on the antioxidant activity and the effect of the leaf on mitochondrial redox status. In the present study, we have assessed the antioxidant activity, angiotensin-converting enzyme inhibition and effect of the aqueous-methanolic extract of *P. biglobosa* leaf on isolated mitochondrial integrity.

Methods

Chemicals

2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) or ABTS and dichlorofluorescin diacetate (DCFHDA) were obtained from Sigma (St. Louis, MO, USA). 6-hydroxy-2,5,7,8-tetramethychroman-2-carboxylic acid (Trolox) and 2,2-diphenlyl-1-picrylhydrazyl (DPPH) were obtained from Fluka, Buchs, Switzerland. Potassium persulfate ($K_2S_2O_8$), ethylenediaminetetraacetic acid (EDTA), ascorbic acid, 2- deoxy-2-ribose, trichloroacetic acid (TCA), and quercetin were obtained from Sisco Research Laboratories Pvt. Ltd., Mumbai, India. Hydrogen peroxide, Ferrous sulfate, potassium hexacyanoferrate, Folin-Ciocalteu reagent, sodium carbonate (Na_2CO_3) and butylated hydroxytoluene (BHT) were obtained from Merck, Mumbai, India. Thiobarbituric acid (TBA) was obtained from Loba Chemie, Mumbai, India.

Plant material

Fresh leaves of *Parkia biglobosa* were collected in Isua-Akoko, Ondo State, Nigeria. Botanical identification and authentication was carried out by Dr. Ugbogu A.O and Mr. Shasanya O.S at the herbarium of the Forestry Research Institute (FRIN) Ibadan, Oyo state, Nigeria where a voucher specimen (no 109603) was deposited.

Parkia biglobosa Extract (PBE) preparation

The leaves were air-dried for 28 days at room temperature and ground to fine powder using a blender. A 500 g sample of the powdered material was macerated in 1200 mL of a mixture of methanol and water (4:1) for 48 h. This was filtered and concentrated to a small volume to remove the entire methanol using rotary

evaporator. The concentrated extract was then lyophilized. The residue was kept at −20 °C for future use. Extract yield was approximately 11%.

Animals

Male Wistar rats (±3 months old), weighing between 270 g and 320 g, from the University breeding colony (Animal House Holding, UFSM, Brazil) were kept in cages with free access to foods and water in a room with controlled temperature (22 °C ± 3) and in 12-h light/dark cycle with lights on at 7:00 a.m. The animals were maintained and used in accordance to the guidelines of the Brazilian Association for Laboratory Animal Science.

Preparation of tissue homogenates

Rats were sacrificed by decapitation on the day of experiment and rapidly dissected to harvest the whole brain and liver which were then placed on ice and weighed. Tissues were immediately homogenized in ten volumes of cold (4 °C) Tris–HCl (10 mM, pH 7.4). The homogenate was centrifuged for 10 min at 4000 g to yield a pellet that was discarded and a low-speed supernatant that was used in thiobarbituric acid reactive substances (TBARs) quantification.

In vitro antioxidant/radical scavenging and metal ion chelating activities assays

Total antioxidant activity

Total antioxidant activity was determined by the ABTS test described by Re et al. [13]. The $ABTS^{\cdot+}$ radical cation was generated by mixing 7 mM ABTS stock solution with 2.45 mM potassium persulfate (final concentration) and incubating for 12–16 h in the dark at room temperature. The absorbance of the $ABTS^{\cdot+}$ solution was equilibrated to 0.70 (± 0.02) by diluting with distilled water. $ABTS^{\cdot+}$ solution (1 ml) was mixed with 10 μl of PBE dissolved in distilled water (0, 10, 25, 50, 100 and 150 μg/ml final concentration) or Trolox standard dissolved in deionized water (0, 1, 2.5, 5.0, 7.5, and 10 μg/ml final concentration). The absorbance was measured at 734 nm after 6 min. All experiments were carried out in replicates. The percentage inhibition of absorbance was calculated and plotted as a function of the concentration of standard and sample to determine the Trolox equivalent antioxidant concentration (TEAC). To calculate the TEAC, the straight line gradient of the plot for the sample was divided by that of Trolox (Additional file 1: Figure S1).

DPPH radical scavenging activity of extract

DPPH radical-scavenging activities of *P. biglobosa* extract and reference compound (Ascorbic acid) were determined as described by Batool et al. [14]. The capacity of extracts to scavenge the lipid-soluble 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical, which results in the bleaching of the purple colour exhibited by the stable DPPH radical, could be monitored at 517 nm.

Briefly, PBE (0, 10, 25, 50, 100, and 250 μg/ml) or the reference compound, ascorbic acid (0, 10, 20, 30, 40 and 50 μg/ml) was added to an ethanol solution of DPPH (0.03 mM). The mixture was shaken and left to stand at room temperature for 30 min. The absorbance of the resulting solution was measured spectrophotometrically at 517 nm. The radical scavenging activity was calculated as a percentage of $DPPH^{\cdot}$ discolouration.

Reducing power

The Fe^{3+}- reducing power of the extract was determined as described by Oyaizu [15] with a slight modification. Different concentrations (0.0–200 μg/mL) of the extract (0.5 mL) were mixed with 0.5 mL phosphate buffer (0.2 M, pH 6.6) and 0.5 mL potassium hexacyanoferrate (0.1%), followed by incubation at 50 °C in a water bath for 20 min. After incubation, 0.5 mL of TCA (10%) was added to terminate the reaction. The upper portion of the solution (1 mL) was mixed with 1 mL distilled water, and 0.1 mL $FeCl_3$ solution (1%) was added. The reaction mixture was left for 10 min at room temperature and the absorbance was measured at 700 nm against an appropriate blank solution. All tests were performed three times. A higher absorbance of the reaction mixture indicated greater reducing power. Butylated hydroxytoluene (BHT) was used as a positive control.

Fe^{2+} chelation

The ferrous ion chelating activity of extract was evaluated by a standard method [16] with minor changes. The reaction was carried out in Tris-HCL buffer (0.1 M, pH 7.5). Briefly, various concentrations (0–200 μg/mL) of plant extract were added to 100 μM ferrous sulfate solution. The reaction mixture was incubated for 30 s, before the addition of 1, 10-Phenanthroline (0.25% *w*/*v*). The absorbance was subsequently measured at 510 nm in a spectrophotometer. EDTA was used as a positive control.

Hydroxyl radical scavenging

This was assayed by a standard method [17]. Deoxyribose is degraded by hydroxyl radicals with the release of thiobarbituric acid (TBA) reactive materials. The assay was based on the generation of hydroxyl radical by the Fe^{2+}-H_2O_2 system (the Fenton reaction) and quantification of the degradation product of 2-deoxyribose by condensation with TBA.

The reaction mixture contained 120 μl of 2-deoxy-2-ribose (3 mM); 80 μl of potassium phosphate buffer (50 mM, pH 7.4); 80 μl of $FeSO_4$ (100 μM); 80 μl of H_2O_2 (1.0 mM) and 40 μl of PBE (0, 25, 50, 100, 150,

and 200 μg/ml) of the test sample and distilled water to make up a final volume of 1 ml. After incubation for 1 h at 37 °C, 0.5 ml of the reaction mixture was added to 1 ml of 2.8% (*w*/*v*) TCA, then 1 ml of 1% aqueous TBA was added. The mixture was incubated at 90 °C for 15 min. After cooling, the absorbance was measured at 532 nm against an appropriate blank solution. The percentage inhibition was evaluated by comparing the test and blank solutions.

Evaluation of membrane lipid peroxidation

Quantification of thiobarbituric acid reactive substances (TBARs) production, an index of biological membrane peroxidation, was determined as described by Puntel et al. [16]. Briefly, 20 μL of PBE (50–250 μg/mL) and pro-oxidant agent (100 μm Fe^{2+}) were added to 100 μL of rat liver or brain tissue homogenate in Tris-HCL buffer (10 mM; pH 7.4). The reaction mixture was incubated at 37 °C in a water bath. Color reaction was developed by adding 200 μL of 8.1% sodium dodecyl sulfate (SDS) to the reaction mixture. This was subsequently followed by the addition of 500 μL of acetic acid/HCl buffer (1.34 M; pH 3.4) and 500 μL 0.6% thiobarbituric acid (TBA). The mixture was incubated at 100 °C for 1 h. TBARs produced were measured at 532 nm and the absorbance was compared with a malondialdehyde (MDA) standard curve.

Angiotensin-converting enzyme (ACE) inhibition assay

The assay was based on the hydrolysis of N-hippuryl-His-Leu hydrate (HHL) by the angiotensin-converting enzyme as described by Cushman and Cheung [18]. Briefly, the enzyme source was prepared with freshly removed rat lung. The tissue was homogenised in cold 125 mM Tris buffer, pH 8.3 (1/10, *w*/*v*) and centrifuged at 4 °C for 10 min at 4000 g to yield a low-speed supernatant. The reaction mixture contains 40 μL Tris-HCl buffer (125 mM, pH 8.3), enzyme source (50 μL) and 10 μL extracts/drug (PBE- 25, 50, 100 μg/mL; ramipril-0.04, 0.2, 0.4 μg/mL). This was incubated at 37 °C for 15 min. Thereafter, ACE substrate, HHL (8.3 mM; 150 μl) was added and further incubated for 30 min at the same temperature in a shaker. The enzymic reactions were terminated by addition of 1 ml of 1 M HCI. The hippuric acid formed by the action of the angiotensin-converting enzyme on HHL was extracted from the acidified solution into l-2 ml of ethyl acetate by vortex mixing for 15 s. After centrifugation at 3000 *g* for 5 min, an l ml aliquot of each ethyl acetate layer was transferred into a clean tube. The ethyl acetate fractions were evaporated by heating at 120 °C for 30 min in a Temp-Blok module heater. The hippuric acid was re-dissolved in 1 ml distilled water and the amount formed was determined from its absorbance at 228 nm wavelength.

Isolation of hepatic mitochondria

Liver mitochondria were isolated as previously described [19]. Wistar rats were killed by decapitation and the liver tissues were rapidly removed and placed on ice-cold isolation buffer containing 225 mM mannitol, 75 mM sucrose, 1 mM ethylene glycol-bis(β-aminoethyl ether)-N,N,N′,N′-tetraacetic acid (EGTA), 0.1% bovine serum albumin (BSA; free fatty acid) and 10 mM HEPES pH 7.2. The tissues were then homogenized and the resulting suspension centrifuged for 7 min at 2,000Xg. Next, the supernatant was centrifuged for 10 min at 12,000Xg. The pellet was re-suspended in isolation buffer II containing 225 mM mannitol, 75 mM sucrose, 1 mM EGTA and 10 mM HEPES pH 7.2 and centrifuged at 12,000Xg for 10 min. Finally, the last supernatant was discarded, and the pellet was re-suspended and maintained in buffer III (sucrose 100 mM, KCl 65 mM, K^+-HEPES 10 mM and EGTA 50 μM pH 7.2) to a protein concentration of 0.5 mg/mL for subsequent analyses. Protein concentration was measured by the method described by Lowry et al. [20] using bovine serum albumin (BSA) as standard.

Measurement of mitochondrial membrane potential (Δψm)

Mitochondrial membrane potential was estimated by fluorescence changes of safranine recorded by spectrofluorimeter [19]. The cuvette inside the spectrofluorimeter contains 3 mL of buffer III to which an aliquot (30 mL) of the isolated mitochondria (approximately 500 mg protein) was added. The reaction was started by the addition of safranine (67 mM) and succinate (1.5 M) added after 10 s. The fluorescence was monitored for 200 s after which 10 μL of extract/drug (PBE: 25, 50, 100 μg/mL; catechin: 1, 5, 10 μg/mL) or distilled water (for control) was added and allowed for additional 150 s. Finally 2,4-dinitrophenol, DNP (100 mM, 30 μL) was added to uncouple oxidative phosphorylation and inhibit adenosine triphosphate production. The change in fluorescence was recorded by a RF-5301 Shimadzu spectrofluorimeter (Kyoto, Japan) operating at excitation and emission wavelengths of 495 nm and 586 nm, respectively, with slit widths of 3 nm. The potential difference ($\Delta\Psi m$) was obtained by the difference between the fluorescence intensity prior to and after DNP addition.

Evaluation of reactive species (RS) formation with DCH (dichlorofluorescein-reactive species (DCH-RS)

RS levels were measured using the oxidant sensing fluorescent probe, 2′,7′-dichlorofluorescein diacetate (DCHF–DA) [21]. The oxidation of DCHF–DA to fluorescent dichlorofluorescein (DCF) was determined at 488 nm for excitation and 525 nm for emission. An aliquot of 5 μL (50 μg/protein) of the homogenate of the isolated mitochondria was added to 3 mL of buffer III (containing

5 mM succinate). The reaction medium was exposed to PBE (25, 50, 100 μg/mL) or catechin (1, 5, 10 μg/mL) with or without Ca^{2+} (80 μM) or SNP (150 μM). After 10 s, DCHF–DA (10 μM, in absolute ethanol) was added to the mixture and the fluorescence intensity from DCF was measured for 300 s. Values of mitochondrial membrane potential (Δψm) were expressed as percent of control.

Statistical analysis

Results calculated from triplicate data were expressed as means ± standard error of means (SEM). With the exception of data on mitochondrial ROS production which was analysed using two-way ANOVA followed by Bonferroni post test, data were analyzed using one-way analysis of variance followed by Neuman-Keuls comparison of means. The significance level was set at $p < 0.05$. Statistical analysis, graphing and EC_{50}/IC_{50} (median effective concentration/median inhibitory concentration) determination were done using Graph Pad Prism (ver.5.0a).

Results

Radical scavenging, ferric reducing and metal-chelating properties of *P. biglobosa* extract (PBE)

The suppression of ABTS radical cation following interaction with PBE or trolox was concentration-dependent and statistically significant ($P < 0.001$; Fig. 1a). The ABTS scavenging potency of PBE was reflected by its trolox equivalent antioxidant concentration (TEAC) value (0.06 ± 0.001), just as its EC_{50} (45.30 ± 0.1 μg/ml) further showed the extract was less potent than the pure reference compound (4.5 ± 0.1 μg/ml). PBE produced concentration-dependent discolouration of the purple colour due to DPPH radical with an EC_{50} value of 98.33 ± 1.0 μg/ml compared with 12.5 ± 0.4 μg/ml for the reference ascorbic acid (Fig. 1b, Table 1). As shown in Fig. 1c, the reduction of $Fe^{3+}$to Fe^{2+} increased with increasing concentration of PBE and was highest (0.89 ± 0.06) at 200 μg/mL final concentration. The extract of *P. biglobosa* possesses moderate but dose-dependent Fe (II) chelating activity with over 40% chelation at 200 μg/mL extract concentration. This was however considerably lower than that of the standard EDTA with about 54% chelation at 50 μg/mL concentration (Fig. 1d).

Effects of PBE on hydroxyl radical ($OH^{\cdot}$) generation and Fe^{2+}/SNP-induced membrane peroxidation

P. biglobosa caused only a weak inhibition of deoxyribose degradation (Fig. 2a; $P < 0.001$). PBE (50 μg/mL) caused a maximal inhibition of 15%, whereas higher concentrations of extract had lesser efficacy. Fe(II) significantly increased TBARs content to about 88% and

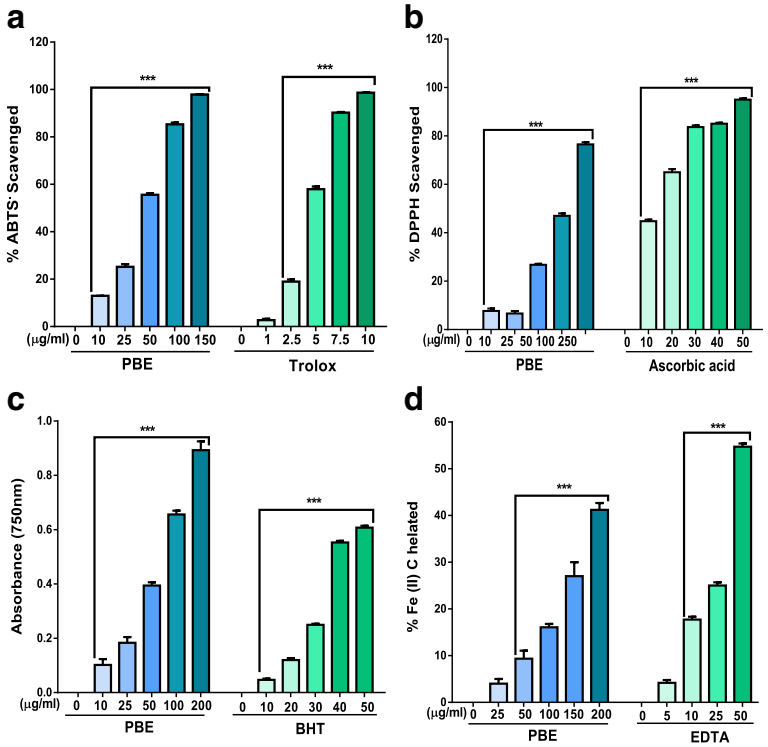

Fig. 1 ABTS (**a**), DPPH (**b**) radicals scavenging, reducing property (**c**) and Fe(II)-chelating potential (**d**) of *P. biglobosa* leaf extract. Results are mean ± SEM of three parallel measurements. *P* value: $^{***}P < 0.001$ vs 0 μg/ml. PBE = *Parkia biglobosa* leaf aqueous-methanol extract; BHT = butylated hydroxytoluene; EDTA = ethylenediaminetetraacetic acid

Table 1 *EC_{50} values of aqueous-methanolic extract of P. biglobosa leaf for ABTS, DPPH radicals scavenging, toxicant-induced lipid peroxidation and IC_{50}* for Angiotensin-1-converting enzyme inhibition in vitro

Parameter	EC50/IC50 (μg/ml)
ABTS Radical Scavenging	45.30 ± 0.1
DPPH Radical Scavenging	98.33 ± 1.0
Fe2 + –Induced lipid peroxidation	
Liver	75.87 ± 2.1
Brain	89.34 ± 2.5
SNP-Induced lipid peroxidation	
Liver	28.10 ± 1.6
Brain	17.25 ± 0.8
Angiotensin-1-converting enzyme	51.30 ± 5.1

Values are mean ± SD of three parallel measurements

85% of the basals in isolated rats' liver and brain homogenates respectively (Fig. 2b, c; P < 0.001). Treatment of tissue homogenates with SNP (5 μM) also caused the accumulation of lipid peroxides as manifested by up to 90% and 85% increase in MDA content of the liver and brain respectively when compared to the respective basal homogenates. The extract showed EC_{50} values (μg/ml) of 75.87 ± 2.05 and 89.34 ± 2.52 in liver and brain respectively for the mitigation of Fe (II) peroxidation and EC_{50} values of 28.1 ± 1.59 and 17.25 ± 0.78 in the respective tissues for that of SNP. It is therefore noteworthy that on the basis of median effective concentrations (EC_{50}), the extract afforded greater protection against SNP-induction when compared to iron-induced lipid peroxidation.

Effect of *P. biglobosa* extract on angiotensin-I-converting enzyme

The inhibition of angiotensin-I-converting enzyme by *P. biglobosa* aqueous-methanolic extract was dose-dependent and significant ($P < 0.01$/$P < 0.05$) in vitro (Fig. 3). The crude extract showed IC_{50} value of 51.30 ± 5.1 μg/mL for the inhibition of ACE (Table 1). This was considerably higher than the value (0.17 ± 0.04 μg/mL) observed for the reference standard.

Modulatory effect of *P. biglobosa* extract on hepatic mitochondrial membrane potential

Catechin at the study doses had no significant effect on the membrane potential of isolated liver mitochondria. PBE caused slight depolarization of the membrane potential of rats' liver mitochondria. Reduction of the mitochondrial potential difference by PBE was however statistically significant ($P < 0.05$) at 100 μg/mL final concentration (Fig. 4).

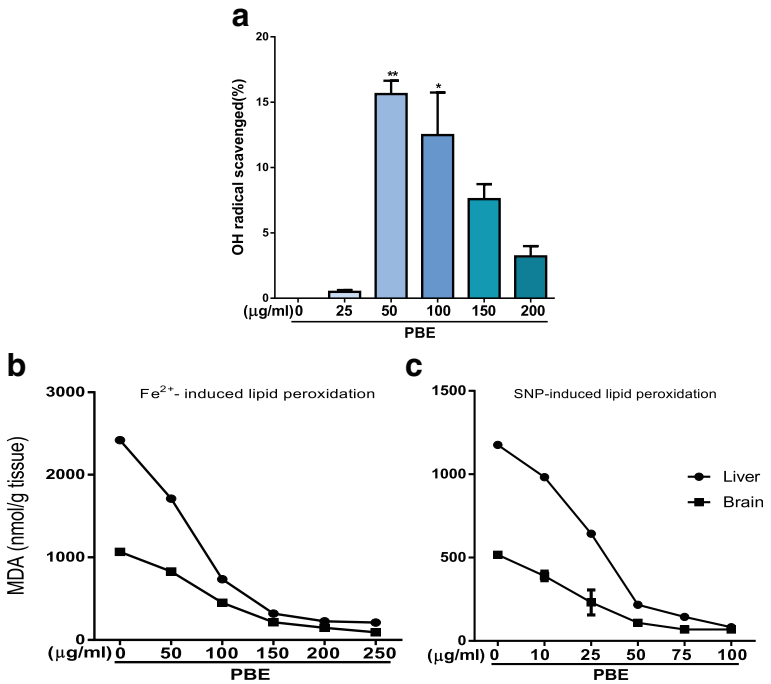

Fig. 2 Hydroxyl radical scavenging activity (**a**) and inhibition of Fe^{2+}- (**b**) and SNP-induced (**c**) lipid peroxidation in rat tissues by *P. biglobosa* leaf extract. Results are mean ± SEM of three parallel measurements

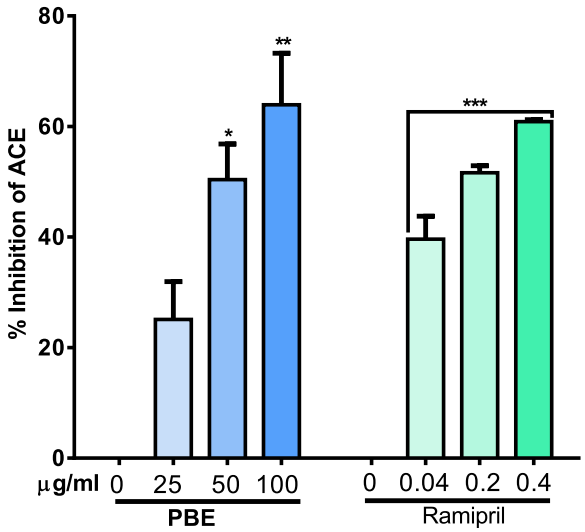

Fig. 3 Inhibition of angiotensin-1-converting enzyme by *P. biglobosa* leaf extract in vitro. Results are mean ± SEM of three parallel measurements.$^{***}P < 0.001$ vs 0 µg/ml

Effects of PBE on Ca^{2+}/SNP-induced mitochondrial reactive species (RS) generation

As shown in Fig. 5, basal mitochondrial RS formation in the liver was significantly reduced ($P < 0.001$) by treatment with all concentrations of PBE under study when compared to the level found in the control. Ca^{2+} and SNP- dependent surge in mitochondrial production of RS was more efficiently attenuated by PBE than catechin. Concentration of catechin used in the present study was based on the proportion of catechin in crude extract as previously documented [11].

Discussion

Many of the therapeutic actions of phytochemicals are thought to be associated with their biologically active polyphenol components, such as flavonoids and phenolic acids, which possess powerful antioxidant activities [22]. In the present study, the radical scavenging activity of *P. biglobosa* extract was evaluated using different radical systems in vitro as it has been proposed that the efficacy and the antioxidant potency of some extracts may vary depending on the pro-oxidant used [23]. As a principle, spectrophotometric measurement of the colour change of the DPPH solution from purple to yellow as the radical is quenched by the antioxidant gives a quantitative estimation of the antioxidant. Hence it could be proposed that reactions of antioxidant, hydrogen donors in *P. biglobosa* with DPPH radicals reduce the latter to the corresponding hydrazine [19]. A similar proposition could also be put forward for the decolourization of the ABTS radicals following exposure to PBE. Potential antioxidant activity has been reported to be concomitant with the reducing power of a plant extract. *P. biglobosa* leaf extract possesses considerable reducing property as demonstrated by its concentration-dependent ability to reduce Fe^{3+} to Fe^{2+}. In this case, the phenolics in the leaf could act as reducing agents by donating electrons to free radicals and terminating the free radical-mediated chain reactions [24]. Iron can promote lipid peroxidation by decomposing lipid hydroperoxides into peroxyl and alkoxyl radicals that can perpetuate the chain reaction and via the Fenton reaction. Metal chelating agents reduce the concentration of metal ions in the Fenton-type reaction and thus would protect the system from oxidative damage through inhibition of metal-dependent processes [25]. Understandably, *P. biglobosa* extract was not as potent as EDTA in iron chelation because the metal-chelating efficiency of the phytochemicals (notably polyphenols) involved are usually lower than those of standard chelators like EDTA [26].

In the present study, the inhibitory effects of PBE against $Fe^{2+}$and SNP-induced lipid peroxidation were assessed. Lipid peroxidation is a free radical-mediated process involving lipid-derived radicals, such as alkoxyl and peroxyl radicals, wherein oxidative damage is propagated to polyunsaturated fatty acids. The toxicity of Fe (II) proceeds via the Fenton reaction where iron catalyses one-electron transfer reactions that generate reactive oxygen species, such as the $OH^{\cdot-}$ from H_2O_2. Iron is capable of decomposing lipid peroxides leading to the generation of peroxyl and alkoxyl radicals and favouring the propagation of lipid oxidation [25]. Sodium nitroprusside (SNP), on the other hand, has been reported to cause cytotoxicity through the release of cyanide and/or nitric oxide [27]. The released NO is capable of causing neuronal damage in cooperation with other reactive oxygen species (ROS) notably superoxide radical to form peroxynitrite radical [28]. The considerable inhibition of SNP-induced lipid peroxidation by PBE could be attributed to the ability of the extract to scavenge $NO^{\cdot}$ radical produced by the SNP, thus protecting the tissues against oxidative insults [28].

The present study revealed that *P. biglobosa* extract exerts concentration-dependent and inhibition of both iron- and SNP-induced peroxidation in liver and brain homogenates. The protections offered by *P. biglobosa* suggest that the hydromethanolic extract may protect the liver and brain against toxicities resulting from a potential overload of iron and nitric oxide. Angiotensin-converting enzyme (ACE) is responsible for producing the potent vasoconstrictor and trophic angiotensin II (AII). AII increases blood pressure by its action as a potent vasoconstrictive substance and by stimulating the production of aldosterone, which promotes sodium and water retention in the body. ACE-inhibitory substances are thus desirable when the aim is to achieve lower blood pressure. In the present study, PBE demonstrated

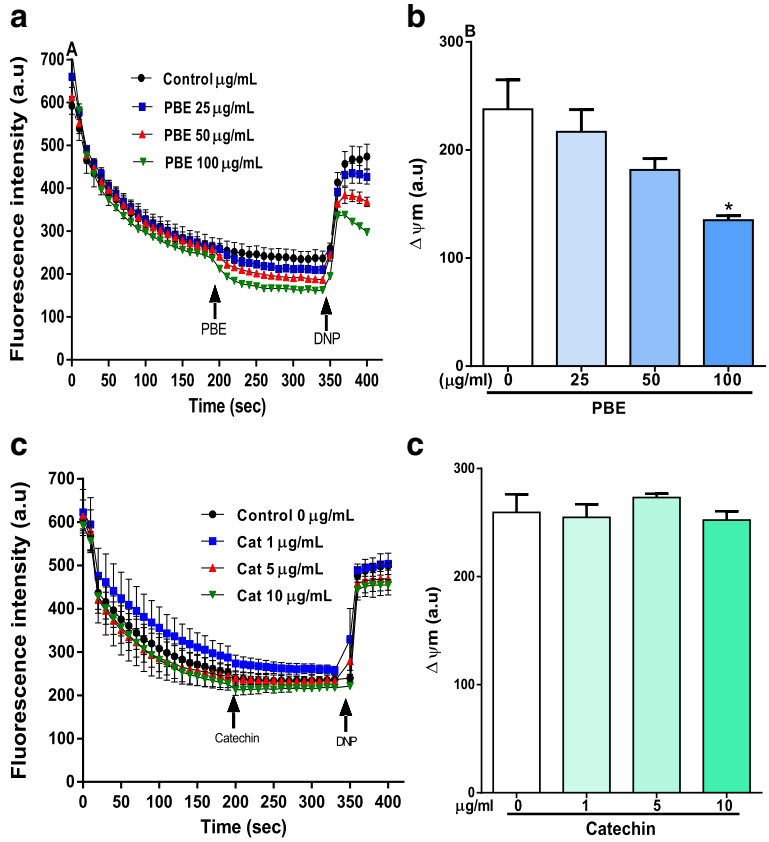

Fig. 4 Effects of *Parkia biglobosa* leaf extract (PBE) (**a** and **b** and catechin (**c** and **d**) respectively on membrane potential difference of isolated liver mitochondria. Data is presented as mean ± SEM of three assays performed in triplicates using independent mitochondrial preparations. *$P < 0.05$ vs 0 μg/ml

ACE- inhibitory effect, a phenomenon that could be involved in its documented hypotensive potential under experimental condition [12]. The phytochemicals responsible for the ACE-inhibitory effect of still remains to be elucidated. Hypothetically, however, the polyphenolic constituents of the leaf could be involved. Free hydroxyl groups of phenolic compounds are important structural moieties capable of chelating the zinc ions in ACE active sites, thus inactivating the enzyme [29]. Several flavonoids have thus been shown to demonstrate competitive inhibition towards ACE [30]. It is noteworthy that the ACE inhibitory activity of the crude extract is much lower than that recorded for the standard drug, Ramipril. Crude extracts are known to contain various phytochemical constituents capable of interfering with one another to produce antagonistic or synergistic effects. Pure compounds, on the other hand, are devoid of negative interactions. A higher ACE-inhibitory potency could, therefore, be recorded with the pure form(s) of the phytochemical(s) responsible for the effect.

SNP is a NO donor compound that reportedly exhibited a deleterious effect on mitochondrial function and survival of synoviocytes [31] while reducing the activity of complex IV of the mitochondrial electron transport chain (MTC) with consequent apoptotic cell death [32]. The toxicity of NO is dependent on its reaction with the superoxide anion radical ($O2^{\cdot-}$) which yield peroxynitrite, a highly cytotoxic ROS. Peroxynitrite is further decomposed to the hydroxyl radical which eventually leads to lipid peroxidation, protein oxidation, and DNA damage [33]. Mitochondrial dysfunction has been suggested as the underlying mechanism of NO-mediated toxicity [34]. In the present study, PBE and its major polyphenolic constituent, catechin attenuated SNP-induced increase in the rate of mitochondrial reactive species formation possibly due to their free radical scavenging effects.

Disruption of calcium homeostasis and free radicals generation are among the detrimental effects associated with the toxicity of some compounds [35]. Formation of ROS by mitochondria is enhanced as a consequence of increased cytosolic calcium concentrations ([Ca2+]) [36]. In the present study, calcium-induced reactive species formation in the mitochondria was attenuated by PBE and catechin. Catechin was selected as the reference phenolic because it was previously reported to be

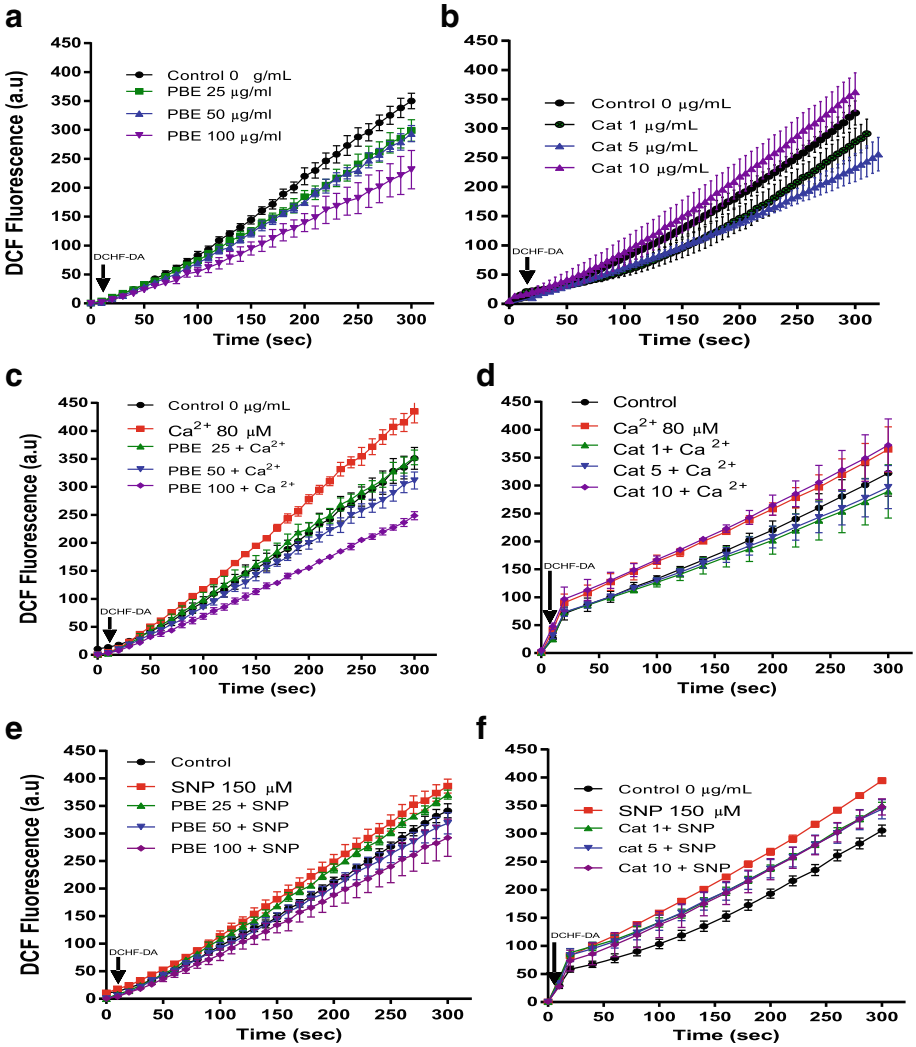

Fig. 5 Respective effect of *Parkia biglobosa* leaf extract (PBE) and catechin on basal ROS production (**a** and **b**), Ca^{2+} – induced (**c** and **d**) and SNP-induced (**e** and **f**) aggravation of ROS generation in isolated rats' liver mitochondria. Data is presented as mean ± SEM of three assays performed in triplicates using independent mitochondrial preparations. **a** PBE 25-, 50-, 100 µg/ml ($P < 0.001$ vs Control), (**b**) Cat 1-, 5-, 10 µg/ml non-significant vs control ($P > 0.05$), (**c**) Ca^{2+} 80 µM, PBE 100 µg/ml ($P < 0.001$ vs control), (**d**) Ca^{2+}80 µM, Cat 10 µg/ml ($P < 0.01$ vs control), (**e**) SNP ($P < 0.01$ vs control), PBE 25 µg/ml ($P < 0.05$ vs control), (**f**) SNP ($P < 0.001$ vs control)

present in high amount in PBE [11]. Catechins possess strong metal ion chelating potentials due to their catechol structures and have been shown to form stable complexes with ions, including $Fe^{2+}$and Ca^{2+} [37]. Therefore, the reduction of a calcium-induced surge in mitochondrial ROS production by PBE might be related to the ability of the constituent phenolics to scavenge free radicals and chelate metal ion. Mitochondrial membrane potential (ΔΨm) contributes to determining a driving force for calcium to enter the mitochondria. ROS formation in mitochondria occurs at high membrane potentials. Mild depolarization of the mitochondrial potential by PBE does not involve the constituent catechin as revealed in the present study. Some activities in herbs may be attributable to other unidentified substances or to synergistic interactions among constituents. Even though the mechanism controlling the mitochondrial membrane potential ΔΨm in vivo are complex and not fully understood, 'mild un-coupling' of mitochondria are turned on in vivo to diminish the formation of ROS [38]. Decreased ΔΨm, due to the uncoupling of electron flow from ATP synthesis by increased proton permeability of the inner mitochondrial membrane, can reduce ROS production at complex I by decreasing $NAD(P)H/NAD(P)^+$ and possibly by decreasing the life span of the semiquinone radical [39]. Such decrease in the mitochondrial membrane potential (ΔΨm) primarily attenuates mitochondrial RO production with a consequential decrease in mitochondrial Ca^{2+} uptake [40], preventing mitochondrial calcium overload and the

subsequent apoptosis [41]. This might account for the superior mitochondrial ROS mitigating property of PBE over catechin. Such mild depolarization has been attributed to the neuroprotective effect of a plant extract [42] and the protection of cardiomyocytes from oxidative stress [40]. In the case of PBE, further studies are warranted to identify the active molecules and the underlying mechanisms involved in its mild mitochondrial potential depolarization propensity.

Conclusion

In summary, the results presented here suggest that *Parkia biglobosa* leaf did exhibit considerable antioxidant activity and ACE inhibition in vitro. It also modulated mitochondrial functions by attenuating toxicant induced ROS generation, with slight mitochondrial membrane depolarization propensity. The phenolic constituents could be actively involved in these effects. Since the findings are largely under in vitro conditions, further studies are needed to make appropriate assumptions about the safety and effectiveness of the plant as hypotensive agent in mammals under in vivo conditions, and to fully characterize the biological potential of the plant.

Additional file

Additional file 1: Figure S1. Determination of the trolox equivalent antioxidant concentration (TEAC) value for PBE. Values are mean ± SEM of three parallel measurements. (DOC 61 kb)

Abbreviations

ABTS: 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid); ACE: Angiotensin-l-converting enzyme; BHT: Butylated hydroxytoluene; DCFH: 2′, 7′-dichlorodihydrofluorescin; DCFHDA: dichlorofluorescin diacetate; DPPH: 1,1-diphenyl-2-picrylhydrazyl; EDTA: Ethylenediaminetetraacetic acid; EGTA: Ethylene glycol-bis (β-aminoethyl ether)-N,N,N′,N′-tetraacetic acid; PBE: Aqueous-methanolic extract of *P. biglobosa* leaf; SNP: Sodium nitroprusside; TBA: Thiobarbituric acid

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Authors' contributions

MTO, JBTR and AAA were involved in the planning of the research as well as the technical design. KK collected sample, performed the experimental works in the laboratory, analyzed the data and drafted the manuscript. RTK and ACA assisted in the laboratory work and data analysis. JBTR and MTO were also involved in the interpretation of the results and revision of the whole manuscript with grammatical checking.

Competing interests

The authors declare that they have no competing interest.

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