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Attenuation of glycation and biochemical aberrations in fructose-loaded rats by polyphenol-rich ethyl acetate fraction of *Parkia biglobosa* (jacq.) Benth. (Mimosaceae) leaves

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Abstract

Background: Different parts of the *Parkia biglobosa* plant are employed in traditional medicine in different African communities. However, information ratifying its use and biochemical influence on health is still scanty in literature. Thus, the present study assessed the influence of the ethyl acetate fraction of *Parkia biglobosa* leaves (EAFPB) on some biochemical parameters of sub-chronic fructose-loaded rats.

Methodology: Twenty-five Wistar rats were randomized into five groups ($n = 5$). The normal control group was maintained on normal diet only while the high fructose solution (HFS) control (placebo), reference and treatment groups received high fructose solution (3 g/kg/d b.w of fructose) for 30 days before treatment. Based on pilot study, two doses (100 and 200 mg/kg/d b.w) of EAFPB were selected and were administered to two groups of test animals while the reference group received 300 mg/kg/d b.w. of metformin for 14 days. Thereafter, blood was collected from fasted animals for biochemical analyses for the examination of level of glycated hemoglobin (HbA1c), liver status (alanine and aspartate aminotransferases (ALT and AST) and alkaline phosphatase (ALP) activities, and bilirubin level), lipid profile (total cholesterol, triglyceride, and low- and high-density lipoproteins levels) and lipid peroxidation (malondialdehyde – MDA level).

Results: EAFPB was shown to have a good DPPH radical scavenging activity ($EC_{50} = 0.395$ mg/ml). Chromatographic analysis of EAFPB revealed 28 known flavonoids (mainly kaempferol (21.31 mg/100 g), quercetin (12.84 mg/100 g), and luteolin (6.75 mg/100 g)), four hydrocinnamic acids derivatives (mainly *P*-coumaric acid (6.73 mg/100 g)), and 11 phenolic acids derivatives (mainly chlorogenic acid (48.18 mg/100 g) and protocatechuic acid (21.58 mg/100 g)). Relative to normal control, it was observed that fructose overload significantly increased serum activities of ALP, ALT, and AST, and levels of MDA, total cholesterol, low density lipoprotein and triglyceride in placebo. However, EAFPB significantly tapered the elevated serum activities of ALP, ALT, and AST. In addition, relative to placebo, the increased levels of HbA1c, MDA, and lipid health markers were also rebated by EAFPB.

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Conclusions: Ethyl acetate fraction of *Parkia biglobosa* leaves attenuates biochemical aberrations in fructose-loaded rats, an effect attributable to the rich store of polyphenolic compounds in the fraction.

Keywords: *Parkia biglobosa*, Fructose, Polyphenolic compounds, Dyslipidemia, Glycated hemoglobin, Antioxidant

Introduction

Nutrition is largely recognized to play a major role in the health and wellbeing of individuals. Thus, intake of high calorie foods, with increased sedentary lifestyles, is widely recognized as the major contributor to the epidemic of many metabolic diseases. Several reports have linked high calorie foods (in the form of sugars) to the alarming increases in cases such as non-alcoholic fatty liver disease and the metabolic syndrome (a group of interconnecting factors which enhance the risk of cardiovascular diseases and type-2 diabetes) [1, 2]. Sugar in the form of sucrose (glucose and fructose in equal amounts) or high-fructose corn syrup (consisting up to 55–60 % of fructose in its free form) are incorporated into many diets worldwide, including soft drinks and many pre-packaged foods such as breakfast cereals and baked goods [3]. Studies have reported the adverse effects of excess circulating sugars on both intra- and extracellular macromolecules [4, 5]. Although there are no specific data indicating daily thresholds of calories from sugars which could negatively affect human health, several experimental results indicate negative health effects of high sugar intake. Prolonged ingestion of high fructose (from foods and drinks) is associated with negative metabolic effects including increased hepatic lipogenesis with a concomitant hepatic and extrahepatic fat deposition. It is also implicated in incidences of hypertriglyceridemia, and insulin resistance, increased oxidative stress, and increased plasma and tissue accumulation of intermediate and advanced glycation products [6].

A number of phytochemicals from natural sources are associated with positive health outcomes in many disease conditions with many of them applied in modern medicine [7–9]. *Parkia biglobosa* (Jacq.), popularly known as African locust bean tree belongs to Leguminosae family and is a native to West Africa. It is known as *Dorowa* (Hausa), *Ogiri* (Igbo) and *Iru* (Yoruba) in some Nigerian languages. The fermented seeds are commonly used as seasoning for soups in many parts of Nigerian. Previous studies have reported some biological activities of different parts of this plant and its anti-hyperglycemic effects have been documented [10, 11]. Ibrahim et al. [12] also reported potent antioxidant activity of the different parts of *P. biglobosa* *in vitro*. There are, however, no reports on its biochemical effects in calorie-induced aberrations. Hence the assessment of the effect of ethyl acetate fraction of *P. biglobosa* leaves on some biochemical markers of fructose-loaded Wistar albino rats.

Materials and methods

Chemicals and Instruments

Instruments used were weighing balance (Vickas Ltd, England), centrifuge (Vickas Ltd, England) and spectrophotometer (E312 Model) (Jenway, UK) while reagents for the biochemical experiments were products of Randox (USA). Other chemicals were methanol and n-hexane (Loba Chemie, India), ethyl acetate and 2,2-diphenyl-1-picrylhydrazyl (DPPH) (Sigma Aldrich, Germany), Silica gel (JHD, China), fructose (Qualikems, India) and STANDARD A1cCare Test Kit (SD BIOSENSOR, Korea). All chemicals were of analytical grade.

Experimental animals

The animals used in this study were inbred healthy male adult Wistar rats (75–150 g) used for the intervention studies. They were maintained on standard animal feed (Grand Cereals Limited, Nigeria) and water *ad libitum*.

Plant sample

Fresh leaves of *P. biglobosa* were collected from a farm in Mkpok, Nsukka LGA of Enugu State, Nigeria and authenticated by John Nwachukwu, a taxonomist at the University of Nigeria, Nsukka and voucher specimen was submitted at the herbarium of Department of Plant Science and Biotechnology (UNH/147). Air-dried and powdered sample was defatted using n-hexane and then extracted by maceration in methanol for 48 h. The filtrate was dried at 40°C to obtain the crude methanol extract which was fractionated by vacuum-liquid chromatography as described by Nwankwo [13] using silica gel as stationary phase. The solvent fractions were collected and evaporated to dryness and stored at 2–8°C. Pilot study showed that ethyl acetate fraction (EAFPB) was more active and was then chosen for intervention study.

Preliminary Phytochemical analyses

The method of Harborne [14] and Trease and Evans [15] were adopted for the preliminary phytochemical analyses of the fraction.

Preparation of the Sample for Gas Chromatography analysis

Hydroxycinnamic acids

following the description of Ortan et al. [16], the hydroxycinnamic constituents of the sample were extracted

with methanol and the resulting extract filtered and concentrated to 1 ml for analysis.

Flavonoids

As described by Millogo-Kone et al. [17], 0.5 g of the sample was boiled in deionized water for 10 min followed by the addition of 100 ml boiling methanol: water (70:30 v/v) to obtain the flavonoid extract. The homogenate was left for about 4 h before filtration and derivatization for volatility in GC analysis. The mixture was then concentrated to 2 ml ready for analysis.

Phenolic Acids

50 mg of the sample was extracted with 5 ml 1 M NaOH for 16 h at ambient temperature as described by Kelley et al. [18] and Provan et al. [19]. This was followed by centrifugation (5000 \times g), rinsing, and further centrifugation. The supernatants were pooled and then heated for 2 h at 90°C to liberate the conjugated phenolic acids [20]. After cooling, the extract was titrated with 4 M HCl to pH < 2.0, diluted to 10 ml with deionized water, followed by centrifugation and further purifying of the resultant supernatant.

Purification of phenolic acids and GC conditions for sample analysis

A fraction (15 ml) of the supernatant obtained above was passed through a Visiprep-attached PPL solid-phase extraction tube (3-mL size with 200 mg packing) (conditioned with 2 mL each of ethyl acetate and water (pH < 2.0)) at 5-mL min⁻¹. Then the resin was completely dried by vacuum treatment (-60 kPa) and the phenolic acids eluted into gas chromatography Autosampler vials with ethyl acetate (1 mL). Chromatographic analysis was carried out on an HP 6890 (Hewlett Packard, Wilmington, DE, USA) GC apparatus, fitted with a flame ionization detector (FID), and powered with HP Chemstation Rev. A 09.01 [1206] software to quantify and identify compounds. The column was a capillary HP INNOWax Column (30 m \times 0.25 mm \times 0.25 μ m film thickness). The inlet and detector temperatures were 250°C and 320°C respectively, split injection with a split ratio of 20:1 was used and the carrier gas was Nitrogen. For flavonoids detection, the hydrogen and compressed air pressures were 22 psi and 35 psi respectively. The oven was programmed to 50°C initial temperature, 8°C/min first ramping for 20 min, and maintained for 4 min, followed by a second ramping at 12 C/min for 4 min, maintained for 4 min. similarly, for the detection of hydroxycinnamic acids derivatives, the hydrogen and compressed air pressures were 25 psi and 38 psi respectively. The oven was programmed to 50°C initial temperature, 8°C/min first ramping for 20 min, and maintained for 4 min, followed by a second ramping at

10 C/min for 4 min, maintained for 3 min. in addition, for the detection of phenolic acids derivatives, hydrogen and compressed air pressures were 28 psi and 32 psi respectively. The initial oven temperature was 60°C for 5 min, with first and second ramping maintained at 15°C/min for 1 min and 10 C/min for 4 min, respectively.

2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity of the ethyl acetate fraction

The DPPH radical scavenging activity of the ethyl acetate fraction was determined as previously described [21], with slight modification. Briefly, a solution of 0.135 mM DPPH in methanol was prepared and 1 ml of this solution was mixed with 1 ml of the fraction and standard separately (BHT) (0.025-0.5 mg/ml concentrations) prepared in methanol. The reaction mixture was kept for 30 min in the dark and at room temperature after thorough mixing. The absorbance of the resulting mixture, read at 517 nm with a spectrophotometer, was used to calculate the radical-scavenging efficacy of the sample as follows:

$$\text{Radical scavenging efficacy} = \frac{\text{Abs control} - \text{Abs sample}}{\text{Abs control}} \times 100$$

Intervention study design

The study protocols were approved by Faculty of Biological Sciences Committee on Research and Bio-Ethics (UNN/FBS/EC/1056). A total of five groups of experimental rats (n = 5) were used: all the animals, except those in group 1 (normal control), were administered high fructose solution (HFS) (3 g/kg body weight fructose dissolved in distilled water) for 30 days before drug administration. Group 2 served as HFS control group (without treatment), the reference group (group 3) was treated with 300 mg/kg/d b.w., *p.o* of Metformin, while the treatment groups (groups 4 and 5) were administered 100 and 200 mg/kg/d b.w., *p.o* EAFPB, respectively for two weeks. After the intervention period, the 12-hr fasted experimental rats were exsanguinated via cardiac puncture under mild anesthesia into both anticoagulant-containing and clot activator-containing containers. The containers were spun at 4000 g for 10 min and plasma derived from the anticoagulant-containing container were used for glycated hemoglobin (HbA1c) test while sera from clot activator-containing containers were used for the liver function, lipid profile, lipid peroxidation and antioxidant tests.

Determination of HbA1c levels of experimental animals

The HbA1c levels of the animals were determined by the immunoassay and reflectometry method using the STANDARD™ A1cCare test kit. The principle is based

on the immunoassay and reflectometry. The kit uses an anti-HbA1c (%) antibody which is specific for the first few amino acid residues of the glycosylated N-terminus of the beta chain of hemoglobin A0. The kit contains the test panel (nitrocellulose membrane), latex tablet (blue dyed latex micro-particles conjugated to specific antibodies) and buffer solution (hemolysis reagent). When whole blood is added to the buffer solution and is mixed with the latex tablet, the erythrocytes are instantly lysed to release the hemoglobin (HbA1c in this case). When sample mixture is loaded onto the sample port of the test panel, the mixture fluid migrates along the membrane of test panel by capillary action and the HbA1c gets immobilized onto the anti-HbA1c antibody-coated line. At the same time, latex conjugate binds to HbA1c. A photometric sensor measures the LED light reflected and then HbA1c is calculated.

Determination of biochemical indices

Total bilirubin concentration was determined by colorimetric method described by Jendrassik and Grof [22]. Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities were assayed following the protocol of Reitman and Frankel [23] while alkaline phosphatase (ALP) activity was assayed by the method of Englehardt [24]. Lipid peroxidation was determined by measuring malondialdehyde concentration following Wallin et al. [25] method. In addition, reduced glutathione concentration was determined using the method of Habig et al. [26]. Furthermore, serum total cholesterol, high density lipoprotein (HDL) and triglyceride (TRIG) concentrations concentration was determined using the methods of Allain et al. [27] and Albers et al. [28], respectively as contained in QCA commercial kits. Similarly, serum low density lipoprotein (LDL) was determined by modified method of Friedwald et al. [29], as the difference between total cholesterol and the cholesterol content of the supernatant after precipitation of the LDL fraction by polyvinyl sulphate in the presence of polyethylene glycol monomethyl ether.

Results

Phytochemical constituents of EAFPB

The analyses of some phytoconstituents of the ethyl acetate fraction of *P. biglobosa* leaves (EAFPB) (Table 1) showed relatively high concentration of phenolics and tannins, moderate concentrations of flavonoids and terpenoids, while alkaloids concentration was very low compared to the other phytochemicals tested.

DPPH radical-scavenging efficacy of EAFPB

It was recorded that the percentage inhibition of DPPH radical by EAFPB is concentration-dependent. The DPPH radical scavenging expressed as the efficient

Table 1 Phytochemical constituents of EAFPB

Phytochemical	Concentration
Tannins	39.93 ± 1.03 (mg/100 g)
Phenolics	20.77 ± 0.4 (mgGAE)
Terpenoids	12.7 ± 0.45 (mg/100 g)
Flavonoids	10.83 ± 0.72 (mg/100 g)
Alkaloids	1.5 ± 0.06 (mg/100 g)

Values are mean ± standard deviation (n = 3)

concentration of sample which is able to scavenge 50 % of DPPH free radicals (EC_{50}) was 0.395 mg/ml (Table 2).

Flavonoids, hydrocinnamic acids and phenolic acids derivatives of ethyl acetate fraction of *Parkia biglobosa* leaves

The GC chromatograms of the flavonoids, hydrocinnamic acids derivatives and phenolic acids derivatives are presented in Figs. 1, 2 and 3. Twenty-eight (28) flavonoids consisting mainly of kaempferol (21.31 mg/100 g), quercetin (12.84 mg/100 g), luteolin (6.75 mg/100 g), myricetin (5.54 mg/100 g), naringenin (2.58 mg/100 g), and (+)-catechin (1.02 mg/100 g) were detected. Four (4) hydrocinnamic acids derivatives namely P-coumaric acid (6.73 mg/100 g), chicoric acid (0.66 mg/100 g), P-coumarin (0.113 mg/100 g), and scopoletin (0.002 mg/100 g) were detected, while eleven (11) phenolic acids derivatives mainly chlorogenic acid (48.18 mg/100 g), protocatechuic acid (21.58 mg/100 g), ferulic acid (14.12 mg/100 g), P-hydroxybenzoic acid (13.84 mg/100 g), sinapinic acid (11.78 mg/100 g), caffeic acid (8.94 mg/100 g), and salicylic acid (7.74 mg/100 g) were detected in the plant sample.

Effect of EAFPB on glycosylated hemoglobin and oxidative status of fructose overloaded rats

Fructose ingestion elevated glycosylated hemoglobin (HbA1c) and malondialdehyde (MDA) levels of the HFS control compared to normal control (Table 3). The graded doses of EAFPB reduced the HbA1c and MDA levels in the intervention groups (groups 4 and 5) relative to placebo (HFS control). Treatment with fructose, and reference and herbal drugs however, had no

Table 2 DPPH radical-scavenging capacity of the ethyl acetate fraction of *Parkia biglobosa* leaves

Concentration (mg/ml)	% Inhibition
0.16	14.75
0.32	35.33
0.48	61.41
0.64	73.01
0.80	85.78

EC_{50} = 0.395 mg/ml

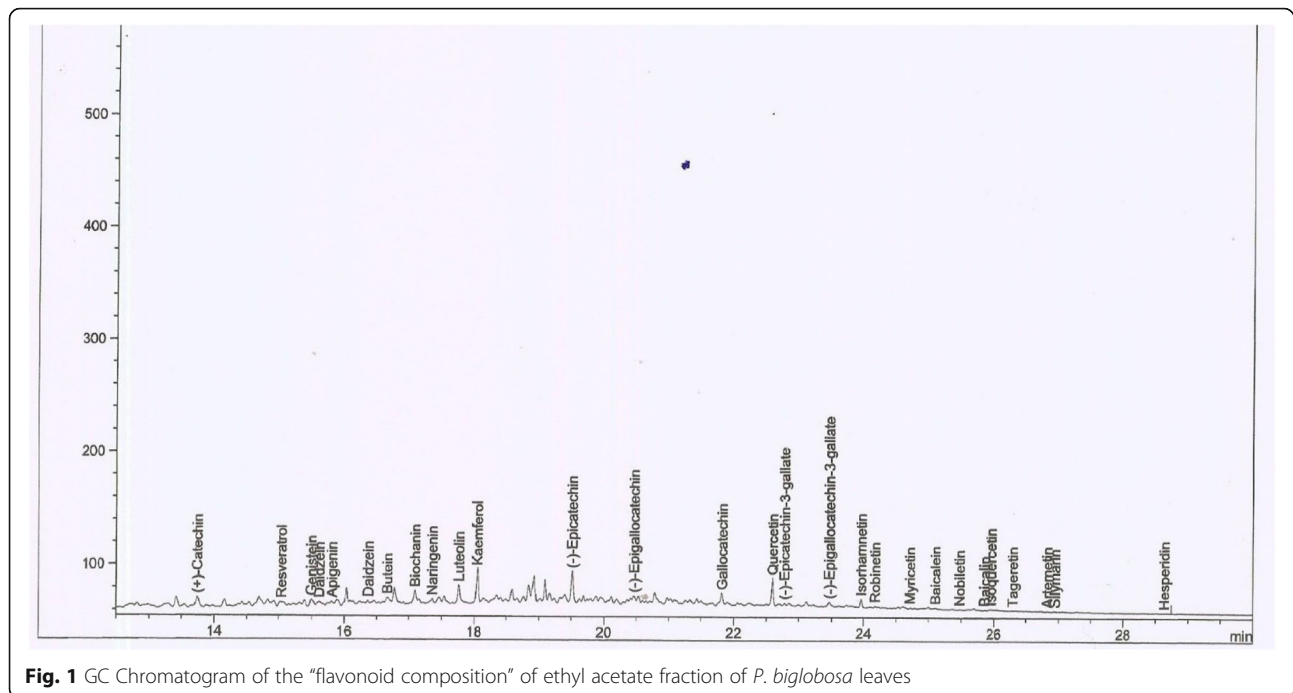


Fig. 1 GC Chromatogram of the "flavonoid composition" of ethyl acetate fraction of *P. biglobosa* leaves

significant effect on serum reduced glutathione concentration in all the groups.

Effect of EAFPB on liver status of fructose overloaded rats

Assay of liver enzymes' activities is important in monitoring aberrations in liver function. From the study, high fructose consumption caused elevation in serum activities of alkaline phosphatase (ALP), alanine aminotransferase (ALT), and aspartate aminotransferase (AST) but caused no significant change in bilirubin concentration when compared to control (Table 4). Compared with placebo, treatment with 100 mg/kg/d b.w. of EAFPB decreased the serum total bilirubin concentration, and

ALP and AST activities but not ALT activity. However, treatment with 200 mg/kg/d b.w. dose of EAFPB decreased serum total bilirubin, ALP and ALT activities but not AST activity when compared to placebo.

Effect of EAFPB on lipid profile of experimental rats

The untreated rats had higher serum cholesterol, triglyceride, and LDL, but no significant change in serum HDL compared to the normal control (Table 5). Treatment with 100 mg/kg/d b.w. of EAFPB caused a reduction in serum cholesterol, triglyceride, and low density lipoproteins concentrations, and an increase in high density lipoprotein concentration of the treated rats

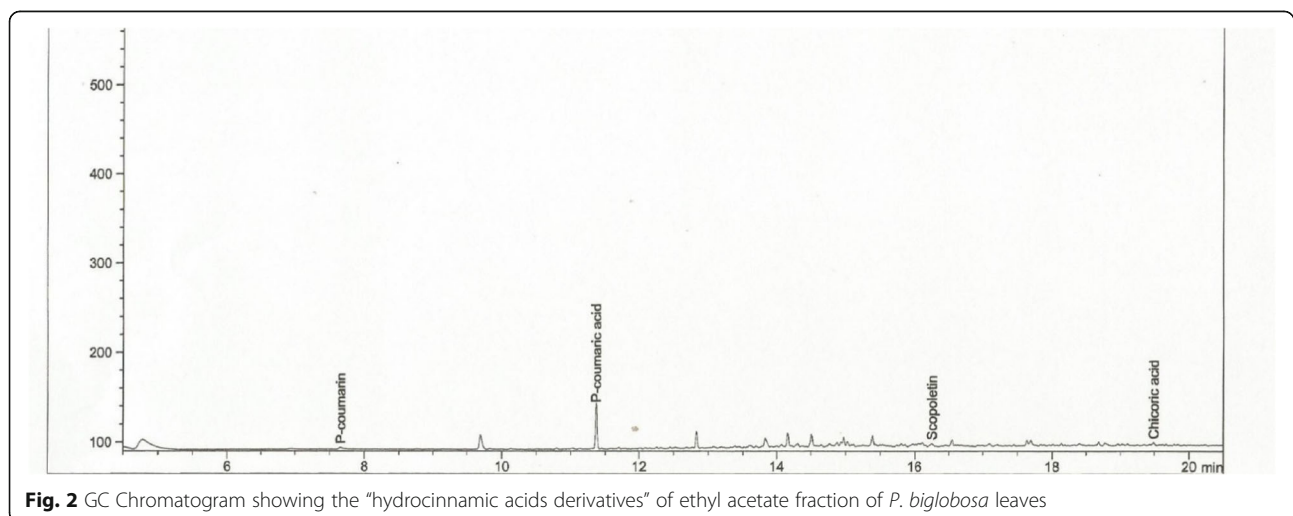


Fig. 2 GC Chromatogram showing the "hydrocinnamic acids derivatives" of ethyl acetate fraction of *P. biglobosa* leaves

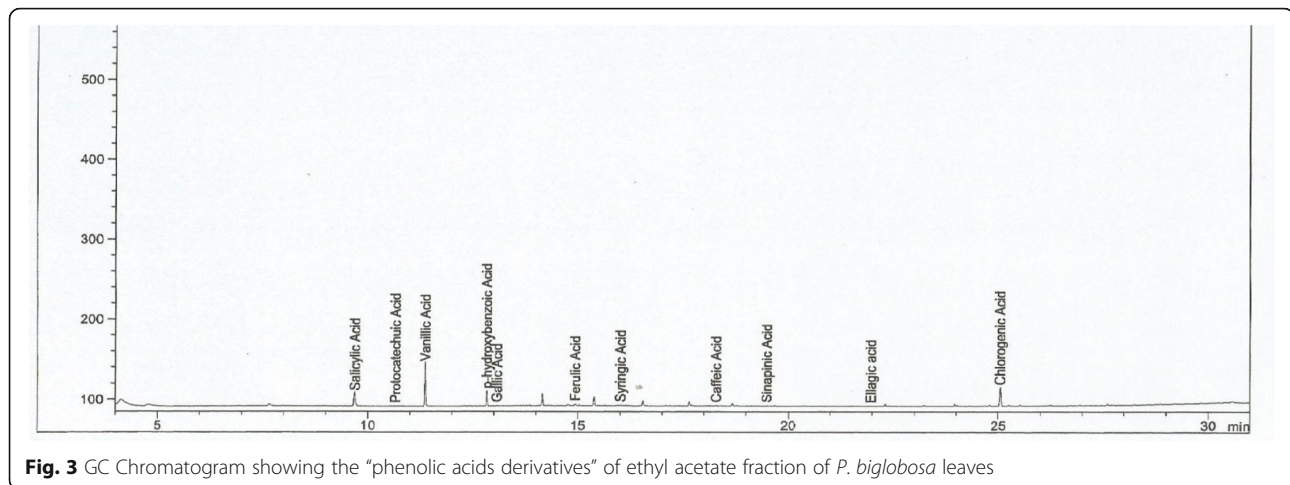


Fig. 3 GC Chromatogram showing the "phenolic acids derivatives" of ethyl acetate fraction of *P. biglobosa* leaves

compared to the untreated group. On the other hand, the 200 mg/kg/d b.w. of EAFPB reduced the serum cholesterol, triglyceride, and LDL concentrations but had no effect on HDL concentration of the treated rats compared to placebo.

Discussion

The therapeutic effects of many plant products are often associated with their anti-oxidative properties which is attributed to the biological activities of constituent polyphenolic compounds such as flavonoids and phenolic acids. In this present study, the primary phytochemical evaluation of the ethyl acetate fraction of *P. biglobosa* leaves (EAFPB) revealed the presence of phenolics, tannins, flavonoids, terpenoids and alkaloids, with the tannin and phenolic contents substantially higher than the rest. The gas chromatography analysis of the fraction revealed 28 known flavonoids (mainly kaemferol, quercetin, and luteolin), four hydrocinnamic acids derivatives (mainly *P*-coumaric acid), and 11 phenolic acids derivatives (mainly chlorogenic acid and protocatechuic acid). Polyphenolic compounds are potent disruptors of the damaging effects of free radicals via their antioxidant activities [21, 30], therefore, the presence of these

compounds in the fraction suggests that EAFPB could have antioxidant capacity. In the presence of an antioxidative compound, the violet color of DPPH in ethanol solution is de-colored to yellow [31]. In this study, a concentration-dependent increase in percentage inhibition of DPPH radicals was observed. This radical scavenging property demonstrated by EAFPB could be linked with some of the detected compounds such as chlorogenic acid, quercetin, kaempferol, and ferulic acid whose antioxidant potentials are known [32]. Furthermore, this result agrees with the finding of Komolafe and Oyelade [33] that attributed the anti-oxidative effect observed in their study to the phenolic and flavonoid contents of the leaf extract, although the authors did not identify the particular compounds suspected.

The link between biological products of glycation and the pathophysiology of many conditions like neurodegenerative disorders, diabetes and its complications, aging and progression of some tumors are known [34]. Relative to normal control, significant elevation in HbA1c levels were observed in placebo rats. HbA1c levels were however lower in fructose-fed rats that received EAFPB than in placebo. High level of circulating fructose is positively correlated to the glycation of both intra- and extracellular proteins including hemoglobin. The rate of non-enzymatic reactivity of fructose with hemoglobin is reported to be 7.5-fold higher than glucose because fructose metabolism generates intermediates that easily undergo glycation [6, 35]. Our result showed markedly reduced HbA1c levels in the EAFPB-treated rats, which may be attributed to the polyphenolic compounds in EAFPB. Wu et al. [36] reported that chlorogenic and ferulic acids, which are among the polyphenolics detected in EAFPB, inhibited glucose mediated protein modification and subsequent cross-linking. Furthermore, luteolin and quercetin which were also present in EAFPB were earlier reported to reduce

Table 3 Effect of EAFPB on glycosylated hemoglobin and some antioxidant parameters of fructose overloaded rats

Groups	HbA1c	MDA	GSH
Normal control	3.39 ± 0.58 ^b	18.13 ± 1.88 ^b	5.04 ± 0.74 ^a
HFS control (placebo)	4.95 ± 0.21 ^a	21.31 ± 0.75 ^c	4.69 ± 0.76 ^a
Reference group	3.28 ± 0.35 ^b	13.24 ± 1.40 ^a	5.46 ± 1.35 ^a
100 mg/kg/d EAFPB	2.80 ± 0.34 ^b	14.56 ± 0.80 ^a	5.83 ± 0.74 ^a
200 mg/kg/d EAFPB	3.20 ± 0.62 ^b	19.46 ± 0.95 ^b	5.05 ± 0.78 ^a

Data represent mean ± SD (n = 5); values with dissimilar alphabets as superscripts are significant at $p < 0.05$

Table 4 Effect of EAFPB on some liver function parameters of fructose overloaded rats

Groups	ALP (IU/L)	ALT (IU/L)	AST (IU/L)	T. Bilirubin
Normal control	49.60 ± 7.16 ^a	14.80 ± 4.32 ^a	36.40 ± 1.82 ^a	1.85 ± 0.25 ^{abc}
HFS control (placebo)	64.20 ± 14.65 ^b	21.80 ± 3.90 ^b	56.20 ± 4.66 ^c	2.29 ± 0.14 ^c
Reference group	50.80 ± 4.97 ^a	14.60 ± 1.82 ^a	35.60 ± 5.55 ^a	2.02 ± 0.21 ^{bc}
100 mg/kg/d EAFPB	50.60 ± 8.44 ^a	18.00 ± 2.35 ^{ab}	44.80 ± 5.26 ^b	1.61 ± 0.50 ^{ab}
200 mg/kg/d EAFPB	46.00 ± 8.28 ^a	15.20 ± 4.15 ^a	50.40 ± 9.04 ^{bc}	1.40 ± 0.72 ^a

Data represent mean ± SD (n = 5); values with dissimilar alphabets as superscripts are significant at $p < 0.05$

HbA1c levels and different stages of advanced glycation end-product (AGE) production [37]. The anti-glycation activity of polyphenols is postulated to occur through nucleophilic attack on the side chain of AGE structures, thus inhibiting glycooxidation of proteins [38, 39].

Apart from the generation of toxic intermediates such as glyoxal and methylglyoxal, increased fatty acid synthesis and overload to mitochondria increases the generation of reactive species that result in enhanced lipid peroxidation and consequent oxidative stress [6]. The increased serum concentration of malondialdehyde (MDA) in the placebo is an indication of elevated peroxidation of lipids and oxidative stress. However, the significant reduction in MDA concentration in the EAFPB-treated groups indicates good anti-oxidative property of the plant fraction as a result of its rich polyphenolic constituents such as kaempferol and quercetin which have been shown to effectively counter intracellular oxidative stress [40]. The positive effect of *P. biglobosa* extract on toxicant-induced mitochondrial dysfunction and reactive oxygen species (ROS) generation as reported by Komolafe et al. [31] also supports the result of this study. Additionally, the presence of luteolin which has been shown to suppress the expression inflammatory cytokine, boost antioxidant status and decrease lipid peroxidation in diabetic condition [41], might be responsible for the health benefits recorded in this study. Due to their electron-donating capacities, polyphenols are capable of transferring electron(s) to free radicals to stabilize them and prevent their oxidative damage to cellular components and peroxidation of lipids [42]. Meanwhile, the non-significant effect of EAFPB on serum reduced glutathione concentration however, indicates that its antioxidant effect that might be independent of the glutathione

pathway. In agreement with the present findings, polyphenols-rich extracts have been shown to abrogate experimentally-induced glycation and tissue oxidative damage via antioxidant potentials, metal-complexing properties, protein interaction, methylglyoxal trapping, and inhibition of receptor for AGE (RAGE)/NF- κ B pathway activation, as well as boosting of antioxidant defence system [43, 44]. Furthermore, the antiglycation potentials of herbal entities have been documented to be directly correlated with the polyphenolic content [45, 46]. This observation strongly suggests that bioavailable polyphenol-rich plant-based diets have potential application in the prevention and treatment of glycation-mediated conditions such as diabetes, atherosclerosis, and neurodegenerative and end-stage renal diseases [47–49].

Monitoring changes in biochemical profile such as the transaminases is one of the commonest methods of detecting liver dysfunctionality. High calorie diets are implicated in the development of non-alcoholic hepatic abnormalities such as fibrosis, cirrhosis, steatosis, and even hepatocellular carcinoma [50]. The result of this study shows significantly elevated activities of liver function enzymes (ALP, ALT, and AST) after prolonged administration of HFS without treatment (placebo). Elevated serum activities of these enzymes are suggestive of fructose overload-related hepatotoxicity. Earlier studies reported increased inflammation and hepatic endoplasmic reticulum (ER) stress in high fructose supplemented rats, further highlights the toxic effects of fructose overload on hepatic cells [51, 52]. Elevated fructose metabolism increases *de novo* lipogenesis with consequent hepatic fat accumulation along with ER and mitochondrial stresses, increasing lipotoxicity [50, 53]. When compared with placebo, treatment with EAFPB

Table 5 Effect of EAFPB on lipid profile of high fructose-fed rats

Groups	T. CHOL	TRIG	HDL	LDL
Normal control	74.20 ± 16.74 ^a	72.20 ± 7.44 ^a	66.60 ± 7.23 ^{ab}	50.60 ± 14.05 ^a
HFS control (placebo)	93.00 ± 14.14 ^b	86.80 ± 9.21 ^b	52.00 ± 12.27 ^a	80.60 ± 7.16 ^b
Reference group	60.60 ± 8.85 ^a	66.20 ± 9.37 ^a	65.60 ± 10.57 ^{ab}	45.60 ± 9.24 ^a
100 mg/kg/d EAFPB	65.80 ± 6.61 ^a	73.00 ± 7.25 ^a	69.60 ± 9.10 ^b	47.60 ± 9.61 ^a
200 mg/kg/d EAFPB	60.00 ± 12.67 ^a	60.40 ± 9.79 ^a	62.80 ± 14.34 ^{ab}	46.00 ± 6.52 ^a

Data represent mean ± SD (n = 5); values with dissimilar alphabets as superscripts are significant at $p < 0.05$

significantly attenuated the fructose-induced up-regulation of serum activities of these enzymes, indicating a hepatoprotective property. This observation is suspected to result from the presence and bioactivity of the polyphenols in EAFFB. This class of compounds are associated with potent antioxidant activities albeit via different mechanisms. For example, *p*-coumaric acid reduces hepatic fat accumulation by increasing AMPK expression [54], while quercetin improves lipid metabolism via activation of Akt signalling [55]. In addition, kaempferol has been shown to prevent lipid accumulation while enhancing antioxidant defense in hepatocytes [56]. Although no significant change in serum bilirubin concentration in the HFS group relative to normal control, the result suggests that prolonged fructose overload could result in hyperbilirubinemia. Similarly, only the highest dose of EAFFB (200 mg/kg) caused a significant reduction in serum bilirubin levels compared to placebo, suggesting improved bilirubin metabolism and excretion by the liver. The overall improvement of hepatic markers agrees with the reports of Gasparotto et al. [57] that polyphenols from peach-derived products attenuated CCl₄-generated oxidative stress and liver damage by down-regulating AGE receptor expression. Ibrahim et al. [11] also reported improvements in hepatic markers of diabetic rats treated with *P. biglobosa* butanol fraction, which further supports the result of this study.

Fructose overload causes aberrations in lipid metabolism via glycation- and oxidative stress-dependent mechanisms. Fructose upregulates the expression of transcriptional regulators of hepatic lipogenesis, the sterol regulatory element-binding protein-1c (*SREBP-1c*) and carbohydrate response element binding protein (*ChREBP*), consequently up-regulating their downstream targets, acetyl Coenzyme A carboxylase (*ACC*) and fatty acid synthase (*FASN*), the key enzymes of fatty acids biosynthetic pathway [5, 58]. High fructose consumption thus causes dyslipidemia by increasing hepatic lipogenesis and triglyceride synthesis while counteracting the β -oxidation of fatty acids [6]. Significant increase in serum levels of lipid markers (cholesterol, triglyceride, and low-density lipoproteins) was observed in this study when placebo is compared with normal control. This further confirms the detrimental effect of fructose overload. Treatment with the plant fraction caused significant improvement in lipid profile of the experimental rats. Earlier studies by Ibrahim et al. [11] also reported improvements in lipid profile of type-2 diabetic rats treated with butanol fraction of this plant, linking it to the insulinotropic effect of the plant in diabetic rats. *P*-coumaric and ferulic acids are reported to decrease the expression of adipocyte differentiation markers, thus reducing unwanted lipid accumulation [59].

Conclusions

The result of this study showed that ethyl acetate fraction of *Parkia biglobosa* leaves contains a good number of health-promoting polyphenolic compounds which are inferred to be responsible for the observed beneficial effects on the biochemical status of fructose overloaded rats. To the best of the knowledge of the researchers, this is the first-time efforts were made to investigate the phytochemical constituents of *Parkia biglobosa* leaves. The exact mechanism of action of *Parkia* polyphenols in reversing sugar-induced biochemical aberrations is, however, still a subject of further research.

Abbreviations

EAFFB: Ethyl acetate fraction of *Parkia biglobosa* leaves; HFS: High fructose solution; HbA1c: Glycated hemoglobin; AST: Aspartate aminotransferase; ALT: Alanine aminotransferase; ALP: Alkaline phosphatase; MDA: Malondialdehyde; DPPH: 2,2-diphenyl-1-picrylhydrazyl; GC-FID: Gas chromatography coupled flame ionization detector; LDL: Low density lipoprotein; HDL: High density lipoprotein; TRIG: Triglyceride; EC50: Efficient concentration of sample that can inhibit 50% of the reaction; AGE: Advanced glycation end-product; RAGE: Advanced glycation end-product receptor; ROS: Reactive oxygen species; SREBP-1c: Sterol regulatory element-binding protein-1c; ChREBP: Carbohydrate response element binding protein; ACC: Acetyl Coenzyme A carboxylase; FASN: Fatty acid synthase

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not applicable.

Authors' contributions

CCC and ABC conceived and designed the work, CCC, ARO, OIU and NCC collected and analyzed the data, CCC and OIU drafted the manuscript. All the authors read and approved the final manuscript.

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Availability of data and materials

The dataset supporting the conclusions of this article is included within the article.

Declarations

Ethics approval and consent to participate

The study protocols were approved by the Faculty of Biological Science Committee on Research Ethics, University of Nigeria, Nsukka (UNN/FBS/EC/1056). Other national and international guidelines for the care and use of animals for research were strictly followed in this study.

Consent for publication

not applicable.

Competing interests

The authors declare that they have no competing interests.

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