Influence of *Bambusa Vulgaris* Leaves Extract on Endoplasmic Reticulum Stress Induced By Streptozotocin in Wistar Rats

Bolade Victoria Olubodun*, Olusola Olalekan Elekofehinti, Akeem Olalekan Lawal, Idowu Waheed Ajibade, Bukunmi Akinleye Sarah, Motunrayo Margaret Tayelolu, Faith Oghuansuan Oluwatosin, Olumide Victor Olowolafe and Tosin Samuel Olawumi

Bioinformatics and Molecular Biology Unit, Department of Biochemistry, Federal University of Technology, Akure, Ondo State, Nigeria

Corresponding Author*

Bolade Victoria Olubodun

Bioinformatics and Molecular Biology Unit, Department of Biochemistry, Federal University of Technology, Akure, Ondo State, Nigeria

E-mail: boladeric08@gmail.com

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Abstract

Abstract Diabetes mellitus is a silent killer that claims about 2 million lives annually. Endoplasmic-reticulum (ER) stress has been linked to type-2 diabetes. This study evaluated the effect of Bambusa vulgaris leaf ethanolic extract (B.V.E.E.) on ER-stress induced by Streptozotocin (STZ) in rats. Rats were divided into six groups and induced with the diabetes using streptozotocin (65 mg/kg) except the control group. Bambusa vulgaris (100, 200 and 400 mg/kg) were administered to the induced rats for twenty-one (21) days. The rats were sacrificed and blood collected for haematology while the liver was harvested for biochemical analysis. The expression of [(activating transcription factor 4 (ATF4), activating transcription factor 6 (ATF6), protein kinase RNA-like endoplasmic reticulum kinase (PERK), tribbles 3 (TRB3), transcription factor C/EBP homologous protein (CHOP), IRE1, interleukin 6 (IL-6), interleukin 1? (IL-1?) and tumor necrosis factor alpha (TNF-?)] genes was done using reverse transcriptase polymerase chain reaction (RT-PCR). Result showed that B.V.E.E. reduced fasting blood glucose in diabetic rats and lowered the activity of AST, ALT and ALP when compared to diabetic un-treated rats. Administration of B.V.E.E. promoted mitochondria biogenesis, reversed inflammation and glucose insensitivity in the pancreas of diabetic ER stress rats via modulation of GADPH, TRB3, CHOP, TNF-?, IL-1? and IL-6 mRNA expressions. B.V.E.E. administration inhibited diabetic ER stress-induced cell apoptosis by depleting the mRNA expression of ER stress sensors (IRE1, ATF4, ATF6 and PERK). Furthermore, B.V.E.E. ameliorated the increased lipid profiles in diabetic rats and restored renal function as shown by lowering the activity of AST, ALT and ALP. as well as restore distorted full blood in diabetic rats. Finally, in silico studies showed that taxiphyllin had the highest binding affinity of all the compounds present in the B. vulgaris leaf and may be the potent compound responsible for mitigation ER stress in the liver. Hence, B.V.E.E. may be deployed as medicinal plant in diabetic ER-stress management and safe for consumption, while taxiphyllin was implicated for the anti-diabetic and anti-inflammatory properties.

Keywords: Diabetes; Medicinal plant; Therapy; Oxidative stress; Bamboo

Abbreviation: BVEE: Bambusa vulgaris ethanolic extract; STZ: Streptozotocin; DM: Diabetes mellitus; ATF4: Activating transcription factor 4; IL-1β: Interleukin-1β; IL-16:Interleukin-16; IRE-1: Inositol-requiring enzyme 1; CHOP: CCAAT homologous protein binding protein; TRB3: Tribbles 3;ATF6:Activating transcription factor 6; PERK: Protein kinase like endoplasmic reticulum protein kinase; TNF-α: Tumour necrosis factor-alpha; RT-PCR: reverse transcriptase-polymerase chain reaction

Introduction

Diabetes mellitus is a chronic diseased state that is characterized by hyperglycaemia (an elevated postprandial blood glucose level) [1]. Chronic hyperglycaemia in synergy with the other metabolic aberrations in patients with diabetes mellitus can cause damage to various organ systems; leading to the development of disabling and life-threatening health complications; most prominent of which are microvascular (retinopathy; nephropathy; and neuropathy) and macrovascular complications leading to a 2-fold to 4-fold increased risk of cardiovascular diseases [2].

The global prevalence of diabetes among adults was reported to be 108 million in 1980; 285 million in 2010; 422 million in 2014; 463 million in 2019; 536.6 million in 2022; and estimated to reach 700 million by 2045 alongside 1.5 million deaths directly attributed to it yearly [3-6].

Endoplasmic reticulum stress leads to an increase in protein misfolding or a decrease in the ability of the cell to handle proteins which can result to cellular dysfunction and disease. Endoplasmic reticulum stress occurs as a result of oxidative insult. Therefore; endoplasmic reticulum stress leads to insulin resistance; autophagy; and apoptosis etc [7].

The endoplasmic reticulum interacts with many organelles within the cytoplasm; such as the mitochondria; plasma membrane (PM); endosomes; Golgi apparatus; peroxisomes; and lipid droplets; as depicted in Figure 1. The physical interaction between the ER and mitochondria is known as the mitochondria-associated ER membrane (MAM); an association that plays a crucial role in maintaining Ca^{2+} stability [8]. The endoplasmic reticulum (ER) functions as a tool for synthesizing and folding 30% of the proteome; but its usual function is easily affected by external factors [9].

Drug and diet therapies are the most popular approaches applied in the management of diabetes mellitus (DM). However; synthetic drugs used in the treatment of DM are relatively expensive and associated with various side effects; they include; metformin; acarbose; sulfonylureas; thiazolidinedione; and meglitinides. Despite the global 536.6 million prevalence of diabetes mellitus and the ever rising associated-deaths; synthetic medications have provided little hope as they come mostly with adverse effects [10].

Traditionally; *Bambusa vulgaris* has been used for the management of diabetes but it has not been validated scientifically; hence this study aims to investigate the anti-diabetic and antioxidant properties of *Bambusa vulgaris*. Bamboo leaves have been established to have anti-diabetic properties with little or no side effect Figure 2 which spelt out as one of the pharmacological advantages they have over synthetic drug.

Many ailments have been treated with edible plants. Antioxidant-rich meals can boost the body's antioxidant systems and lowering the impacts of free radical production. *Bambusa vulgaris* have been used in various traditional treatment systems for the treatment of various diseases in human [11]. *Bambusa vulgaris* has been reported to have analgesic; antipyretic; antidiabetic; antioxidant and antiviral properties.

The effects of *Bambusa vulgaris* on the expression of genes associated with ER stress genes and inflammatory genes were also investigated in the liver of the experimental rats used in this study. Compounds previously characterized from *Bambusa vulgaris* were further docked into the active site of insulin using Schrödinger suites to investigate their binding modes and binding affinities.

Materials and Methods

Plant collection and preparation

Fresh leaves of Bamboo leaves were harvested at University of Ibadan College

Hospital forestry and nursery garden; Ibadan; Oyo state. Identification and authentication of the plant were done. The leaves were separated from the stem; rinsed; air-dried and pulverized. Samples were subjected to ethanolic extraction procedure. The bioactive components were determined using HPLC (High performance liquid chromatography).

Chemicals and reagents

Streptozotocin (STZ) was procured from Santacruz's; Ethanol; Citrate Buffer (0.1 M; pH 4.5); Metformin; Formalin; distilled water; cotton wool; methylated spirit Aqueous solution and Primer sets (forward and reverse) procured from Inqaba biotech.

Laboratory equipments

UV absorbance spectrophotometer (Model AE-560-20; A & E LAB-UK); Centrifuge (Model (D-37520 Osteorode); Kendro Laboratory products; Germany) Microwave oven(model no: H2OMOWH; Hisense China);thermocycler/PCR Machine (EPPENDORF-AG 22331 Hamburg; Germany); Pipette (Labmate & Lichen- South Africa); Blue Box Transilluminator (Model {QP - 1700-01 Rev. 2}; USA); Camera Phone (Redmi note 9 pro); Glucometer machine; Gloves; Weighing balance; insulin syringe; Cannula; Whatman filter paper; Weighing balance; Eppendorf tubes; Dissection set and Glucometer and strip.

Feed preparation; animal grouping; and management

Thirty-five (35) Wistar rats of average weights ranging from 150–180g were purchased from the Department of Medical Biochemistry; College of Medicine; Ekiti State University; Ado-Ekiti; Nigeria. The animals were housed in clean aluminium cages placed in well-ventilated housing conditions and maintained at 25 °C on a 12h light/dark cycle. They were allowed free access to rat's pellets and water. They were handled and used by the National Institute of Health (NIH) Guidelines for the Care and Use of Laboratory Animals.

Experimental design

After two weeks of acclimatization; the rats were allocated into different groups and other groups except the non-diabetic control group were induced with diabetes mellitus by administering a single intraperitoneal injection of STZ (60mg/kg body weight) freshly prepared in citrate buffer (0.1 M; pH 4.5). After 72h prior the induction with STZ; the blood samples were taken by tail vein puncture; glucose level was monitored using automatic auto-analyzer (Fine test Auto-coding). Animals with blood glucose ≥200mg/dl after 72h were considered diabetic and used in the study. Rats were weighed accordingly and recorded. The rats were divided into six groups (n=5) each as illustrated below:

Group 1: Normal control rats fed with rat's pellets and water

Group 2: Untreated diabetic control rats fed with rat's pellets and water

Group 3: Diabetic control rats fed with rat's pellets and water with a standard drug given (metformin) orally (25mg/kg body weight).

Group 4: Diabetic control rats fed with rat's pellets and water with B.V.E.E (100mg/kg)

Group 5: Diabetic control rats fed with rat's pellets and water with B.V.E.E (200mg/kg)

Group 6: Diabetic control rats fed with rat's pellets and water with B.V.E.E (400mg/kg)

Gene expression study

RNA isolation

The animals were sacrificed via cervical dislocation as described previously [12-27]. The rats were sacrificed via cervical dislocation. Thereafter; the desired tissue/organs were removed and dissected on ice. The liver tissues were homogenized in Eppendorf tubes containing Trizol reagent (Thermofisher Scientific). The homogenate was added to gradient separation medium.

(chloroform) and 0.1M phosphate buffer (pH 7.4); and were centrifuged using a high-speed cold centrifuge at 12;000 rpm for 10 min; the supernatants were separated and then stored at -20˚C for biochemical assays. The RNA pellet was washed three times with 70% alcohol before being treated with DNA se.

The concentration and purity of the RNA were measured at 260nm and 280nm after it was reconstituted in nuclease-free water. RNA was converted to cDNA using the ProtoScript first-strand cDNA synthesis kit (NEB) according to the manufacturer's instructions.

Polymerase chain reaction (PCR): The cDNA was amplified using the OneTaq 2x Master Mix (NEB) and the gene-specific primer sets described below in a polymerase chain reaction Figure 1. Primer Express v2.0 was used to create the primers (Applied Biosystem; Foster City; Calif). - During the experiment; actin was utilized as a control. The expressions of nuclear factor Inositol-requiring enzyme 1(IRE1); Protein kinase RNA like ER-protein kinase (PERK); Tribbles homolog 3(TRB-3); CCAAT enhancer binding protein homologous protein (CHOP); Activating transcription factor 4 (ATF-4); Figure 3 Activating transcription factor 6 (ATF-6); Tumour necrosis factor-α (TNF-α); Interleukin 6 (IL-6); and Interleukin1β (IL-1β) Figure 4 genes were investigated in the liver of the experimental rats.

Agarose gel electrophoresis

Polymerase chain reaction amplicons were subjected to 1% agarose gel electrophoresis. Snapshots revealing the relative density of DNA bands were taken with a camera under a blue box transilluminator.

Molecular docking

Ligand preparation

Compounds previously characterized from *Bambusa vulgaris* were sketched

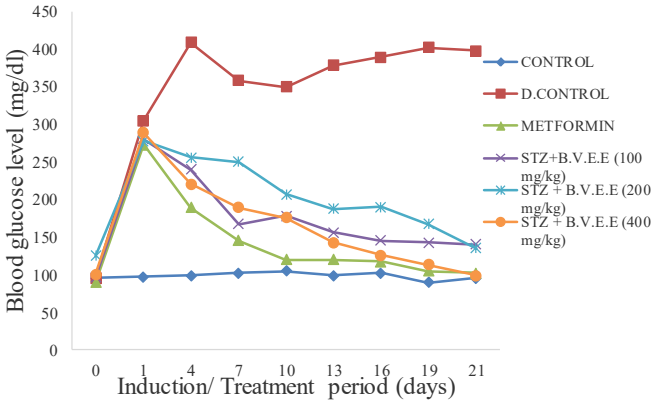

Figure 1: Effect of *Bambusa vulgaris* on the weight patterns and fasting blood glucose.

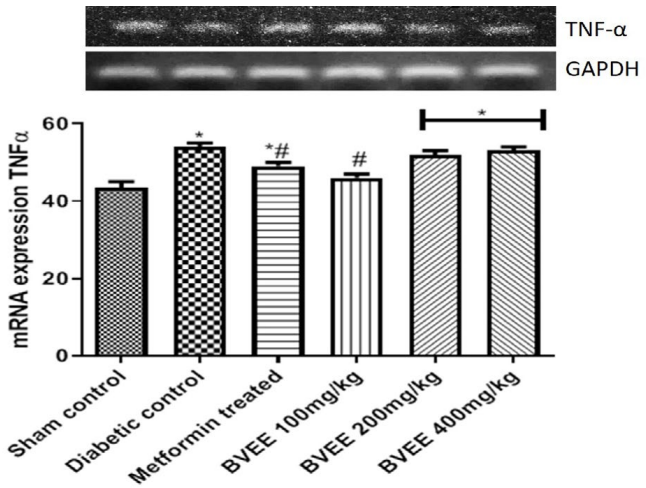

Figure 2: Effect of *Bambusa vulgaris* Ethanolic extract (BVEE) on the mRNA expression of TNF-α in the liver of experimental rats. Data represented as mean ± SEM; n = 5 per treatment. Sham control = 10 ml/kg dH_2O; Diabetic control = 60 mg/kg STZ; Metformin treated = 100 ml/kg metformin; BVEE = 100 mg/kg, 200 mg/kg and 400 mg/kg. Superscript of *, # represent $p < 0.05$ significance when compared against sham control and diabetic control respectively. (Effect of *Bambusa vulgaris* on the expression of Tumor necrosis factor (TNF-α) in the liver of STZ-induced diabetic and non-diabetic rats).

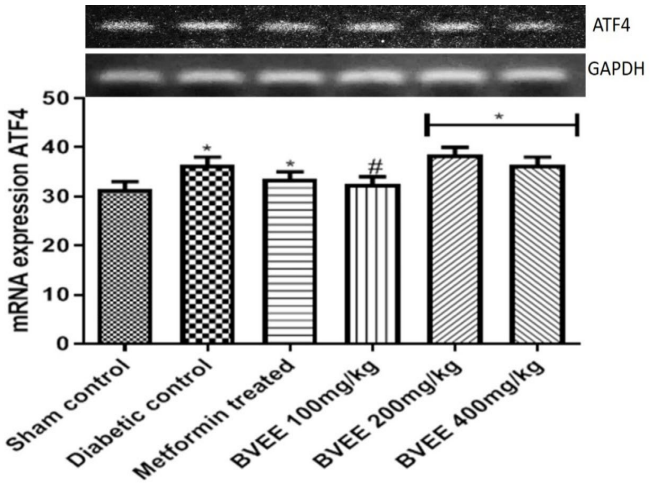

Figure 3: Effect of *Bambusa vulgaris* Ethanolic extract (BVEE) on the mRNA expression of ATF-4 in the liver of experimental rats. Data represented as mean ± SEM; n = 5 per treatment. Sham control = 10 ml/kg dH2O; Diabetic control = 60 mg/kg STZ; Metformin treated = 100 ml/kg metformin; BVEE = 100 mg/kg, 200 mg/kg and 400 mg/kg. Superscript of *, # represent p < 0.05 significance when compared against sham control and diabetic control respectively. (Effect of *Bambusa vulgaris* on the expression of activating transcription factor-4 (ATF-4) in the liver of STZ-induced diabetic and non-diabetic rats).

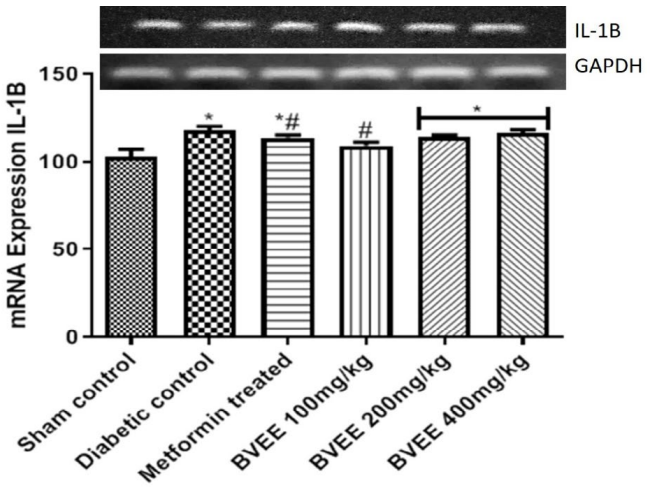

Figure 4: Effect of *Bambusa vulgaris* Ethanolic extract (BVEE) on the mRNA expression of IL-1β in the liver of experimental rats. Data represented as mean ± SEM; n = 5 per treatment. Sham control = 10 ml/kg dH2O; Diabetic control = 60 mg/kg STZ; Metformin treated = 100 ml/kg metformin; BVEE = 100 mg/kg, 200 mg/kg and 400 mg/kg. Superscript of *, # represent p < 0.05 significance when compared against sham control and diabetic control respectively. (Effect of *Bambusa vulgaris* on the expression of Interleukin-1β (IL-1β) in the liver of STZ-induced diabetic and non-diabetic rats).

in SDF format using Marvin Suite and prepared for docking using LigPrep [28;29] module in maestro; Schrödinger suites. Low-energy 3D structures with correct chirality were generated. The possible ionization states for each ligand structure were generated at a physiological pH of 7.2±0.2 and minimized using the OPLS3 force field [29].

Molecular docking study

The receptor grid was created around the binding site of the insulin receptor (PDB ID: 3EKK) using the "receptor grid generation" option in the glide-v7.5 programme of Maestro-v11.5. Afterward; the prepared ligands were docked into the receptor grid using the extra precision (XP) workflow module of the Schrödinger suite with default parameters. The virtual Screening Workflow in Maestro was used to dock and score *Bambusa vulgaris* derived compounds.

Biochemical assays (in-vivo)

Ferric reducing antioxidant property

The reducing property of the extracts was determined by assessing the ability of the extract to reduce $FeCl_3$ solution as described by Oyaizu (1986). Briefly; 2.5 mL aliquot was mixed with 2.5 mL 200 mM sodium phosphate buffer (pH 6.6) and 2.5 mL 1 % potassium ferricyanide. The mixture was incubated at 50°C for 20 min. and then 2.5 mL 10 % trichloroacetic acid was added. This mixture was centrifuged at 650 rpm for 10 min. 5 mL of the supernatant was mixed with an equal volume of water and 1 mL 0.1 % ferric chloride. The absorbance was measured at 700 nm using the JENWAY UV-Visible spectrophotometer. Then; the ferric reducing antioxidant property was subsequently calculated as ascorbic acid equivalent (AAE).

Determination of DPPH radical scavenging ability

The radical scavenging ability of the extracts against DPPH free radical was evaluated as described by [12]. In brief; appropriate dilution of the extracts (1 mL) was mixed with 1 mL of 0.4 mM methanolic solution containing $DPPH^*$; the mixture was left in the dark for 30 min; and the absorbance of the remaining $DPPH^*$ was measured at 516 nm. The percentage $DPPH^*$ scavenging ability of the extracts was subsequently calculated as percentage of the control.

Molecular interaction of *Bambusa vulgaris* with targets proteins involved in ER stress

The potential of *Bambusa vulgaris* in mitigating STZ-induced Figure 5 ER stress was assessed in *silico* using molecular docking approach.

Statistical analysis

Analysis of data collected would be evaluated using one-way analysis of variance (ANOVA) using SPSS version 20 and were expressed as mean ± SEM. Duncan's multiple ranges will be used to separate the means. Differences were considered to be statistically significant when ($p < 0.05$). Graph padprism version 7.04 will be used to plot the graph. The intensities of the bands form agarose gel electrophoresis will be quantified densitometrically using ImageJ software. Pymol will be used to visualize the protein interaction while Ligplot will be used to analyse the protein-ligand binding interaction.

Biochemical assays (*in-vitro*)

Effect of *Bambusa vulgaris* on the weight patterns Figure 1 **and fasting blood glucose**

Significant increase in blood glucose ranged between 289 – 398mg/dL was observed in the untreated STZ-stressed diabetic rats compared to the normal control; while that of B.V.E.E. treated only ranged between 135 to 99mg/dL after the experimental period. Interestingly; diabetic rats administered B.V.E.E. at 100mg/kg had competitive glucose reduction effect in comparison with the standard drug (metformin). Specifically; significantly ($p < 0.05$) reduction of blood glucose level was observed to be concentration and duration dependent in comparison with normal control and untreated STZ-stressed rats.

Effect of *Bambusa vulgaris* on the expression of Tumour necrosis factor in the liver

The expression of TNF-α Figure 2 in the liver Figure 6 was significantly up-

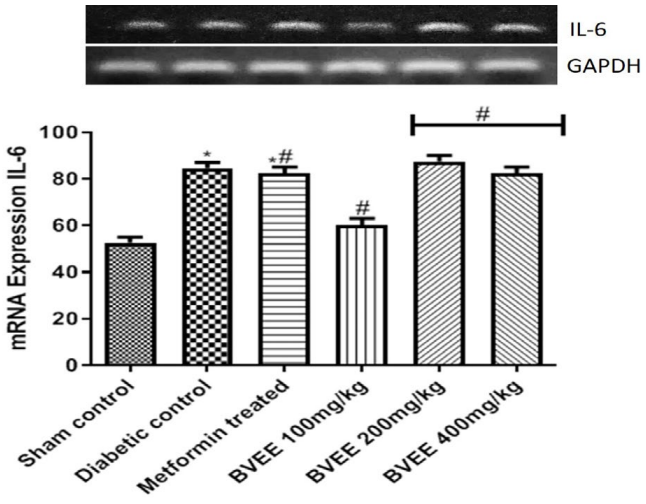

Figure 5: Effect of *Bambusa vulgaris* on the expression of Interleukin-16 (IL-16) in the liver of STZ-induced diabetic and non-diabetic rats.

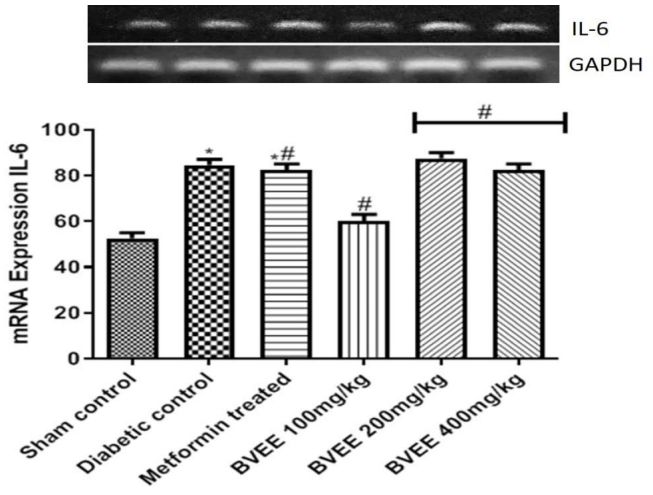

Figure 6: Effect of *Bambusa vulgaris* Ethanolic extract (BVEE) on the mRNA expression of IL-16 in the liver of experimental rats. Data represented as mean ± SEM; n = 5 per treatment. Sham control = 10 ml/kg dH2O; Diabetic control = 60 mg/kg STZ; Metformin treated = 100 ml/kg metformin; BVEE = 100 mg/kg, 200 mg/kg and 400 mg/kg. Superscript of *, # represent p < 0.05 significance when compared against sham control and diabetic control respectively.

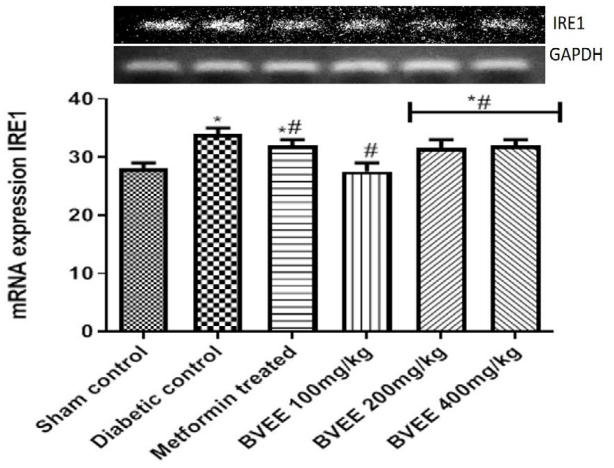

Figure 7: Effect of *Bambusavulgaris* Ethanolic extract (BVEE) on the mRNA expression of IRE-1in the liver of experimental rats. Data represented as mean ± SEM; n = 5 per treatment. Sham control = 10 ml/kg dH2O; Diabetic control = 60 mg/kg STZ; Metformin treated = 100 ml/kg metformin; BVEE = 100 mg/kg, 200 mg/kg and 400 mg/kg. Superscript of *, # represent p < 0.05 significance when compared against sham control and diabetic control respectively. (Effect of *Bambusa vulgaris* on the expression of Inositol requiring enzyme-1 (IRE-1) in the liver of STZ-induced diabetic and non-diabetic rats).

regulated in the diabetic state when compared with control but upon the treatment with the extract it was significantly down-regulated. This revealed the possible potential of *Bambusavulgaris* in mitigating diabetes-induced inflammation.

Effect of *Bambusa vulgaris* on the expression of Endoplasmic reticulum stress genes

Endoplasmic reticulum stress genes expression was significantly up-regulated in the diabetic state when compared with control but upon the treatment with the extract it was significantly down-regulated. This revealed the possible potential of *Bambusa vulgaris* in mitigating diabetes-induced inflammation Figure 7.

Docking results of *Bambusa vulgaris* extract derived compounds as potential anti-diabetics

Molecular docking simulation of compounds previously characterized from *Bambusa vulgaris* extract with anti-diabetic targets insulin revealed that they are potent ligands of this target (Figure 8; Figure 9; Figure 10 and Figure 11).

Discussion

2; 2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging ability of *Bambusa*

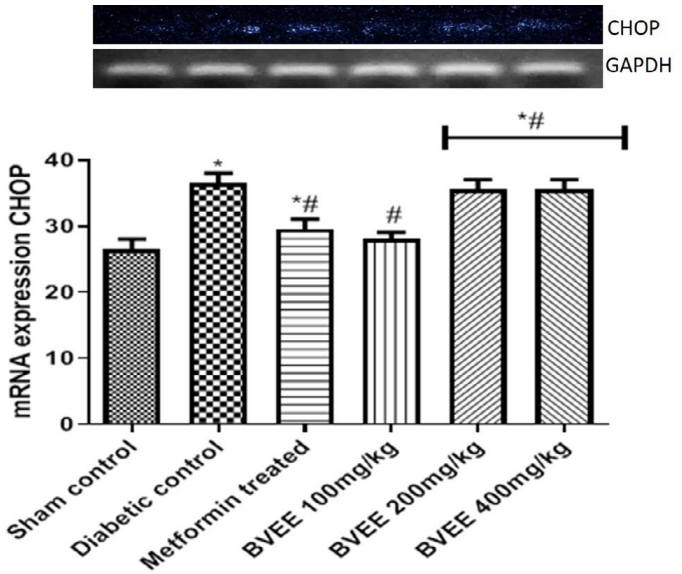

Figure 8: Effect of *Bambusa vulgaris* Ethanolic extract (BVEE) on the mRNA expression of CHOP in the liver of experimental rats. Data represented as mean ± SEM; n = 5 per treatment. Sham control = 10 ml/kg dH2O; Diabetic control = 60 mg/kg STZ; Metformin treated = 100 ml/kg metformin; BVEE = 100 mg/kg, 200 mg/kg and 400 mg/kg. Superscript of *, # represent p < 0.05 significance when compared against sham control and diabetic control respectively. (Effect of *Bambusa vulgaris* on the expression of CHOP (CCAAT enhancer binding protein homologous protein) in the liver of STZ-induced diabetic and non-diabetic rats).

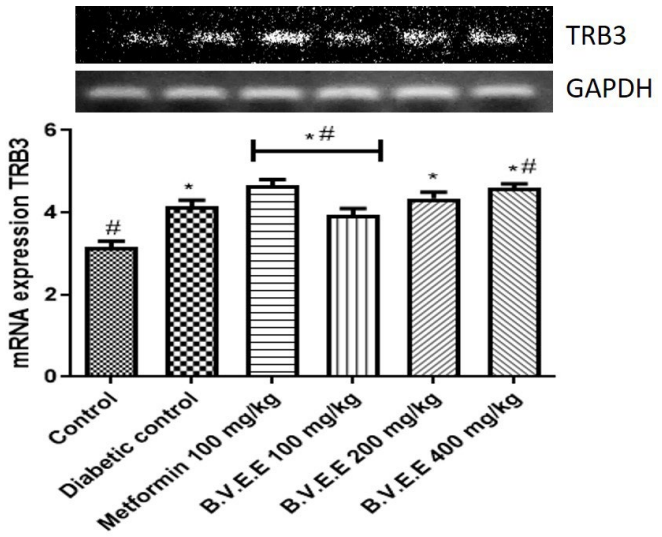

Figure 9: Effect of *Bambusa vulgaris* Ethanolic extract (BVEE) on the mRNA expression of TRB3 in the liver of experimental rats. Data represented as mean ± SEM; n = 5 per treatment. Sham control = 10 ml/kg dH2O; Diabetic control = 60 mg/kg STZ; Metformin treated = 100 ml/kg metformin; BVEE = 100 mg/kg, 200 mg/kg and 400 mg/kg. Superscript of *,# represent p < 0.05 significance when compared against sham control and diabetic control respectively.

vulgaris leaf sample is as shown in Figure 4. It was observed that the leaf sample has appreciable DPPH radical scavenging ability that increased in a concentration dependent manner. This was in line with previous study which observed that plant phytochemicals exert their antioxidant potentials by inhibiting production of free radicals; scavenging the produced free radicals or by metal chelation [13]. It was also observed that the ferric reducing antioxidant property (FRAP) of the leaf sample to reduce Fe^{3+} to Fe^{2+} was observed. The result showed that the B. vulgaris leaf sample have appreciable ferric reducing antioxidant property that increased in a concentration dependent manner. The reducing power of a plant sample is an essential anti-oxidation defence system mechanism which the sample scavenges frees radicals via hydrogen atom or electron transfer [14]. Furthermore; the ferric reducing antioxidant property of the leaf sample was consistent with the previous result of DPPH radical scavenging ability as earlier observed in the

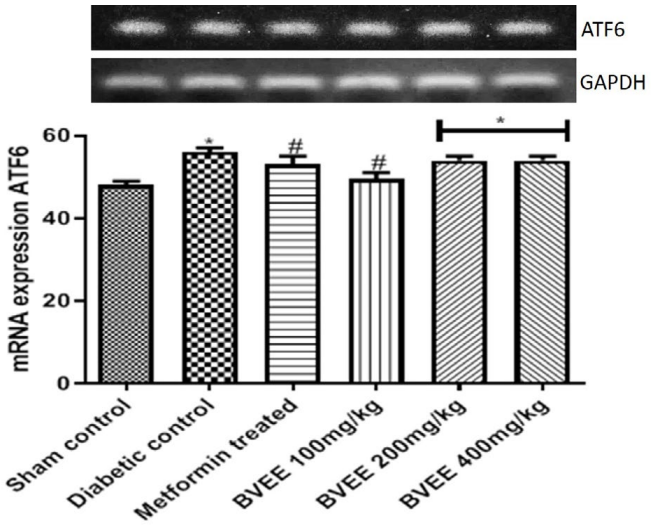

Figure 10: Effect of *Bambusa vulgaris* Ethanolic extract (BVEE) on the mRNA expression of ATF-6 in the liver of experimental rats. Data represented as mean ± SEM; n = 5 per treatment. Sham control = 10 ml/kg dH2O; Diabetic control = 60 mg/kg STZ; Metformin treated = 100 ml/kg metformin; BVEE = 100 mg/kg, 200 mg/kg and 400 mg/kg. Superscript of *, # represent p < 0.05 significance when compared against sham control and diabetic control respectively. (Effect of *Bambusa vulgaris* on the expression of activating transcription factor 6 (ATF-6) in the liver of STZ-induced diabetic and non-diabetic rats).

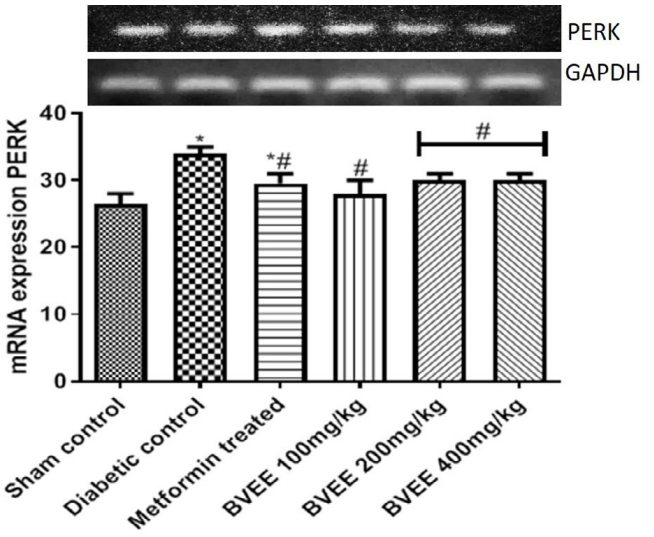

Figure 11: Effect of *Bambusa vulgaris* Ethanolic extract (BVEE) on the mRNA expression of PERK in the liver of experimental rats. Data represented as mean ± SEM; n = 5 per treatment. Sham control = 10 ml/kg dH2O; Diabetic control = 60 mg/kg STZ; Metformin treated = 100 ml/kg metformin; BVEE = 100 mg/kg, 200 mg/kg and 400 mg/kg. Superscript of *,# represent p < 0.05 significance when compared against sham control and diabetic control respectively.(Effect of *Bambusa vulgaris* on the expression of Protein kinase-RNA- like Endoplasmic reticulum kinase (PERK) in the liver of STZ-induced diabetic and non-diabetic rats).

present study. This thus confirms correlation between the phenolic contents of a plant and its overall antioxidant potentials [15].

The HPLC quantitative analysis of the *B. vulgaris* leaf revealed abundance of phenolic acids and flavonoids. The polyphenolics present in leaf include: Chlorogenic acid; Benzoic acid; 8-C-Glycosylapigenin; 6-C-Glucosylapigenin; P-coumaric acid; Iso-Orientin; Luteolin; Taxiphyllin; Betain; Oxalic acid; Alpha Amyline Acetate; 1-O-Methyl-3;4-cyclohexy; 1-O-Methyl-5-O-caffeoylquinine; 2-deoxcytidine;2-deoxyadenosine;2-deoxyguanosine;2-O-methyl-adenosine;3-O-(3 methylcaffeoyl)-quinine; 3-O-caffeoyl-1-methylquinine; 5-feruloylquinic acid; 5-O-caffeoyl-4-methylquinine; 5-O-caffeoyl-4-methylquinine; 5;7;3';4'-tetrahydroxy-6; 5;7;3';4'-tetrahydroxy-8; 5;7;4'-trihydroxy-6-C-b-D; adenine; adenosine; cassiaoccidentalin Bcytidine; farobin A; farobin B; guanosine; homoorientin; Isovitexin; Isovitexin; L-tyrosine; ; Methyl 1-O-Methyl-3;4-cyclo; methyl chlorogenate; orientin; thymidine; tricin-5-O-glucoside; tricin; and uridine. Interestingly; these polyphenolics are well-known antioxidants and have been reported for their antidiabetic properties [16-19].

Hyperglycaemia is key to the pathogenesis of diabetes and its complication [20]. Although; the use of synthetic drugs in the management of diabetes has been reported to be effective; but also lack the holistic ability to alleviate diabetic complications [21;22]. Metformin; a class of Biguanides major therapeutic mechanism in diabetes is the improvement of insulin sensitivity by modifying the post-receptor signalling in the insulin pathway; once this is done the skeletal muscle and adipocytes will then experience the up-regulation of the GLUT4 which therefore increases glucose entry into cells [23]. The side effect of metformin is that it can affect renal function; resulting in nephropathy [24]. This side effect has enhanced the constant lookout for other therapeutic options with minimal or no side effects. To this end; emerging evidencesuggests those food-drug interactions has been of tremendous interest over the years with thevarying possibilities it holds. Indeed; foods rich in bioactive compounds; otherwise known asphytochemicals; such as polyphenols; alkaloid; among others; are known to inhibit; synergize;potentiate or antagonize therapeutic properties of antidiabetic drugs [25;26].

Streptozotocin; diabetogenic agent induces pancreatic β-cell apoptosisthrough the activation of ER stress signalling; as reported both in *Vitro* [27] and in vivomodels [28;29]. Moreover; it has been reported that chronically elevated glucose has been associated with ER stress induction in the pancreatic β-cells andincreased reactive oxygen species and $NO^{\cdot}$ production along with othercellular effects. Interestingly; the findings of this study revealed an increase blood glucose beyond the normal level; which depicts diabetic condition. Which coincides with earlier studies demonstrating the role of STZ in arbitrating an irreversible damage to pancreatic β-cells and also instigated loss in its capacity to secrete insulin. However; administration of BVEE was observed to ameliorate the aggravated blood glucose level. Many polyphenolic compounds have been reported to play a major role in the maintenance of glucose homeostasis and render protective effect against diabetes mediated complications. With this in mind; it is obvious that BVEE may have glucose lowering effects in an ER stress model by improving pancreatic β-cell function.

Researches have highlighted the contribution of ER stress to diabetes and its complications. Hence; targeting ER stress is a promising approach to the prevention of diabetes-mediated vascular dysfunction. During stressful conditions; such as an increase in the misfolded protein level; chaperons become overloaded and the ER fails to fold and export newly synthesized proteins;leading to ER stress. Clinically; the increased expression of ER stress markers in β-cells in pancreatic sections from Type 2 diabetic patients provides direct evidence for the activation of the ER stress response.

In response to the ER stress; three ER localized transmembrane signal transducers; namely IRE1; PERK and ATF6; are activated to initiate subsequent responses. Acting as stress sensors; the three transducers act to monitor the condition of the ER. Under physiological conditions; each of these sensors is maintained inactively by binding to GRP78; however; due to ER stress; GRP78 dissociates from each transducer which triggers their activation and the induction of unfolded proteins response (UPR). Considering these roles of IRE1; PERK and ATF6; it is affirmed that these receptors are fundamental to ER stress-induced apoptosis. Interestingly; in the present study; induction of diabetic ER-stress resulted in significant ($P < 0.05$) increase in mRNA expression of IRE1; when compared with the normal control group. However; administration of BVEE at 100 mg/kg was able to significantly ($P < 0.05$) restore the aggravated IRE1 mRNA expression when compared to the untreated diabetic-ER stress rats. A significant ($P < 0.05$) decrease in IRE1 mRNA expression was also experience with BVEE at 200 and 400mg/kg; but the administration of BVEE at the aforementioned concentrations were not able to significantly ($P < 0.05$) restore IRE1 to normal control level.

Additionally; the mRNA expression of ATF 6 was significantly ($P < 0.05$) elevated in untreated diabetic ER-stress rats when compared to the normal control group according to the study of Van De Maele; ATF6 is exported from ER and then translocated to Golgi apparatus during ER stress. After cleavage by Site-1 proteases (S1P) and Site-2 proteases (S2P); the cytosolic domain of ATF6 then translocate to nucleus to initiate transcription of unfolded protein response (UPR) target genes. In general; these genes induced on the proapoptotic phase of ER stress contribute to programmed cell death.

Meanwhile; a recent study also showed that ATF6 had the ability to mediate ER stress-induced apoptosis on its own. However; treatment with metformin and BVEE (100mg/kg) was able to significantly (P <0.05) reduce the aggravated AFT6 mRNA expression. Meanwhile; administration of BVEE at 200 and 400mg/kg was unable to significantly (P <0.05) restore the aggravated AFT6 mRNA expression.

In the same vein; the mRNA expression of PERK was significantly (P <0.05) elevated in untreated diabetic ER-stress rats when compared to the normal control group. Interestingly; administration of metformin and BVEE (100; 200 and 400) were all able to significantly (P <0.05) reduce the aggravated PERK mRNA expression. Typically; the ameliorative effects of BVEE may be indicative of the anti-apoptotic potential of the ethanolic extracts. As observed in this study; PERK activation by autophosphorylation and homo-multimerization in response to ER stress is an established biological mechanism. The activated PERK (p-PERK) then phosphorylates the alpha-subunit of Eukaryotic initiation factor 2 (eIF2α) which then shut down translation initiation of global genes except for ATF4; which expression is stimulated. Interestingly; induction of diabetic ER stress resulted in significantly (P <0.05) elevated ATF4 mRNA expression. Meanwhile; the administration of metformin and BVEE (200 and 400mg/kg) were unable to restore the aggravated AFT4 mRNA expression. However; BVEE administration at 100 mg/kg was able to significantly (P <0.05) reduce the aggravated AFT4 mRNA expression.

Hyperglycaemia perturbs ER homeostasis leading to the chronic activation of unfolded protein responses (UPR) resulting in cell death by triggering the expression of the transcription factor C/EBP homologous protein (CHOP); a proapoptotic UPR effector molecule. CHOP is primarily considered as a pro-apoptotic transcription factor and its expression strongly depends on ATF4. It has also been reported that ATF6 could induce expression of CHOP. In the present study; the induction of ER stress was onfirmed by the significant (P <0.05) upregulation of CHOP mRNA expression in the pancreas of STZ-induced diabetic rats; when compared to the normal control group. This correlates with the study that observed upregulated CHOP mRNA expression in STZ-induced diabetes model; as well as reduction in β-cell mass and promotion of apoptosis. Although CHOP can be activated by all three ER stress sensors (IRE1; PERK and ATF6); it is most strongly induced by phosphorylation of PERK. Interestingly; BVEE administration reduced CHOP expression and inferably ER stress; which coincides with the earlier findings of stating the protective roles of quercetin against pancreatic β-cell dysfunction under ER stress.

Excessive glucose concentrations are shown to increase pro-inflammatory cytokines by inducing oxidative mechanism mediated NF-κB activation. In turn; these cytokines produce NADPH oxidase-generated ROS formation; leading to oxidative stress which eventually results in the destruction of pancreatic β-cells. Additionally; some studies have established increase in inflammation of pancreatic tissues in diabetic rats through the over expression of both pro-inflammatory and inflammatory cytokines. These cytokines (TNF-α; IL-1β andIL-6) are known for their unwanted effect on insulin signalling and sensitivity of glucose. Typically; the upregulation of TNF-α; IL-1β and IL-6 genes observed in this study after induction of diabetes correlates with impaired insulin signalling pathways (through phosphorylation of protein kinaseB) and type-2 diabetes mellitus. The crosstalk between these signal pathways is associated with the apoptosis of β-cells in diabetes. Thus; disrupting the cellular stress-mediated pancreatic β-cell death could be a valuable therapeutic approach to prevent and cure diabetes. Inferably; the anti-inflammatory properties of BVEE were shown through the repression of TNF-α; IL-1β and IL-6 mRNA. Hence; the present result may be suggestive that BVEE has ability to ameliorate inflammation in the pancreas of STZ-induced diabetic ER stressed rats.

Molecular docking is described as the orientation of small-molecular weight compounds with the binding site of protein; which is followed by ranking of the compounds based on docking score/binding energy. The data provided in this computational study showed that taxiphyllin had favourable binding affinity with the crystal structures of IRE-1 and TNF-α from the high docking score as shown in Figure 1. The compound formed hydrogen bond interaction with some of the amino acids within the binding pockets of the proteins; an indication that BVEE can exploited in the design of antidiabetic drugs. Interestingly; strong binding and interaction with these enzymes have been established to enhance glucose uptake and eventually amelioratethe complication of type-2 diabetes in skeletal muscle.

Considering the observed positive modulatory and ameliorative effects of BVEE at 100; 200 and 400 mg/kg on blood glucose; insulin production; gene expression [activating transcription factor 4 (ATF4); activating transcription factor 6 (ATF6); protein kinase RNA-like endoplasmic reticulum kinase (PERK); tribbles 3 (TRB3); transcription factor C/EBP homologous protein (CHOP); IRE1; interleukin 6 (IL-6); interleukin 1β (IL-1β) and tumor necrosis factor alpha (TNF-α)]; and computational docking in this study; further investigation on the toxicity and mitigating effects of the BVEE was also carried out; so as to established the overall safety level via the assessment of liver [Alanine transferase (ALT); Alkaline phosphatase (ALP); aspartate transferase (AST) and total albumin] and kidney (creatinine and urea) function test in serum; as well as lipid profiles and haematological parameters.

In this study; ALT; AST; ALP activities and total albumin level were observed to be significantly (p ≤0.05) increased in the serum of experimental animals induced with diabetic ER stress. The trend of the present result may be indicative that the induction of streptozotocin necrotized the liver; which is accompanied by a leakage of liver enzymes into the serum. ALP is a potent anti-inflammatory mediator that protects tissues from damage. Therefore; the significantly (p ≤0.05) increased ALP activity in the serum of untreated diabetic ER stress rats could have result from diabetes-induced damage to liver tissue. ALT and AST; biomarkers of hepatocellular injury; were also observed to be significantly (p ≤0.05) increased in the serum of untreated diabetic ER stress rats. Inferentially; the toxicity of streptozotocin has been linked to elevated inflammatory markers and oxidative stress; with the study of Yap implicating the cascade a major cause of liver damage in diabetic ER stress. However; the levels of ALT; AST; ALP activities and total albumin in the serum of experimental animals were observed to be significantly (p ≤0.05) reduced in experimental animals administered BVEE at 100; 200 and 400 mg/kg; which may be indicative of their therapeutic potentials at those doses.

Metabolic renal changes were also observed in the serum creatinine and urea of experimental animals induced with diabetic ER stress. Serum creatinine and urea are used to assess kidney impairment and drug induced toxicity. Typically; hyperglycemia-induced secondary mediators; such as protein kinase C and production of inflammatory cytokines are responsible for ER stress induced renal injury. Interestingly; significantly (p ≤0.05) elevated creatinine and urea was observed in the serum of untreated diabetic ER-stress rats; which may be indicative of the toxicological effects of streptozotocin on the kidney of the experimental animals. Nevertheless; all the diabetic ER stress rats fed with BVEE mitigated this effect and reversed the altered renal status. Apparently; the reno-protective effect exhibited by the BVEE at 100; 200 and 400mg/kg could be ascribed to their phytochemicals and/or antioxidant potential(s); therefore indicated the safety of the ethanolic extracts.

Full blood counts of the untreated diabetic ER stress group showed significantly (p ≤0.05) depleted mean cell haemoglobin concentration (MCHC); mean cell haemoglobin (MCH); mean corpuscular volume (MCV); Packed Cell Volume (PCV); Blood Haemoglobin Level (HGB); White Blood Cell Count (WBC); and Red Blood Cell Count (RBC) levels compared to normal control group. This finding is in line with a study by Yap; who indicated that the full blood counts were found to have lower values in diabetic rats and suggested the possibility of anaemic complication. This condition could lead to various complications associated with diabetes mellitus. However; administration of BVEE at 100; 200 and 400 mg/kg was able to significantly (p ≤0.05) restore the depleted MCHC; MCH; MCV; PCV; HGB; WBC and RBC levels. Thus; the result reveals that treatment with BVEE has protective or stimulation effect on the MCHC; MCH; MCV; PCV; HGB; WBC and RBC levels; improving the anaemia-like condition in the diabetic ER stress rats. The improvement of the full blood counts is also indicative of their enhanced immune system. As these blood cells are responsible for invading pathogens by releasing complements or phagocytosis. Inferentially; the observed improved full blood counts can also be linked to the anti-inflammatory ability of the BVEE.

Hyperlipidemia has been proven to be a contributory cause of atherosclerosis in patients with diabetes. Dysregulation of lipid profile is a critical attribute oftype-2 diabetes;and is usually characterized by elevated total cholesterol; lowdensity lipoprotein cholesterol (LDL-c) and triglycerides; and reduced high-density lipoproteins (HDL-c). These alterations are critically involved

in elevated risk of cardiovascular diseases andrapid onset of macrovascular atherosclerotic disease. In this study; induction of diabetic ER stress resulted in significantly ($P < 0.05$) depleted HDL-c concentration as well as significantly ($P < 0.05$) elevated cholesterol; triglyceride; and LDL-c levels. Meanwhile; treatment with BVEE was observed to markedly modulate the components of the lipid profiles relative to the untreated diabetic and normal control animals. This significant lowering of serum cholesterol; triglyceride; and LDL-c concentrations; as well as the significant rise in serum HDL-c concentration agrees with the study by Obafemi; where plant phenols significantly ameliorated the abnormalities in lipid metabolism in an STZ-model of diabetes. In addition; it could be suggested that incidence of cardiovascular events could be prevented after treatment with BVEE as cardiovascular complications associated with Type II diabetes are attributable to reduced HDL-c as well as elevated cholesterol; triglyceride; and LDL-c levels. In addition; hypertriglyceridemia as well as low level of HDL-c adversely affect glucose metabolism. Inferentially; reduction of cholesterol concentration by BVEE may reflect both increases in cholesterol excretion as well as inhibition of its absorption. Thus; improvement in these lipid components as observed in this study could also contribute to the observed reversal of hyperglycaemia after treatment of diabetic rats with BVEE.

Conclusion

The current findings establish the ameliorative effect of B. vulgaris leaf ethanolic extract (B.V.E.E) on STZ-induced diabetic ER stress model rats. The B. vulgaris leaf sample was observed to possess appreciable DPPH scavenging ability and ferric reducing antioxidant power. Typically; the administration of B.V.E.E. showed hypoglycemic effects through its ability to reduce glucose level. Administration of B.V.E.E. promoted mitochondria biogenesis; reversed inflammation and glucose insensitivity in the pancreas of diabetic ER stress rats via modulation of GADPH; TRB3; CHOP; TNF-α; IL-1β and IL-6 mRNA expressions. Also; B.V.E.E. administration inhibited diabetic ER stress-induced cell apoptosis by depleting the mRNA expression of ER stress sensors (IRE1; ATF4; ATF6 and PERK). Furthermore; the ethanolic extract was observed to be safe and ameliorative as it lowered aggravated lipid profiles; renal and hepatic toxicity parameters as well as restore distorted full blood counts of STZ-induced diabetic ER stress model rats. Finally; in silico studies showed that taxiphyllin had the highest binding affinity of all the compounds present in the B. vulgaris leaf. Hence; B.V.E.E. may be deployed as medicinal plant in the management of diabetic ER stress and safe for consumption; while taxiphyllin was implicated for the anti-diabetic and anti-inflammatory properties.

Recommendation

In view of the findings in this present study; the following recommendations are therefore proposed that clinical trial study should be conducted with the B. vulgaris leaf ethanolic extract on diabetic ER stress patients to authenticate the potential of the product as a potential anti-diabetic agent; further evaluate the safety of the ethanolic extract; and utilize the extract as medicinal plant to improve the health wellbeing of diabetic patients.

Authors' Contributions

This work was carried out in collaboration between all authors. Authors OOE and AOL designed the study and supervised the work. Authors BVO; IWA; OOE; MMT; BSA; OVO; TSO and FOO did the laboratories work. OOE provided laboratory facilities. Authors BVO and OOE drafted the manuscript and performed the statistical analysis. Authors OOE and AOL edited the manuscript.

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Availability of Data and Materials

The datasets generated and/or analyzed in this study are available from the corresponding author on reasonable request.

Ethics Approval

The experimental protocol was approved by the Ethical Committee of Federal University of Technology; Akure.

Consent for Publication

Not applicable.

Competing Interests

The authors hereby declare no conflict of interest.

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