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Effect of unripe *Blighia Sapida* fruit on hyperglycaemia, hyperlipidemia, oxido-inflammatory stress and apoptosis in high-fat diet/streptozotocin-induced diabetic rats

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Abstract

Background *Blighia sapida* (ackee) unripe fruit is known for its hypoglycaemic effect at toxic dose, however, its antidiabetic activity at small dose is fairly unknown. This study aimed to investigate antidiabetic potentials from *B. sapida* unripe fruit extract.

Methods Unripe *B. sapida* fruits were air-dried, ground and extracted sequentially with n-hexane and methanol. GC-MS profiles of phytochemical constituents were determined in *B. sapida* fruit extract (BSFE). In vitro antioxidant, α -glucosidase and α -amylase inhibitory activity were also determined. Antidiabetic effect was investigated in 3-weeks High-fat diet fed rats injected with streptozotocin (35 mg/kg). Rats with FBS > 200 mg/dL were randomly grouped into 5 ($n=6$) and treated with vehicle, BSFE (25, 50 and 100 mg/kg) and metformin (250 mg/kg). Treatments were administered orally daily for 14 days. FBS was taken at 7 and 14 days, while blood, brain and liver were collected for biochemical and histological analysis.

Results The GC-MS analysis revealed the presence of various compounds. BSFE showed potent antioxidant, as well as α -glucosidase and α -amylase inhibitory activities. The BSFE (25–100 mg/kg) when compared with diabetic control significantly reduced FBS on day 14 by 64.7%, 61.1%, and 64.2%. BSFE significantly reduced total cholesterol, triglycerides, VLDL and atherogenic index. BSFE treatment significantly reduced MDA and nitrites levels, while increasing GSH, catalase and SOD in the diabetes affected organs, liver and brain. Also, TNF- α , IL-6, caspase-3 and caspase-9 levels were reduced in the liver and brain of diabetic rats treated with BSFE.

Conclusion *Blighia sapida* unripe fruit extract showed promising antihyperglycaemic, hepatoprotective and neuroprotective effects through antioxidant, anti-inflammatory and antiapoptotic mechanisms.

Keywords *Blighia Sapida*, Ackee extract, Diabetic rats, Antihyperglycaemic, Anti-inflammatory, Antioxidant

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Introduction

Type 2 Diabetes Mellitus (T2DM) is a non-communicable disease that has become a global burden. It is regarded as a chronic metabolic disorder that is characterized by impaired metabolic function of insulin, an endogenous hormone synthesized and released from the beta cells of the pancreas when there is an increased blood sugar level [1, 2].

The beta cells of the pancreas secrete the polypeptide, insulin which controls both the in-between meal energy usage (intra-prandial) of food nutrients and after meal (postprandial) storage of glucose. Absolute or relative or both deficiency of insulin secretion leads to a persistent hyperglycemic state [2]. In accordance to the International Federation of Diabetes (IDF), the population of diabetes mellitus' patients as of 2021 is as high as 537 million adults aged between 21 and 79 years, with a projection of increase to 643 million by 2030 and 783 million by 2045 [3]. T2DM is characterized by reduced insulin action as a result of insulin resistance, with an overtime decrease in insulin secretion. It is the more common type of DM accounting for about 90% of cases of DM [2]. The rate of incidence of T2DM is common in obese individuals and usually with other co-morbidities such as hypertension and dyslipidemia [4].

In T2DM, prolonged hyperglycemia elicits oxidative stress and inflammation, which affects internal organs resulting in macrovascular and microvascular complications [5]. Macrovascular complications of T2DM are basically diseases of cerebrovascular system and arteries affecting large blood vessels of the brain, heart and kidney [6]. On the other hand, microvascular complications are damages which are associated with the small blood vessels in some selected areas such as the brain and nephron and retinal and generally categorized as neuropathy, nephropathy and retinopathy [7]. In the liver of T2DM individual, chronic hyperglycemia increases risk of non-alcoholic fatty liver diseases, wherein fat builds up in the liver which may results in coronary artery disease, stroke or peripheral arterial disease [8]. These diabetic complications present a unique challenge which is being driven by oxidative stress and inflammation, hence preventive and management strategies require intricate search for new molecules.

Non-pharmacological approaches such as diet modifications and exercise are the initial steps in the management of DM. However more than 90% of the patients do not achieve the diabetic goal using this alone and they require the use of either insulin or oral-hypoglycemic agent (OHA) or a combination of both [9]. OHA has been associated with a wide range of adverse effects (AE), which have necessitated an increase in the need for more effective antidiabetic agents with less AE to be discovered [10]. Some recent findings have shown the ability of

frequent consumption of some high flavonoid-containing fruits and vegetables to reduce the risk of T2DM in early adulthood [11, 12].

Blighia sapida (k. Koenig) is a fruit bearing tree, belonging to the soapberry family, sapindaceae. The tree which originated from the West and Central Africa, is now cultivated in the Central and South America and West Indies [13, 14]. The fruit aril known as ackee is considered as National fruit in Jamaica [15]. In southwest Nigeria, it is called "Isin", it however has other dialectal names in Western Africa countries [13]. While the ripe fruit is delicious, consumption of unripe ackee is known for its toxicity which manifest as vomiting, hypoglycaemia, seizures and death [16, 17]. Ackee arils is considered as food source being used as vegetables in sauce or mature arils being used as snacks [18]. Various preparation and combination of the fruits extract have been made for the treatment of diseases such as dysentery, epilepsy, and diabetes [19]. Analysis of the likely constituents of the unripe ackee to determine the hypoglycaemic causing chemical detected the rich abundance of a toxic substance that is now known as hypoglycin A (HGA) [20].

Several studies have shown the anti-diabetic effects of extract of some unripe fruits both in in vitro and in vivo models of streptozotocin-induced diabetic rats [21, 22]. The results from these studies demonstrated that unripe fruit extracts significantly reduced blood glucose levels, improved insulin sensitivity, and decreased oxidative stress and inflammation in the rats [22, 23]. Various investigations have shown phytochemical, nutritional and antioxidants levels of unripe and ripe ackee fruits [23, 24]. Grande-Tovar et al., [24], for examples showed that the levels of hypoglycin A in unripe ackee differ from region to region and Jamaica showing the highest content, while West Africa country of Bukina Faso showed the least amount. A case report has documented hypoglycemic effects following ackee fruit consumption in a patient with poorly managed type 2 diabetes mellitus [15]. Early investigation on hypoglycin from unripe ackee fruits indicated its oral hypoglycemic effect which peaked at the 3rd hour post administration [20]. In addition, the levels of ascorbic acid and polyphenolic compounds were ten times higher in the unripe compared to the ripe fruits [25]. Despite the hypoglycaemic toxicity arising from consumption of the unripe fruit, there is paucity of information on the protective effects in diabetic rats as a result of other abundant bioactive constituents. Ajayi et al., [26] reported that repeated oral administration of low dose extract of *B. sapida* unripe fruits demonstrated minimal toxic effects in rats. Hence this study was designed to investigate antidiabetic potentials of *B. sapida* unripe fruit extract in high-fat diet/streptozotocin-induced diabetes in rats.

Materials and methods

Chemicals

Streptozotocin (CAS 18883-66-4) was a product of Santa Cruz Biotechnology (San Juan, USA). α -glucosidase (9001-42-7), α -amylase (10065), 5, 5'-Dithio-nitrobenzoic acid (DTNB, 69-78-3), acetylthiocholine iodide (1866-15-5), adrenaline (51-43-4), thiobarbituric acid (TBA, 504-17-6) were products of Sigma Chemicals Co., (St Louis, USA). TNF- α (430904) and IL-6 (431304) ELISA product of Biolegend (San Diego, USA), while caspase-3 (E-EL-R0160) and Caspase-9 (E-EL-R0163) kits were product of Elabscience (Wuhan, China). Petroleum ether, methanol, and all other reagents were of analytical grade and the purest quality available.

Collection of unripe fruits of *Blighia Sapida* and extraction

The unripe fruits of *Blighia sapida* were harvested before the ripening season in May 2021 from a tree on the campus of the University of Ibadan. The fruit was identified and authenticated at Forest Herbarium Ibadan with voucher specimen number FHI-111,210. Arils were separated from the pods of unripe fruit and oven-dried at 55 °C to constant weight for seven days. The extraction method used was a slightly modified version of the protocols previously described [27, 28]. Briefly, about 400 g of the dried powder was soaked in 800 mL n-hexane and left for 72 h, it was then filtered and the residue was left in an open place for the n-hexane to evaporate. Thereafter, the defatted powder was macerated in 1 L of absolute methanol for 72 h. The extract was filtered and concentrated under reduced pressure and temperature at 40 °C in a rotary evaporator and dried to a constant weight in a vacuum dry air oven resulting in a percentage yield of 12.32%.

In vitro biological activity assays

Determination of total phenolic content (TPC) and total flavonoid content (TFC)

The folin-Ciocalteu assay method was used to determine the TPC of BSFE using gallic acid as the standard phenolic compound as described [29]. Briefly, BSFE (1 mg/mL) was mixed with an aliquot of 0.4 mL Folin-Ciocalteu phenol reagent and 0.4 mL of 7% Na₂CO₃. Absorbance was read thereafter with a spectrophotometer at 725 nm after 2 h of incubation. TPC in BSFE was expressed in Gallic acid equivalent as μ gGAE/mg sample. Also, TFC was determined using the aluminum chloride colorimetric method. Rutin was used as the standard flavonoid and TFC was expressed as μ gRE/mg sample [30].

2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical assay

The free radical scavenging activity of BSFE was determined using the DPPH assay [31]. BSFE or Ascorbic acid was dissolved in methanol and diluted at graded

concentrations 1000, 500, 250, 125, and 62.5 μ g/mL and added to 96-well plate, to which freshly prepared DPPH solution (0.1 mM) in methanol was also added, and incubated for 30 min in the dark. Absorbance was read at 540 nm in a microplate reader (LT4500, UK). The absorbance of blank was used to calculate free radical scavenging activity as;

% inhibition = [(absorbance of blank – absorbance of test sample)/absorbance of blank] x 100%.

The DPPH scavenging activity was evaluated by determining the IC₅₀ value, which represents the extract concentration required to inhibit 50% of DPPH radicals. IC₅₀ was calculated using linear regression analysis of the percentage inhibition vs. extract concentration plot.

Nitric oxide free radical scavenging assay

Nitric oxide radical scavenging activity assay based on the reaction with sodium nitroprusside was used [31]. Briefly, Sodium nitroprusside (10 mM in 0.1 M sodium phosphate buffer, pH 7.4), was incubated with graded concentrations of BSFE 1000, 500, 250, 125, and 62.5 μ g/mL and ascorbic acid (0–250 μ g/mL) and incubated at room temperature for 150 min. The same reaction mixture without the BSFE but with equivalent amount of water served as control. After incubation period, 100 μ L of Griess reagent (equal volume of 1% sulfanilamide in 5% phosphoric acid and 0.1% N-(1-naphthyl) ethylenediamine dihydrochloride) was added to equal volume of samples in a 96-well plate. The absorbance was read at 540 nm in a microplate reader (LT4500, UK).

Ferric reducing-antioxidant power (FRAP) assay

FRAP assay was determined in BSFE as earlier described with slight modification [32]. This antioxidant activity was evaluated by monitoring the formation of a Fe²⁺–TPTZ complex. FRAP reagents was freshly prepared consisting of sodium acetate buffer (0.3 M, pH 3.6 in glacial acetic acid), 2, 4, 6-Tris (2-pyridyl)-S-triazine (TZPTZ, 10 mM in 40 mM HCl) and ferric chloride (20 mM), in the ratio of 10:1:1. About 50 μ L of BSFE or ascorbic acid at 1000, 500, 250, 125, and 62.5 μ g/mL were incubated with 250 μ L FRAP reagent for 20 min in a 96-well microplate. The absorbance was read at 620 nm in a microplate reader. The antioxidant activity was calculated by subtracting the blank's absorbance from the sample readings. Results were then expressed as Trolox equivalents (μ g/mL) based on a standard curve generated with Trolox.

Total antioxidant capacity (TAC) by phosphomolybdate assay

TAC of BSFE was determined using the phosphomolybdate assay, with ascorbic acid as the standard [33]. The assay involved incubating varying concentrations of BSFE (62.5–1000 μ g/mL) with a reagent mixture containing ammonium molybdate, sulfuric acid, and sodium

phosphate buffer. The reaction mixture was incubated in a water bath at 95 °C for 30 min. Aliquot (200 µL) of the cooled mixture was added into 96-well plate and the absorbance was read at 695 nm in a micro plate reader. Ascorbic acid standard calibration curve was used to determine the total antioxidant capacity equivalent in (µg AAE).

Evaluation of inhibitory activity against carbohydrate metabolizing enzymes

The in vitro inhibitory effect of BSFE against α -glucosidase and α -amylase was determined spectrophotometrically. The inhibition of α -glucosidase activity was determined according to the protocol earlier described [34] with slight modification. α -glucosidase from *Saccharomyces cerevisiae* (100UN) was dissolved in 0.1 M phosphate buffer (pH 7.4). 50 µL BSFE (1000, 500, 250, 125, and 62.5 µg/mL) was added into a 96-well plate, and incubated with 100 µL of enzyme solution (0.2 U/mL) for 20 min. The substrate, p-Nitrophenyl- α -D-glucopyranoside (50 µL; 1 mM) was added and incubated for an additional 20 min. Finally, the reaction was terminated by the addition of 100 µL Na₂CO₃ before absorbance was read at 405 nm in a microplate reader (LT4500, UK). Determination of α -amylase in BSFE was determined using *Aspergillus oryzae* α -amylase (30 U/mg, 100 µL), incubated at 28 °C with 100 µL BSFE (1000, 500, 250, 125 and 62.5 µg/mL) for 10 min. Starch (1% in 0.02 M sodium phosphate buffer) was added as substrate for additional 10 min and reaction stopped after addition of dinitrosalicylic acid by heating in water bath at 100 °C for 15 min. The tubes were diluted with 1.5 mL of buffer, and an aliquot of 200 µL was read at 540 nm. The inhibitory concentration (IC₅₀) value was determined from a non-linear regression curve of percentage inhibition [31].

Evaluation of phytochemical constituents using gas chromatography-mass spectrometry (GC-MS)

The BSFE was subjected to phytochemical characterization using GC-MS. The analysis was conducted using the GCMS-QP2010 PLUS SHIMADZU instrument. Gas chromatography was performed using a Perkin Elmer Elite-5 column (30 m x 0.25 mm, 0.25 µm film thickness) with helium as the carrier gas (0.5 ml/min). A 1 µL sample was injected at an inlet temperature of 250 °C. The GC oven temperature was programmed from 80 °C (4 min) to 280 °C at 20 °C/min, with a total run time of 25 min. The MS transfer line and source temperatures were 200 °C and 180 °C, respectively. Electron impact ionization (70 eV) was used for GC-MS analysis. The resulting data was assessed using the total ion count (TIC) method to identify and quantify the compounds present. The spectra of the constituents were cross-referenced

with the GC-MS library's database of spectra of known constituents.

Experimental animals

Male Wistar rats of eight weeks old, weighing 150–180 g were obtained from the central animal house of the University of Ibadan. The animals were housed in cages kept in a laboratory room under standard environmental conditions of light and darkness cycle, relative humidity, and room temperature (28 ± 4 °C). The animals were allowed *ad libitum* water and feed (Vital Feeds Ltd, Nigeria). The feed composed of crude protein (13%), fat (8%), crude fiber (15%), calcium (0.9%), phosphorus (0.35%), metabolizable energy (2600 Kcal/kg). The experimental procedures followed established guidelines as described by the National Institute of Health guidelines in the "Principle of Laboratory Animal Care" (NIH Publication No. 85–23) and approved by Animal Care and Use Research Ethics Committee, University of Ibadan (UI-ACUREC/066–0721/2).

Evaluation of the antidiabetic effect of BSFE in high-fat diet/streptozotocin-induced diabetic in rats

Induction of diabetes and treatment

Male Wistar rats were fed continuously on HFD for 3 weeks. The high-fat diet was prepared according to the method earlier described [35]. Fresh animal fat was melted, liquefied, and solidified to give a pure animal fat lard. The powdered standard pellet was homogenously mixed with lard (60 / 40% w/w) and supplemented with 20% methionine to make the HFD. Diabetes was induced by injection of streptozotocin (35 mg/kg, i.p) in fifty HFD-fed male rats [36]. The animals were monitored for seven days, after which fasting blood sugar (FBS) was determined by taking blood from the tail vein into the glucometer strip using the accucheck® glucometer (Roche, UK) with a detection limit of 500 mg/dL. Rats showing FBS ≥ 200 mg/dL were selected as diabetic and randomly divided into 5 groups ($n=6$) and treated as follows; diabetic control (normal saline, 10 mL/kg), BSFE (25, 50 and 100 mg/kg) and metformin (250 mg/kg). Non-diabetic rats fed with normal diet and serves as the normal control. The BSFE dose was selected based on previous research, which indicated a safe range of up to 231 mg/kg in rats [37] and minimal toxicity at 100 mg/kg in mice [26]. The animals were treated once daily by oral gavage for 14 days. Body weights were taken every four days. Fasting blood sugars were determined from overnight fasted rats on the 7th and 14th day. The diets were removed overnight after the 14 days of treatment before the animals were anesthetized with deep ether anesthesia. Retroorbital blood was collected in plain tubes for separation of serum. When all the rats were euthanized, brain and liver tissues were harvested, rinsed from blood,

weighed, and stored in iced-cold sodium phosphate buffer (0.1 M, pH 7.4) at -20°C for biochemical analysis. Samples for histopathological analysis were fixed in 10% phosphate buffer formalin.

Biochemical analysis

Brain and Liver tissues were homogenized in ice-cold phosphate buffer using a mechanical grinder. Tissue homogenates were centrifuged at 10,000 rpm in a refrigerated centrifuge at 4°C for 10 min. Supernatants were aliquots and stored at 20°C for later determination of brain and liver markers of oxidative stress, inflammation, and apoptosis.

Measurement of lipid profile and atherogenicity

Serum lipid profile parameters were determined: Triglycerides, total cholesterol (TC), and high-density-lipoproteins-cholesterol (HDL-c) assays were performed using the Randox Kit according to the manufacturer's instructions. Low-density lipoprotein-cholesterol (LDL-C) and very low-density lipoprotein-cholesterol (VLDL-c) were calculated from the values of triglycerides, total cholesterol, and HDL as earlier described [35, 38]. Atherogenicity risk was determined by calculating the atherogenic index ((TC-HDL)/HDL) and coronary risk index.

Measurement of oxidative stress parameters in the liver and nitrites

Malondialdehyde (MDA) was estimated brain and hepatic tissues homogenates supernatants following the Thiobarbituric Acid Reacting Substance (TBARS) assay procedure [39]. 100 μL of supernatant was diluted twenty times in 0.15 M Tris-KCl buffer, and deproteinized with 500 μL trichloroacetic acid (30%). The mixture was centrifuged in a bench top centrifuge at 4000 rpm for 10 min at room temperature. 200 μL of the supernatant was removed into eppendorff tube, followed by addition of 200 μL thiobarbituric acid (0.75%), and the mixture was heated at 80°C for 1 h. The tubes were cooled by placing on ice, 200 μL was removed into microtitre plate and absorbance measured at 532 nm. The concentration of TBARS in the tissues were expressed as $\eta\text{mol MDA/mg protein}$. Hepatic and brain tissue nitrite levels were assayed using the Griess reagent method. 100 μL of twice diluted supernatant was incubated with Griess reagent [mixing equal volumes of 0.1% N-(1-naphthyl) ethylene diamine dihydrochloride and 1% sulphanilamide (in 5% phosphoric acid)], and absorbance was measured at 540 nm.

Reduced glutathione (GSH) was determined in hepatic and brain tissue using Ellman's reagent [40]. Briefly, 100 μL of the deproteinized supernatant was mixed 100 μL of 5¹, 5¹ -Dithios-nitrobenzoic acid (DTNB, 0.0006 M) in a microplate plate. Assay for catalase enzyme activity

in brain and hepatic tissue supernatants was determined using the colorimetric assay based on the yellow complex formation with H_2O_2 and molybdate [41]. Super-oxide dismutase (SOD) enzyme activity was determined in brain and hepatic tissue using the autooxidation of adrenaline assay method [42].

Determination of pro-inflammatory markers and caspase-3 and 9 by ELISA

Tissues levels of tumour necrosis factor-alpha (TNF- α) and interleukin-6 (IL-6) were determined using ELISA MAX[™] Deluxe kit (Biolegend, USA), while caspase-3 and 9 were determined using Elabscience ELISA kits (Wuhan, China) according to manufacturer's instruction. Briefly, 100 μL of tissue supernatants and respective standards were added to plates coated with capture antibody, followed by detection antibody, Horse-raddish peroxidase (HRP) and substrate according to respective incubation duration by respective manufacturer's protocol. The final tissue concentration was extrapolated from the standard curve obtained from standard concentrations of TNF- α , IL-6, Caspase-3 and 9, respectively.

Assay for brain acetylcholinesterase and glutamic acid decarboxylase activities

Acetylcholinesterase (AChE) activity in the brain homogenate supernatant was estimated according to the procedure described by Ellman et al., [43] with slight modification by Ajayi et al., [35]. Briefly, an Aliquot (50 μL) of brain supernatant was incubated with DTNB and acetylcholine and read kinetically at 405 nm wavelength in a microplate reader (LT4500, labteck, UK). Glutamic acid decarboxylase (GAD) activity in the brain supernatant was determined following the procedure described [44] with slight modification. Briefly, 50 μL aliquots of twice diluted brain tissue supernatant was incubated with a reaction mixture containing 20 mM sodium acetate, 70 μM bromocresol, 10 mM pyridoxal-5-phosphate (PLP) and 2 μL glutamate. The increase in absorbance at 630 nm for 5 min was read in a microplate reader (LT4500, UK). The unit of enzyme was expressed as $\mu\text{moles min}^{-1} \text{mg protein}^{-1}$ using the extinction coefficient 23,700 $\text{M}^{-1} \text{cm}^{-1}$ [44].

Histological staining of liver and brain tissues

Rats were subjected to ether anesthesia and the thoracic cavity opened for intracardiac perfusion, first with normal saline and followed with sodium phosphate buffered formalin (10%). The brain and liver were harvested and kept in formalin (10%), cut into blocks and slides. The slides were processed and stained with hematoxylin and eosin (H&E). The photomicrographs of stained slides were captured using Olympus microscope (Olympus,

New Jersey, USA) connected through a digital camera to a computer interface.

Statistical analysis

Data obtained were expressed as mean ± standard errors of means (SEM). Statistical analysis was performed using one-way or two-way ANOVA followed by Tukeys post hoc test for multiple comparisons with Graphpad Prism® software version 5.0 (USA). *P* < 0.05 was set as statistically significant value.

Results

***In vitro* biological activity results**

Quantitative determination of total phenolic content and flavonoid content in BSFE is as shown in Table 1. TPC in BSFE was 0.247 ± 0.007 (mgGAE/ g sample), while TFC was 0.257 ± 0.005 (mgRE/g sample), with a flavonoid: phenolic ratio of 1.038 ± 0.015. The BSFE showed free radical scavenging activities with good IC₅₀ values when compared with ascorbic acid standard in the DPPH (303.8 ± 0.31 vs. 129.46 ± 0.69 µg/mL) and NO (364.81 ± 0.32 vs. 239.12 ± 0.29). Also as shown in Table 1, the antioxidant power of BSFE when compared with ascorbic acid in the FRAP assay was 256.76 ± 0.49 vs. 217.64 ± 0.71 µg/mL TE, while total antioxidant capacity in the phosphomolybdate assay was 305.75 ± 0.38 vs. 246.31 ± 0.33. In addition, the BSFE also demonstrated significant inhibition of carbohydrate metabolizing enzymes activities when compared with acarbose with IC₅₀ values of α-glucosidase inhibition (65.32 ± 1.45 vs. 47.05 ± 0.40 µg/mL), and α-amylase enzymes (20.22 ± 0.64 vs. 25.06 ± 0.69 µg/mL), respectively (Table 1).

Phytochemical constituents in BSFE

Phytochemical profiling of BSFE by GC-MS identified 16 chemical constituents with different retention times as shown in the chromatogram (Fig. 1). Table 2 list the retention time, peak area percentages and the compounds detected. The compounds detected as Methyl

4-O-methyl-d-arabinopyranoside, Decanoic acid (capric acid), 3-methyl-Silane, ethyltrimethyl- showed retention time at 12.117 min with peak area percentage of 28.07. Other compounds with higher peak area greater than 10% was also revealed: unsaturated fatty acids (Cyclododecane, 9-Octadecenoic acid, (E)-, 9-Octadecene, (E)-), saturated fatty acid [n-Hexadecanoic acid (palmitic acid), and Tetradecanoic acid (myristic acid)] and naturally occurring monosaccharides [(D-Allose, beta.-D-Glucopyranose, 1,6-anhydro-Propanedioic acid, (manolic acid)].

***B. sapida* fruit extract improves fasting blood sugar in diabetic rats**

There was persistent elevation of FBS in HFD/STZ diabetic control rats which was reduced in animals treated with BSFE (25, 50 and 100 mg/kg) when measured at days 7 and 14 (Fig. 2). Only 100 mg/kg gave a significant (*p* < 0.05) reduction on day 7. There was a significant reduction in mean FBS on day 14 in diabetic rats treated with BSFE (25, 50 and 100 mg/kg) when compared with diabetic control (70.00 ± 2.35, 77.17 ± 5.29, 71.00 ± 4.51 vs. 198.20 ± 30.13 mg/dL), resulting in percent reduction by 64.7%, 61.1%, and 64.2%, respectively. Metformin significantly reduced FBS by 58.1% on day 14 post treatment.

Effect of BSFE treatment on body and organ weights

The body weight measurement taken at beginning and day 14 is shown in Table 3. There was significant reduction in body weight of diabetic control rats compared with the normal control. However, treatment with BSFE could not significantly reversed this decline in body weight of diabetic rats. The BSFE at 100 mg/kg caused a pronounced loss in body weight, while metformin could also not reverse the body weight loss in diabetic rats during the 14 days treatment. The effect of treatment on relative organ weights of liver, kidney, pancreas and brain is presented in Table 4. The liver and brain weights in diabetic control were significantly (*p* < 0.05) increased when

Table 1 *In vitro* assays on *B. sapida* fruit extract (BSFE)

Parameters	BSFE	Ascorbic acid	Acarbose
TPC (mgGAE/ g sample)	0.247 ± 0.007	-	-
TFC (mgRE/g sample)	0.257 ± 0.005	-	-
Flavonoid: Phenolic ratio	1.038 ± 0.015	-	-
IC ₅₀ DPPH (µg/mL)	303.8 ± 0.31	129.46 ± 0.69	-
IC ₅₀ NO (µg/mL)	364.81 ± 0.32	239.12 ± 0.29	-
FRAP (µg/mL TE)	256.76 ± 0.49	217.64 ± 0.71	-
TAC (µg/mL AAE)	305.75 ± 0.38	246.31 ± 0.33	-
IC ₅₀ Alpha Glucosidase (µg/mL)	65.32 ± 1.45	-	47.05 ± 0.40
IC ₅₀ Alpha amylase (µg/mL)	20.22 ± 0.64	-	25.06 ± 0.69
Mean ± SEM (n = 3)			

TPC- Total Phenolic Content, TFC- Total Flavonoid Content, GAE- Gallic Acid Equivalent, RE- Rutin Equivalent, DPPH- 2,2-diphenyl-1-picrylhydrazyl, NO – Nitric Oxide, FRAP – Ferric Reducing Antioxidant Power, TAC- Total Antioxidant Capacity

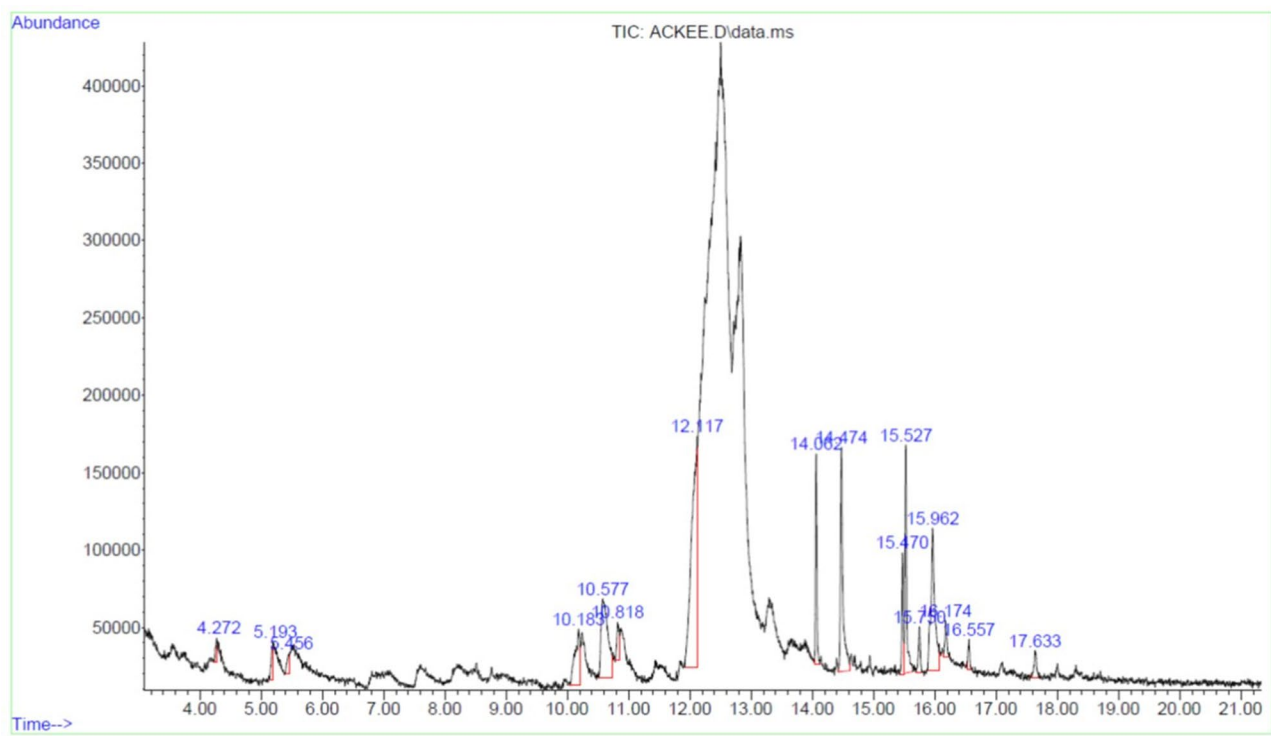


Fig. 1 GC-MS profile of *Blighia sapida* unripe fruit extract

Table 2 Results of GC-MS analysis of the unripe *Blighia Sapida* fruit extract

Peak #	RT	Peak Area %	Compounds Detected
1	4.272	0.84	2-Oxabicyclo[3.2.0]hepta-3,6-diene, Bicyclo[2.2.1]heptan-2-ol, Phenol
2	5.193	1.67	Benzoic acid, methyl ester, Benzoic acid, hydrazide, Butanedioic acid, monohydrazide, N 2-benzoyl-, methyl ester
3	5.456	1.05	1,3-Benzenediol, 2-methyl- Pyrimidine, 2-methoxy-5-methyl- Orcinol
4	10.183	6.02	2-Fluoro-5-trifluoromethylbenzoic acid, 3-hexadecyl ester, 2-Fluoro-3-trifluoromethylbenzoic acid, 5-pentadecyl ester, Phenol, 3,5-bis(1,1-dimethylethyl)
5	10.577	11.89	D-Allose, .beta.-D-Glucopyranose, 1,6-anhydro-Propanedioic acid, propyl-
6	10.818	1.95	Dodecanoic acid, Undecanoic acid, Nonanoic acid
7	12.117	28.07	Methyl 4-O-methyl-d-arabinopyranoside, Decanoic acid, 3-methyl-Silane, ethyltrimethyl-
8	14.062	6.20	Hexadecanoic acid, methyl ester, Pentadecanoic acid, 14-methyl-, methyl ester, Hexadecanoic acid, methyl ester
9	14.474	11.75	n-Hexadecanoic acid, Tetradecanoic acid
10	15.470	4.05	1,8,11-Heptadecatriene, (Z, Z)-, 9,12-Octadecadienoic acid (Z, Z)-
11	15.527	8.54	cis, cis-7,10,-Hexadecadienal,7-Hexadecenoic acid, methyl ester, (Z) 9-Octadecenoic acid (Z)-, methyl ester
12	15.750	1.92	Methyl stearate, Heptadecanoic acid, 16-methyl-, methyl ester, Methyl stearate
13	15.962	12.13	Cyclododecane, 9-Octadecenoic acid, (E)-, 9-Octadecene, (E)-
14	16.174	1.42	Octadecanoic acid, Decanoic acid, silver(1+) salt, Hexadecyl propyl ether
15	16.557	1.06	2(1 H)-Naphthalenone, octahydro-4a-methyl-7-(1-methylethyl)-, (4a.alpha.,7.beta.,8a.beta.)- 4,7,7-Trimethylbicyclo[2.2.1]heptan-2-one O-allyloxime, 9-Octadecenoic acid (Z)-, methyl ester
16	17.633	1.44	3-Hydroxy-3-trifluoromethyl-2-oxa-spiro[5.5]undecan-5-one, Heptadecanolide, Bicyclo[3.3.2]decan-9-one

compared with non-diabetic control. However, treatment with BSFE (100 mg/kg) and metformin significantly reversed the brain enlargement when compared with diabetic control (0.75 ± 0.02 , 0.74 ± 0.08 vs. 0.85 ± 0.03).

B. sapida fruit extract improves atherogenic indices in diabetic rats

AS shown in Fig. 3A-C, there was significant ($p < 0.05$) increase in triglycerides, total cholesterol, LDL-c but change in HDL-c and VLDL-c of diabetic control when compared with normal control rats. In diabetic rats treated with BSFE (25, 50 and 100 mg/kg), the effects

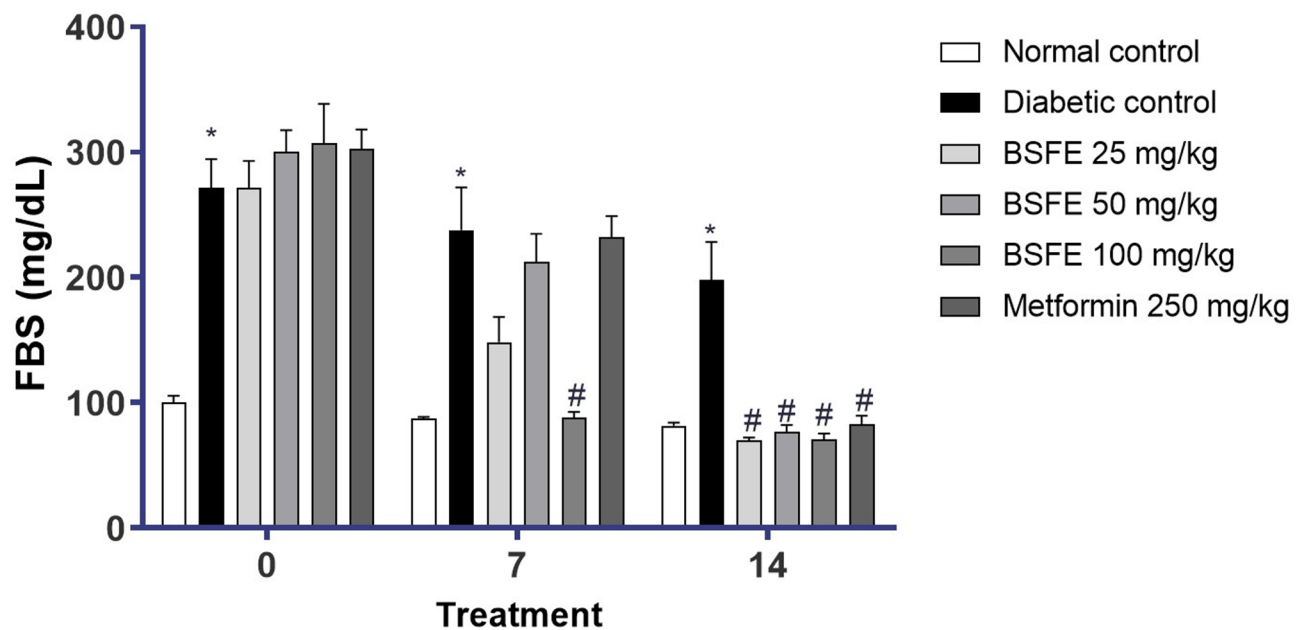


Fig. 2 Antihyperglycaemic effect of BSFE (25–100 mg/kg) in HFD/STZ-induced diabetic rats. Bars represents Mean $\pm$ SEM ($n=6$). * $P < 0.05$ vs. normal control, # $P < 0.05$ vs. diabetic control using two-way ANOVA followed by Tukeys *post hoc* test

Table 3 Effect of *B. sapida* fruit extract (BSFE) on body weight in change on HFD/STZ-induced diabetic rats

Treatment	Weight (g) ^a		
	Day 0	Day 14	Change in weight
Normal control	220.40 $\pm$ 12.75	267.40 $\pm$ 20.49	47.00 $\pm$ 11.57
Diabetic control	239.20 $\pm$ 7.07	194.80 $\pm$ 9.06	-44.40 $\pm$ 5.98*
BSFE 25 mg/kg	230.67 $\pm$ 5.91	198.40 $\pm$ 2.98	-30.67 $\pm$ 8.11
BSFE 50 mg/kg	228.80 $\pm$ 9.88	206.20 $\pm$ 11.18	-10.25 $\pm$ 18.47
BSFE 100 mg/kg	241.80 $\pm$ 11.07	189.67 $\pm$ 12.66	-53.00 $\pm$ 4.71
Metformin 10 mg/kg	220.20 $\pm$ 3.02	197.40 $\pm$ 13.39	-20.40 $\pm$ 9.96

^a Mean $\pm$ SEM ($n=6$). * $P < 0.05$ vs. normal control, # $P < 0.05$ vs. diabetic control using one-way ANOVA followed by Tukey's *post hoc* test

Table 4 Effect of *B. sapida* fruit extract (BSFE) on relative organ weights in HFD/STZ-induced diabetic rats

Treatment	Relative organ weight (%) ^a			
	Liver	Kidneys	Pancreas	Brain
Normal control	2.79 $\pm$ 0.11	0.64 $\pm$ 0.02	0.28 $\pm$ 0.04	0.62 $\pm$ 0.03
Diabetic control	3.10 $\pm$ 0.20#	0.73 $\pm$ 0.03	0.26 $\pm$ 0.01	0.85 $\pm$ 0.03*
BSFE 25 mg/kg	2.99 $\pm$ 0.13	0.69 $\pm$ 0.03	0.27 $\pm$ 0.02	0.76 $\pm$ 0.01
BSFE 50 mg/kg	2.83 $\pm$ 0.19	0.67 $\pm$ 0.02	0.25 $\pm$ 0.12	0.79 $\pm$ 0.05
BSFE 100 mg/kg	3.22 $\pm$ 0.26	0.80 $\pm$ 0.05	0.18 $\pm$ 0.03	0.75 $\pm$ 0.02#
Metformin 10 mg/kg	3.10 $\pm$ 0.39	0.74 $\pm$ 0.09	0.23 $\pm$ 0.04	0.74 $\pm$ 0.08#

^a Mean $\pm$ SEM ($n=6$). * $P < 0.05$ vs. normal control, # $P < 0.05$ vs. diabetic control using one-way ANOVA followed by Tukey's *post hoc* test

on lipid profile (Fig. 3A) showed significant reduction in triglycerides and total cholesterol levels, as well as reduction in LDL-c (Fig. 3B). The extract and metformin demonstrated significant atheroprotective effects by reducing atherogenic index and coronary risk index (Fig. 3C).

***B. sapida* fruit extract reversed liver and brain oxidative/nitrogenic stress parameters in diabetic rats**

Malondialdehyde and nitrites were significantly ($p < 0.05$) increased in the liver (Fig. 4A&B) and brain (4 C&D) tissues of diabetic control when compared with normal control. However, treatment with BSFE (25, 50 and 100 mg/kg) significantly ($p < 0.05$) and dose-dependently reversed diabetes-induced elevation of hepatic malondialdehyde and nitrites levels. In the brain tissues, BSFE (25, 50 and 100 mg/kg) significantly reduced malondialdehyde

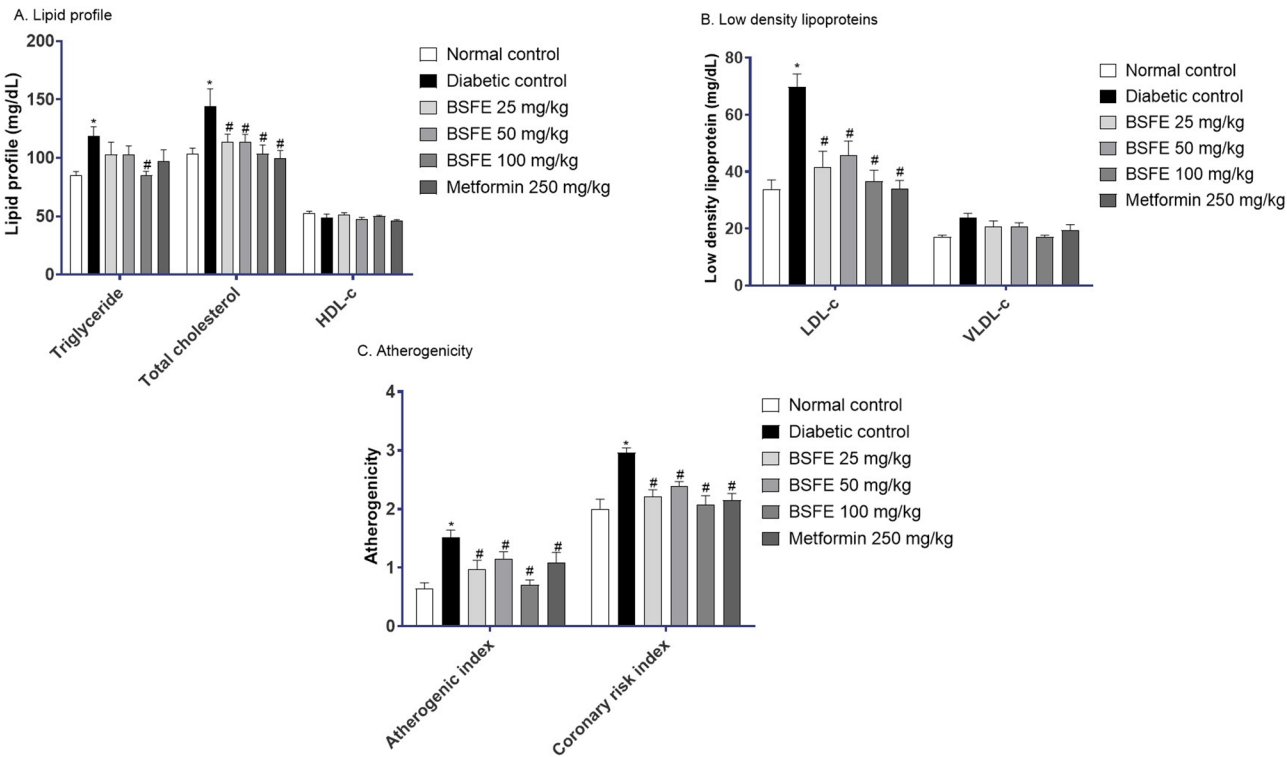


Fig. 3 Post-treatment effect of BSFE (25–100 mg/kg) on (A) fasting lipid profile (B) Low density Lipoprotein and (C) Atherogenicity in HFD/STZ-induced diabetic rats. Bars represents Mean $\pm$ SEM ($n=4$). * $P<0.05$ vs. normal control, # $P<0.05$ vs. diabetic control using one-way ANOVA followed by Tukey's *post hoc* test

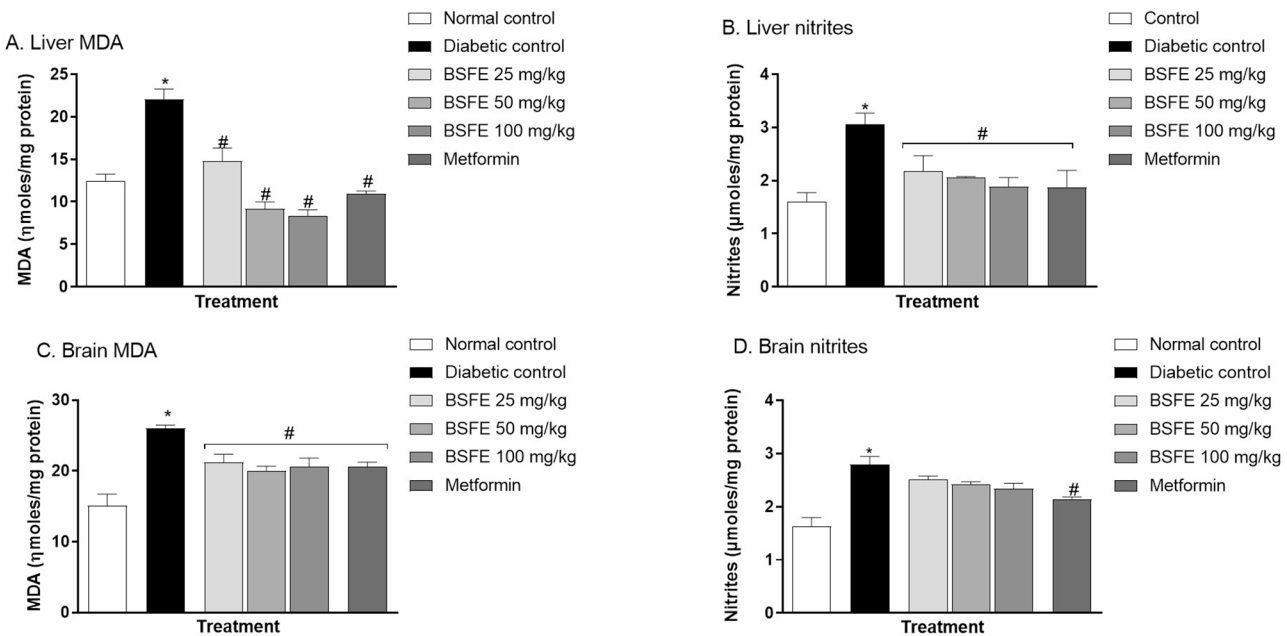


Fig. 4 Post-treatment effect of BSFE (25–100 mg/kg) on (A) Liver Malondialdehyde (MDA), (B) Liver Nitrites (C) Brain Malondialdehyde (D) Brain Nitrites level in HFD/STZ-induced diabetic rats. Bars represents Mean $\pm$ SEM ($n=4$). * $P<0.05$ vs. normal control, # $P<0.05$ vs. diabetic control using one-way ANOVA followed by Turkey's *post hoc* test

but not nitrites when compared with diabetic control. Metformin significantly reduced malondialdehyde and nitrites levels in both hepatic and brain tissues of diabetic rats (Fig. 4).

***B. sapida* fruit extract boost liver and brain antioxidant defense in diabetic rats**

HFD/STZ induction significantly ($p < 0.05$) reduced liver and brain GSH levels, while decreasing catalase and SOD activities in diabetic animals (Fig. 5A–F). Post treatment with BSFE (25, 50 and 100 mg/kg) could not significantly reversed GSH depletion in liver and brains of treated animals when compared with untreated diabetic control. However, there was significant ($p < 0.05$) increase in the catalase (Fig. 5B&E) and SOD (Fig. 5C&F) activities in both the liver and brain of diabetic rats treated with BSFE (25, 50 and 100 mg/kg) when compared with untreated diabetic control. Metformin showed significant improvement of antioxidant defense in the liver, and only brain SOD was significantly increased.

***B. sapida* fruit extract suppressed pro-inflammatory cytokines in liver and brain tissues of diabetic rats**

TNF- α and IL-6 levels in both liver and brain tissues of diabetic rats were significantly elevated when compared with normal control rats (Fig. 6A–D). However, post treatment with BSFE (25, 50 and 100 mg/kg) dose-dependently reduced liver TNF- α (Fig. 6A, $p < 0.05$) and IL-6 (Fig. 6B, $p < 0.05$). Similarly, BSFE (25, 50 and 100 mg/kg) significantly ($p < 0.05$) reduced in non-dose dependent manner brain levels of TNF- α and IL-6 (Fig. 6C&D). Metformin ameliorated significantly both

TNF- α and IL-6 increase in the liver and brain tissues of diabetic rats.

***B. sapida* fruit extract suppressed hepatic apoptosis in diabetic rats**

Apoptotic marker enzymes, caspase-3 and 9 were significantly increased in liver tissues of diabetic control when compared normal control rats (Fig. 7A&B). However, in diabetic rats treated with BSFE (25, 50 and 100 mg/kg), there was significant ($p < 0.05$) and dose-dependent reduction in caspase-3 and caspase-9 markers when compared with diabetic control (Fig. 7A&B). Metformin reduced hepatic caspase-3 ($p > 0.05$) and caspase-9 ($p < 0.05$) in diabetic rats when compared with diabetic control.

***B. sapida* fruit extract modulated neurotransmitter-related enzymes in brain tissues of diabetic rats**

As shown in Fig. 7, diabetes significantly increased acetylcholinesterase activity (Fig. 8A), and reduced GAD (Fig. 8B) in brain tissues. However, post treatment with BSFE (25, 50 and 100 mg/kg) significantly ($p < 0.05$) decreased acetylcholinesterase and increased GAD activities in brain tissues of diabetic rats. Metformin demonstrated significant modulation of the two neurotransmitter-related enzymes in brain tissues of diabetic rats.

***B. sapida* fruit extract improves histological changes in cortical brain tissues of diabetic rats**

The histological changes in cortical brain tissues are presented in Fig. 9 (A–F). Normal neuronal morphology was seen in normal control (Fig. 9A) as opposed to

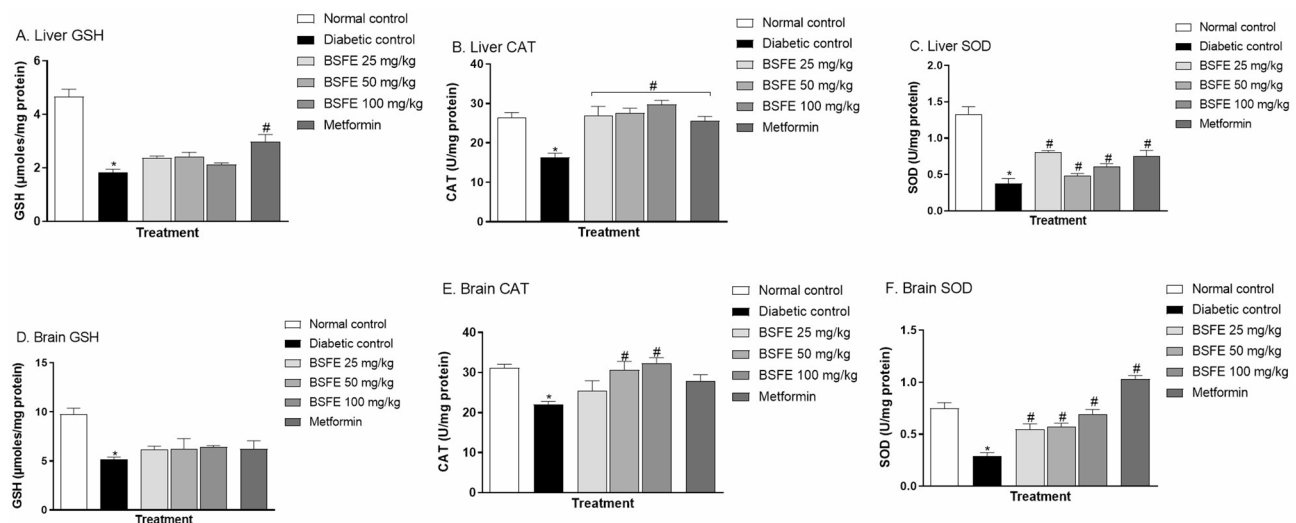


Fig. 5 Post-treatment effect of BSFE (25–100 mg/kg) on (A) Liver Reduced glutathione (GSH) (B) Liver Catalase (CAT) (C) Liver Superoxide Dimutase (SOD), (D) Brain GSH (E) Brain CAT (F) Brain SOD level in HFD/STZ-induced diabetic rats. Bars represents Mean $\pm$ SEM ($n = 4$). * $P < 0.05$ vs. normal control, # $P < 0.05$ vs. diabetic control using one-way ANOVA followed by Tukey's *post hoc* test

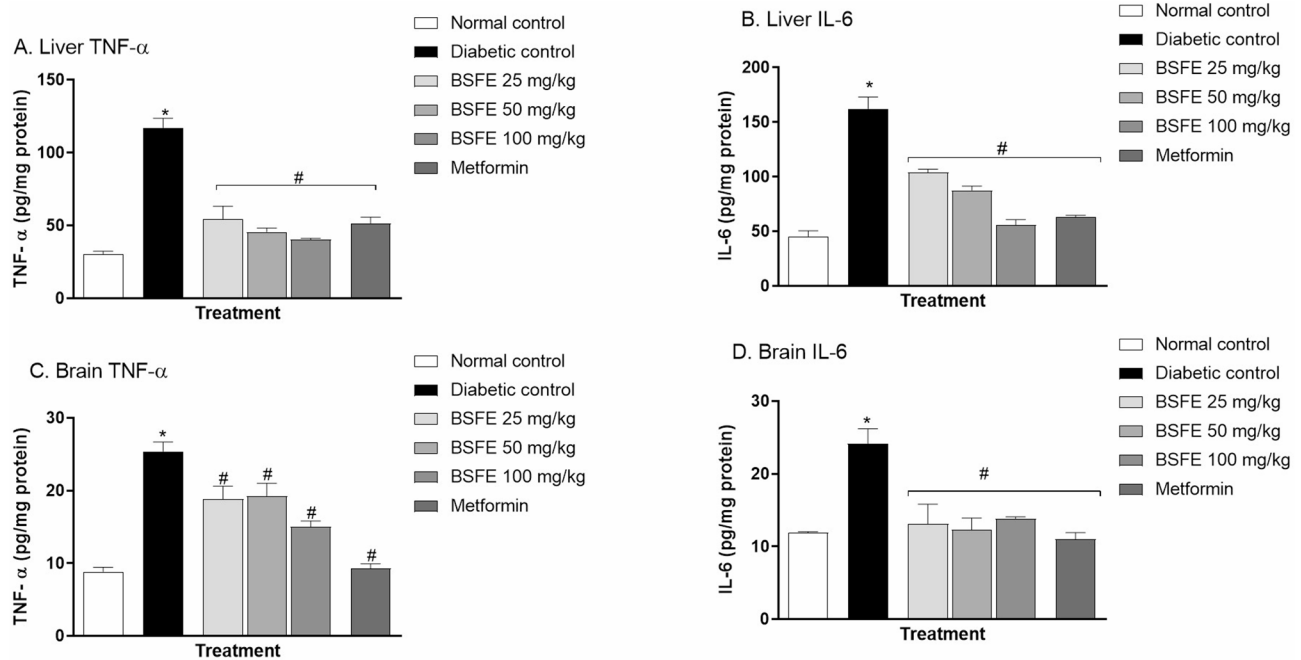


Fig. 6 Post-treatment effect of BSFE (25–100 mg/kg) on Liver and Brain Tumor necrosis factor alpha (TNF- α) and interleukins (IL-6) in HFD/STZ-induced diabetic rats. Bars represents Mean $\pm$ SEM ($n=4$). * $P < 0.05$ vs. normal control, # $P < 0.05$ vs. diabetic control using one-way ANOVA followed by Tukey's *post hoc* test

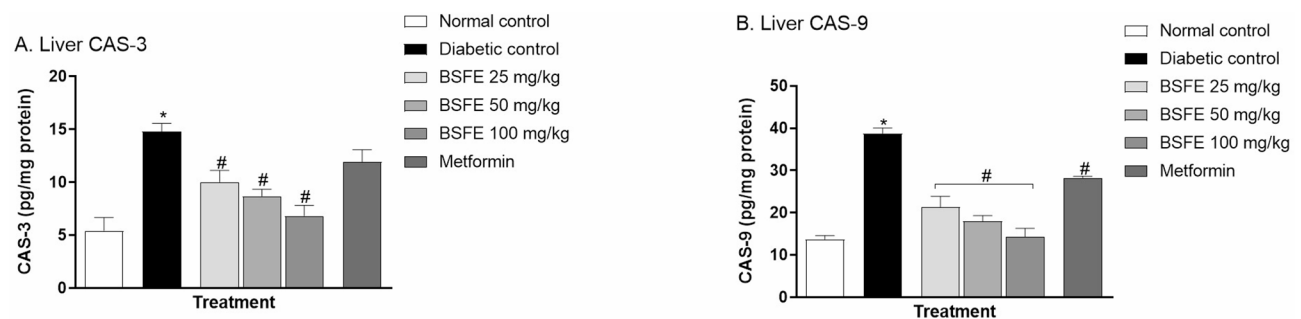


Fig. 7 Post-treatment effect of BSFE (25–100 mg/kg) on Liver Caspase level in HFD/STZ-induced diabetic rats. Bars represents Mean $\pm$ SEM ($n=4$). * $P < 0.05$ vs. normal control, # $P < 0.05$ vs. diabetic control using one-way ANOVA followed by Tukey's *post hoc* test

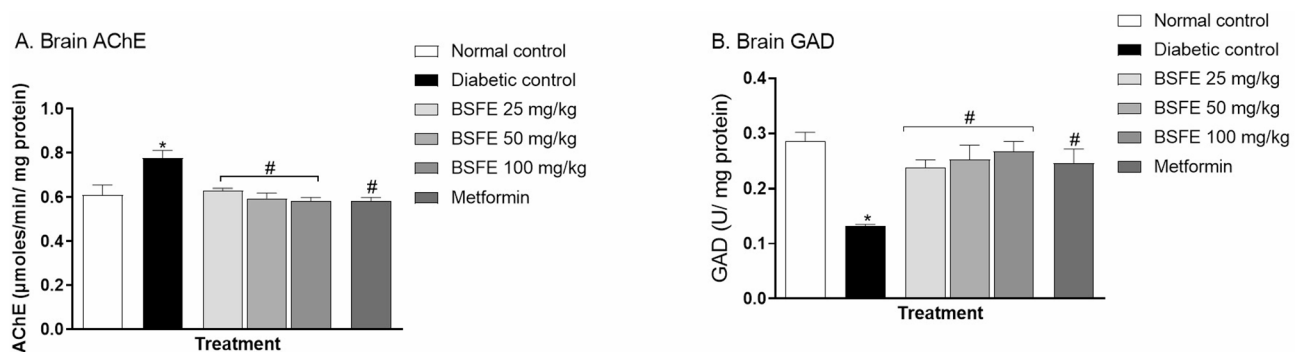


Fig. 8 Post-treatment effect of BSFE (25–100 mg/kg) on (A) Brain Acetylcholinesterase (AChE), and (B) Glutamic acid decarboxylase (GAD) in HFD/STZ-induced diabetic rats. Bars represents Mean $\pm$ SEM ($n=4$). * $P < 0.05$ vs. normal control, # $P < 0.05$ vs. diabetic control using one-way ANOVA followed by Tukey's *post hoc* test

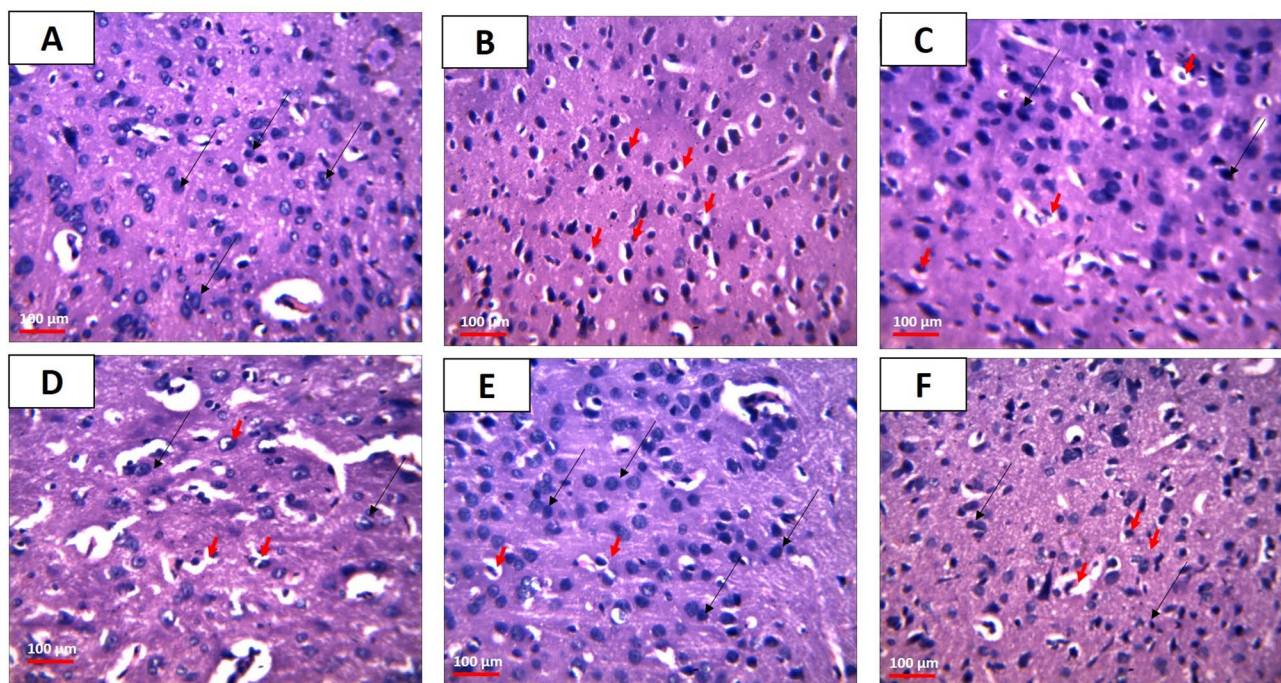


Fig. 9 Representative photomicrographs of H&E stained brain cortex (mag x 400). Black slender arrow indicates normal neuronal morphology, while red colored arrow heads indicates pyknotic cells. (A) Normal control, (B) Diabetic control, (C) BSFE 25 mg/kg, (D) BSFE 50 mg/kg, (E) BSFE 100 mg/kg, and (F) Metformin 250 mg/kg

increased pyknotic cells, with nuclei seen detached in the cortical tissues in diabetic control (Fig. 9B). BSFE (25, 50 and 100 mg/kg) reduced the neuronal damage caused by diabetes (Fig. 9C-E). Metformin (250 mg/kg) showed reduced intensity of pyknotic cells in diabetic rats' brain (Fig. 9F).

***B. sapida* fruit extract restores histopathological changes in hepatic tissues of diabetic rats**

As shown in Fig. 10A, there was normal hepatic cytoarchitecture in non-diabetic control rats. However, in the diabetic groups, histological staining revealed distorted hepatic cytoarchitecture with presence of inflammatory cell infiltration, dilation and congestion of veins in the portal areas, and fatty liver which was more severe in the untreated diabetic rats (Fig. 10B). In diabetic rats treated with BSFE (25, 50 and 100 mg/kg) and metformin, it shows restoration towards normal liver cytoarchitecture, reduced congestion of sinusoids, fatty changes and mild inflammatory cells infiltration (Fig. 10C-F).

Discussion

This study reported the *in vitro* and *in vivo* anti-diabetic potentials of unripe fruits of *B. sapida* extract. The ripe ackee as the fruit is known is popularly consumed fruit snacks while the unripe ackee is associated with hypoglycemic-related toxicities due to presence of hypoglycins [37]. However, studies have shown that the toxicities may

be related to ingestion of the whole fruits and extraction is suggested to reduce the hypoglycin-related toxicity [37].

The phytochemical analysis of phenolic content showed appreciable amount of total phenolic and flavonoid content. This higher content of phenolic and flavonoid is consistent from previous report [24]. GC-MS profiling further revealed other components of the extract to include saturated and unsaturated fatty acids and reducing sugars. However, literature report corroborates the revealed evidence of the presence of these secondary metabolites including phenols and reducing sugars in the unripe fruit of the plant [45]. The compound with the highest peak area percentage found in BSFE was Methyl 4-O-methyl-d-arabinopyranoside which also have been shown to demonstrate antioxidant and alpha glucosidase inhibitory activities in other plant extracts [46]. In addition, recent investigations of the unripe ackee fruit have shown presence of compounds with antidiabetic potentials inclusive of the hypoglycin, triterpenoids (such as friedelane and squalene), and phenolic compounds (catechin, epicatechin and phloridzin) [24].

The *B. sapida* fruit extract demonstrated promising free radical scavenging activity in both DPPH and nitric oxide (NO) assays, though with lower potency than the ascorbic acid standard. While BSFE's IC₅₀ values are higher than ascorbic acid, its activity is still biologically significant. The DPPH assay highlights BSFE's

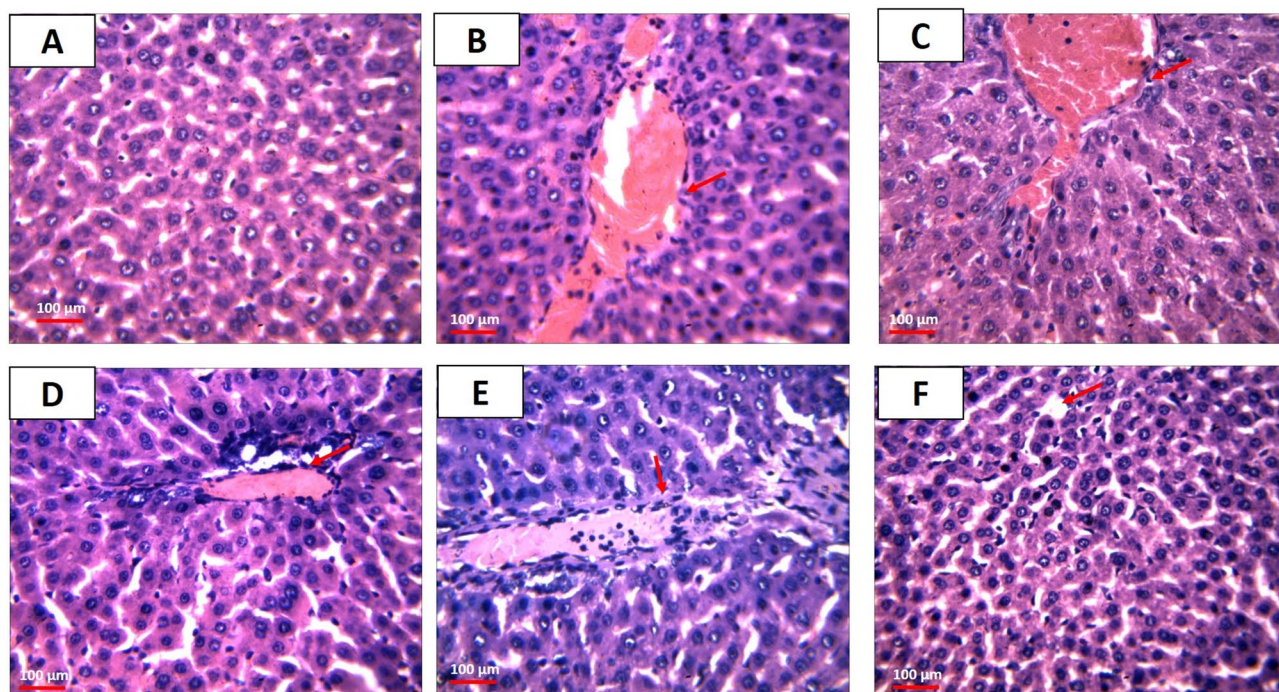


Fig. 10 Representative photomicrographs of H&E stained liver tissue (mag x 400). (A) Normal control showing normal hepatic architecture, (B) Diabetic control showing severe dilation of veins in the portal areas, with fatty changes and inflammatory cells infiltration. (C) BSFE 25 mg/kg showing severe dilation of veins in the portal areas. (D) BSFE 50 mg/kg, (E) BSFE 100 mg/kg, and (F) Metformin 250 mg/kg showed mild dilation of veins in the portal areas. Red arrow indicates central vein

hydrogen-donating ability, critical for neutralizing lipid peroxidation, which is associated with cellular system exposed to ROS, on the other hand, the NO scavenging suggests potential anti-inflammatory properties, as excess NO is linked to chronic inflammation [47]. In addition, BSFE demonstrated notable antioxidant properties in both the FRAP and phosphomolybdate assays, outperforming the ascorbic acid standard. BSFE's superior performance in the phosphomolybdate assay reflects its broad-spectrum antioxidant activity, likely due to synergistic interactions among polyphenols, flavonoids, or other reducing agents in the extract. The difference in antioxidant activity between BSFE and ascorbic acid reflects the inherent complexity of natural extracts, which can result in reduced potency compared to isolated compounds. Nonetheless, the multicomponent nature of natural extracts may offer advantages in terms of polyvalent interactions and synergistic effects, potentially leading to enhanced therapeutic efficacy [48]. The phosphomolybdate result aligns with studies on antioxidant-rich fruits, where multi-component synergy enhances total antioxidant capacity [49]. This broad activity hints at potential applications in nutraceuticals targeting systemic oxidative stress such as reported in hyperglycaemic conditions [50].

Fruits extracts acting as inhibitors of carbohydrate metabolizing enzymes have shown potentials

in managing postprandial elevation of glucose in diabetes [51]. *B. sapida* unripe fruit extract in this study demonstrated inhibitory activity on α -glucosidase and α -amylase enzymes in vitro. This indicates that the extract has potential to suppress postprandial hyperglycaemia. Although, the leaf extract of *B. sapida* has shown anti-amylase and anti-glucosidase activity [52], this is the first report demonstrating this activity in unripe ackee fruit. This is unsuspecting as some unripe tropical fruit extracts have shown α -glucosidase and α -amylase inhibitory activities [53, 54].

Type 2 diabetes mellitus is well modeled in the HFD/streptozotocin-induced diabetes in rats. The model is characterized by persistent hyperglycaemia, hypertriglyceridemia as well as insulin resistance [55]–[56]. In our study, FBS remained elevated throughout the duration of the experiment. Repeated oral treatment with BSFE demonstrated oral hypoglycaemic effect at day 14 post-induction. Ability to reduce FBS in HFD/STZ-induced diabetic rats is an important pointer to antidiabetic property of this extract. This activity is similar to that produced by standard drug, metformin. In addition, body weight was also reduced in rats fed with HFD. Early reports had indicated that weight reduction is associated with better glucose levels, especially in type 2 diabetes which is usually accompanied by lowering glycosylated hemoglobin (HbA1c) levels [55, 57]. Hartmann et al. [58], reported

the benefit of Weight-Beneficial Antidiabetic drugs such as Metformin and Glucagon-like peptide (GLP)-1 analogs in patients with type-2 diabetes. However, the limitation of this study is the inability to measure glucose tolerance test (GTT), insulin levels and to calculate homeostasis model of insulin resistance to determine the further mechanism of antidiabetic effects in a model of type-2 diabetes.

In this study, compared to diabetic control, BSFE significantly reduced TC, Triglycerides and VLDL levels, while increasing HDL-c levels. The LDL-c is often referred to as “bad” cholesterol, high levels of LDL can lead to plaque buildup in arteries. The results show that BSFE lowers LDL levels, which could reduce the risk of atherosclerosis. Atherogenicity is defined as the tendency to form fatty deposits in the arteries. A lower ratio of LDL to HDL and a reduction in VLDL suggest that BSFE may decrease atherogenicity, potentially offering a protective effect against cardiovascular complications associated with diabetes. The data suggests that BSFE could be beneficial in managing lipid-related diabetic conditions by improving the lipid profile and reducing atherogenic risk. Giglio et al. [59], has earlier reported the atherogenic activity of *Opuntia ficus-indica* which reduces the effects of Small Dense Low-Density Lipoproteins in Patients with risk factors for certain metabolic Syndrome.

Furthermore, the antioxidant effects of *Blighia sapida* fruit extract (BSFE) on hyperglycaemia-induced oxidative stress markers in the liver and brain of diabetic rats was significant. Malondialdehyde, a marker of lipid peroxidation and oxidative stress, is crucial in antidiabetic research. BSFE treatment significantly reduced MDA levels in a dose-dependent manner, indicating a reduction in oxidative damage in the liver. It also led to a decrease in brain nitrites levels, suggesting a protective effect against oxidative/nitrogenic stress in the brain. The results suggest BSFE has antioxidative properties potentially preventing or reducing diabetes-related complications. Nimse and Pal [60] reported that natural antioxidants has the ability to scavenge for free radicals by neutralizing free radicals (reactive oxygen species, ROS) and donating electrons or hydrogen atoms. This scavenging action prevents oxidative damage to cellular components. In addition, the results showed BSFE may bolster the antioxidant defense system in diabetic conditions, potentially reducing oxidative stress-related damage in both the liver and brain. BSFE treatment increases liver GSH levels, a crucial endogenous antioxidant, indicating enhanced antioxidant defense. It also elevates liver CAT activities, an enzyme that converts hydrogen peroxide into water and oxygen, reducing oxidative stress. The increase in SOD activity suggests improved cellular ability to neutralize superoxide radicals. Similar to the liver, an increase in CAT levels in the brain indicates a protective effect

against oxidative stress. Wu et al. [61], reported that one major mechanism of action of natural compounds with antioxidant and anti-inflammatory potential is their ability to reduce oxidative stress by enhancing endogenous antioxidant defenses such as SOD, CAT, and Glutathione peroxidase, and modulate signaling pathways such as Nuclear factor-kappa B (NF- κ B), p38 mitogen-activated protein kinase (MAPK), and Janus kinase/signal transducer and activator of transcription-3 (JAK/STAT-3). In vitro, BSFE has shown strong antioxidant activity including free radical scavenging activities, reducing power and enhanced total antioxidant capacity, hence increasing its overall antioxidant defenses. There in vitro properties may contribute to the observed in vivo antioxidants effects suggesting BSFE potential as an antioxidant agent.

The TNF- α is a pro-inflammatory cytokine involved in immune responses and inflammation. Research has shown that increased TNF- α levels are associated with chronic inflammation, insulin resistance, and diabetes. Interleukin-6, another key pro-inflammatory cytokine, contributes to immune regulation and inflammatory processes [62]. However, Increased IL-6 levels are linked to insulin resistance and contribute to the pathogenesis of diabetes [63]. The result in this study showed that BSFE reduces TNF- α and IL-6 level in a dose dependent manner in both hepatic and brain tissues. Increased pro-inflammatory markers such as TNF- α and IL-6 has been shown to correlate well with weaker cognitive performance [64]. Also, the glial cells when activated by persistent hyperglycaemia releases TNF- α and IL-6 which are potent mediator of diabetic retinopathy [65]. Also, increased levels of hepatic pro-inflammatory cytokines promotes insulin resistance, glucose dysmetabolism and apoptosis, hence contributing to the progression of hepatic diabetic complication [62]–[63]. In this study, BSFE caused significant reduction in TNF- α and IL-6 in both the hepatic and brain tissues, hence should be considered as having potential to improve insulin sensitivity and reversing other associated diabetes-induced complications.

Further, we investigated hepatic markers of apoptosis in diabetic rats and the effects of BSFE treatment. Chronic hyperglycaemia in diabetes has been shown to activate tissue senescence and apoptosis, which accelerates progression into diabetic complications [66]. Apoptosis contributes to development of diabetic micro vascular complications such as retinopathy, neuropathy, nephropathy and cardiomyopathy [67]. In this study, caspase-3 and caspase-9 levels were elevated in hepatic tissues of diabetic rats. These caspases are early markers of inflammation and cell death in hepatic tissues of diabetes and are considered as potential therapeutic targets in mitigating diabetic complications [68, 69]. Treatment with BSFE significantly ameliorated hyperglycaemia-induced

apoptosis in liver tissue by reducing caspase-3 and caspase-9 levels, suggesting its anti-apoptotic mechanisms.

Brain neurotransmission such as dopaminergic, glutamatergic and cholinergic pathways have been demonstrated to be impaired in DM [70]. In this study, two neurotransmitter-related enzymes –acetylcholinesterase and glutamic acid decarboxylase were investigated in the brain of diabetic rats. Acetylcholinesterase is the marker enzyme for cholinergic activity in the brain, being responsible for the hydrolysis of acetylcholine, an important neurotransmitter involved in vital function of the CNS, as memory, learning and sleep control [71]. Some previous reports have shown that chronic hyperglycaemia alter brain acetylcholinesterase activity, thereby inhibiting cholinergic brain functions [72, 73]. Persistent hyperglycaemia is positively correlated with cognitive deficits, which is estimated to present in 20–70% of diabetic mellitus patients [73]. In this present work, AChE was increased in brain tissues of diabetic rats, but was significantly reduced by BSFE treatment. GAD is the rate limiting enzyme in the conversion of the major inhibitory neurotransmitter, gamma amino butyric acid (GABA) from glutamate. It serves an important function of maintaining balance between the two neurotransmitters to ensure proper brain functioning [74]. However, inhibition of GAD has been associated with dementia in diabetes [75]. It has been established that streptozotocin is capable of eliciting release of glutamic acid decarboxylase autoantibodies (GADA) which inhibits GAD65 and thereby impaired synthesis of GABA [76, 77]. In this study, GAD activity reduced significantly in the diabetic control, however, BSFE treatment increased GAD activity. The combined effect of BSFE on AChE and GAD suggests its potential in maintaining glutamatergic and cholinergic neurotransmission in diabetic state, hence improving cognitive functions.

Furthermore, histological examination of the brain and liver tissues showed improved histomorphology features of the brain and liver tissues of diabetic rats treated with BSFE. Markers of neuronal death of the brain cortex such as pyknotic cells were reduced, demonstrating the neuroprotective effect of BSFE. Features of hepatic vascular congestion, fatty liver and marked disseminated infiltration of inflammatory cells were significantly reduced in BSFE treated diabetic rats. Although, fulminant hepatic failure has been attributed to ingestion of unripe ackee fruit in a sickle cell patient [78], and liver cirrhosis was detected in unripe ackee extract administered to rodents [26, 37, 79]. In contrast, BSFE reversed histomorphology disorganization of the hepatic tissues in diabetic rats in this study. This may be as a result of the hepatoprotective properties of the polyphenol-rich extract of BSFE.

Conclusion

The unripe fruit extract of *Blighia sapida* exhibits promising antidiabetic properties, mediated through multiple mechanisms including lipid profile modulation, antioxidant effects, cytokine regulation, and potential influences on apoptotic pathways and neurotransmitter function. While these findings are encouraging, further investigation is warranted to elucidate the precise molecular mechanisms underlying these effects and to explore their potential clinical applications in the management of diabetes.

Abbreviations

BSFE	Blighia sapida fruit extract
GC-MS	Gas chromatography- mass spectrometry
T2DM	Type 2 Diabetes mellitus
FBS	Fasting blood sugar
IDF	International federation of diabetes
OHA	Oral hypoglycemic agent
HGA	Hypoglycin A
TPC	Total phenolic content
TFC	Total flavonoid content
DPPH	2,2-diphenyl-1-picrylhydrazyl
FRAP	Ferric reducing-antioxidant power
TAC	Total antioxidant capacity
HFD	High fat diet
TC	Total cholesterol
HDL-c	High density lipoproteins-cholesterol
LDL-c	Low density lipoproteins-cholesterol
VLDL-c	Very low density lipoproteins-cholesterol
TBARS	Thiobarbituric acid reacting substance
SOD	Superoxide dismutase
TNF- α	Tumour necrosis factor- α
IL-6	Interleukin-6
AChE	Acetylcholinesterase
GAD	Glutamic acid decarboxylase
H&E	Hematoxylin and eosin

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Author contributions

Conceptualization TOA, JJO, AMA, Data curation TOA, JJO, AMA, Writing original draft manuscript TOA, JJO, AMA, supervision TOA and AMA, Funding acquisition TOA and AMA. All authors have read and agreed to publishing of the manuscript.

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Data availability

No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate

The experimental procedures followed established guidelines as described by the National Institute of Health guidelines in the “Principle of Laboratory Animal Care” (NIH Publication No. 85–23) and approved by Animal Care and Use Research Ethics Committee, University of Ibadan (UI-ACUREC/066–0721/2).

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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