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# Apigetrin-enriched *Pulmeria alba* extract prevents assault of STZ on pancreatic β-cells and neuronal oxidative stress with concomitant attenuation of tissue damage and suppression of inflammation in the brain of diabetic rats

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ABSTRACT

In the present study, in vitro, *in vivo*, and *in silico* models were used to evaluate the therapeutic potential of *Pulmeria alba* methanolic (PAm) extract, and we identified the major phytocompound, apigetrin. Our in vitro studies revealed dose-dependent increased glucose uptake and inhibition of α-amylase (50% inhibitory

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Dyslipidemia
Attenuation
Phytoconstituent

concentration ($IC_{50}$)= 217.19 μg/mL), antioxidant (DPPH, ferric-reducing activity of plasma (FRAP), and lipid peroxidation (LPO) [$IC_{50}$ = 103.23, 58.72, and 114.16 μg/mL respectively]), and anti-inflammatory potential (stabilizes human red blood cell (HRBC) membranes, and inhibits proteinase and protein denaturation [$IC_{50}$ = 143.73, 131.63, and 198.57 μg/mL]) by the PAm extract. In an in vivo model, PAm treatment reversed hyperglycemia and attenuated insulin deficiency in rats with streptozotocin (STZ)-induced diabetes. A post-treatment tissue analysis revealed that PAm attenuated neuronal oxidative stress, neuronal inflammation, and neurocognitive deficiencies. This was evidenced by increased levels of antioxidants enzymes (superoxide dismutase (SOD), catalase (CAT), and reduced glutathione (GSH)), and decreased malondialdehyde (MDA), proinflammatory markers (cyclooxygenase 2 (COX2), nuclear factor (NF)-κB and nitric oxide (NOx)), and acetylcholinesterase (AChE) activities in the brain of PAm-treated rats compared to the STZ-induced diabetic controls. However, no treatment-related changes were observed in levels of neurotransmitters, including serotonin and dopamine. Furthermore, STZ-induced dyslipidemia and alterations in serum biochemical markers of hepatorenal dysfunction were also reversed by PAm treatment. Extract characterization identified apigetrin (retention time: 21,227 s, 30.48%, *m/z*: 433.15) as the major bioactive compound in the PAm extract. Consequently, we provide *in silico* insights into the potential of apigetrin to target AChE/COX-2/NOX/NF-κB Altogether the present study provides preclinical evidence of the therapeutic potential of the apigetrin-enriched PAm extract for treating oxidative stress and neuro-inflammation associated with diabetes.

## 1. Introduction

Diabetes is a rapidly increasing metabolic disease with more than half a billion global cases, and about 240 million people living with this disease are undiagnosed [1]. There are different types of diabetes but type 2 diabetes mellitus (DM; T2DM) accounts for 90% of the total reported cases [2]. Several factors can lead to the initial onset of this disease, such as genetic and sedentary lifestyle factors or defects in insulin signaling pathways [3]. Prolonged undiagnosed diabetes or improper management can lead to complications such as osteoporosis and osteoarthritis, micro- and macrovascular complications, retinopathies, nephropathies, and neuropathies [4,5]. Atherosclerosis and hyperlipidemia are also associated with high blood glucose levels as well as weight gain especially in type 2 diabetes [6].

Oxidative stress and inflammation are widely recognized as intimately associated pathological mechanisms of diabetes and its complications [7,8]. Excessive free radical generation induces oxidative damage to cellular macromolecules [9] and promotes pancreatic β-cell destruction and insulin resistance (IR), and thus the development of DM. Furthermore, excessive free radicals modulate several intracellular and redox-signaling networks [10] such as nuclear factor (NF)-κB, resulting in the release of proinflammatory mediators, cellular dysfunction, and injury [11] and thus the development of DM complications, such as coronary artery disease, neuropathies, nephropathies, retinopathies, and stroke [12].

Conventional antioxidants and antidiabetic drugs are expensive and associated with side effects such as weight gain, gastrointestinal disturbances, edema, and anemia [13]. These effects call for alternative remedies. Over the years, several orthodox and traditional interventions have been employed to treat various diseases due to their affordability, availability, and few or no side effects [14,15]. Plants are rich in secondary metabolites and antioxidants, and hence their pharmacological properties have been widely explored [16–22]. Besides, the conventional medicinal systems chiefly rely on the use of multiple natural product scaffolds for the development of a new drug, in an unmodified state or for-property optimization by modifying the natural-product pharmacophores [23,24]. As a reflection of the high significance of natural products for the discovery and development of new pharmaceuticals, a continual evaluation of medicinal plants as a source of new therapeutic agent is highly recognized [23,24].

*Pulmeria alba* (PA) belongs to the Apocynaceae family; it is a flowering plant native to the USA, Mexico, and the Caribbean region but is also widely distributed in tropical and subtropical regions [25]. The plant is commonly used in folk medicine against respiratory infections, skin diseases, liver diseases, and diabetes and its associated complications [26]. Phytochemical screening of the leaf revealed the presence of phenolic compounds, flavonoids, and alkaloids [27]. The plant was also shown to possess anti-repellant [19], antimicrobial [28], antioxidant [29], and hypoglycemic properties [30]. However, despite the paucity of scientific proof of efficacy and safety of this plant to treat diabetic complications, it increasing use is supported by the promising results in the traditional medical system. Herein, we provide preclinical evidence of the therapeutic potential of the PA methanolic extract (PAme) against oxidative stress and inflammatory events associated with diabetes. Our findings also revealed that the PAme is a rich source of useful antioxidant compounds, such as apigetrin and cinnamic acid. The bioactive compounds were hence attributed to the displayed effects which provides further insights into the plant's potential for developing drug candidates against diabetes. Our findings may serve as a foundation for antidiabetic drug development, and a study is currently ongoing to fully ascertain the therapeutic effects of the extract and its bioactive compounds.

## 2. Materials and methods

### 2.1. Assay kits, chemicals, and reagents

The assay kits used for the liver and kidney function analyses were products of Randox Laboratories-Atrium while the enzyme-linked immunosorbent assay (ELISA) kits for neurotransmitters and inflammatory markers were from Elabscience. The insulin ELISA kit was from Calbiotech (El Cajon, CA, USA). The ELISA reader was a product of Sunrise, Tecan (Austria). All chemicals and reagents used in the study were of analytical grade and were products of Sigma Aldrich (St. Louis, MO, USA).

### 2.2. Collection and preparation of the extract

*Pulmeria alba* plants were collected within the Bosso Area of Minna, Niger State, Nigeria, and were identified and authenticated at the Biological Science Department of Federal University of Technology, Minna (FUTMinna; Minna, Nigeria). The plants were rinsed with water, air-dried for 2 weeks to obtain a constant dry weight. The dried plant was pulverized with a blender into fine powder. Extraction was done by adding 1500 mL of methanol to 300 g of plant powder for 72 h, during which time the mixture was shaken intermittently for exhaustive extraction. The extract was filtered and concentrated under reduced pressure to obtain the *P. alba* methanol extract (PAme), which was stored for further use.

### 2.3. Extract characterization by liquid chromatography mass spectrometry (LC-MS)

The PAme was subjected to an LC-MS analysis using the Shimadzu

LC-MS-8040 ultrafast mass spectrometer. The LC-MS-8040 offers high sensitivity while maintaining ultrafast performance of the LC-MS-8030. The column consisted of a Shim-pack FC-ODS analysis column (2.0 mm ID × 150 mm L, 3 μm); mobile phase A of 5 mmol/L ammonium acetate and water, mobile phase B of 5 mmol/L ammonium acetate and methanol, with a gradient program of 15% B (0 min), 40% B (1–3.5 min), 50% B (6 min),55% B (8 min), 95% B (17.5–30 min), and 15% B (30.01–40 min); a flow rate of 0.2 mL/min; and a column temperature of 40 °C. The following parameters were used for MS: probe voltage of + 4.5 kV (ESI-positive mode), nebulizing gas flow of 1.5 L/min, drying gas flow of 10 L/min, a DL temperature of 250 °C, and heat block temperature of 400 °C. Base peaks were obtained at a scan speed of 5000 u/s from 100 (start *m/z*) to 1000 (end *m/z*) atomic mass units in the positive [M+H+ ] ionization mode. The spectra generated within the retention time frame of 0.00–50.00 s was monitored using Shimadzu Lab solution software for LC-MS. The results obtained were exported in CDF format and used for successive steps required for compound identification with mzmine software (vers. 2.53). The software provides a graphical interface that allows for result filtering and noise minimization to ensure a better peak analysis. Prior to identification, five preliminary steps of data analysis were performed viz, mass detection, chromatogram building, peak deconvolution, peak de-isotyping, and data filtering. The data obtained following these preliminary steps were then used for identification using mzmine.

### 2.4. Maximum tolerated dose (MTD) analysis of PAme

Preliminary safety evaluation of PAme in vivo was evaluated at increasing extract concentrations (10–5000 mg/kg BW) in a 7-day toxicity study as described by Lorkes [31]. The Maximum Tolerated Dose (MTD) was defined as the maximum extract concentration that cause ≤ 10% body weight loss and produces no death, or external signs of toxicity that would be predicted to adversely affect animal's health or shorten the animal's natural lifespan [32–34]. The animals were afterward monitored for mortality and adverse effect over a period of 2 weeks.

### 2.5. In vitro antioxidant, anti-inflammatory, and hypoglycemic analysis

First, the well-established protocols of Singleton et al. [35] and Chang et al. [36] were used to quantify total phenolic and flavonoid contents of the extract. Calibration curves were plotted using standard gallic acid and quercetin equivalents, respectively. The in vitro antioxidant, anti-inflammatory, and hypoglycemic effects of the extract were evaluated at varying concentrations of 50, 100, 200, 300, and 400 μg/mL. A method of Tsado et al. [37] was used to determine the 2, 2-diphenyl-1-picrylhydrazyl (DPPH) free radical-scavenging effect, while the Oyaizu [38] method was used to determine the ferric-reducing activity of plasma (FRAP) of the extract. An in vitro anti-diabetic analysis based on the α-amylase inhibitory effect was conducted as described by Worthington [39], while an assay of glucose uptake by yeast cells was conducted with glucose concentrations of 5, 10, and 20 mM and extract concentrations of 50–400 μg/mL. The in vitro anti-inflammatory effect of the extract was assessed using a human red blood cell (HRBC) membrane stability test according to the protocol described by Thenmozhi [40]. The established protocol of Mizushima and Kobayashi [41] was used to determine the percentage inhibition of protein denaturation by the extract, while a proteinase inhibitory assay was performed as described by Oyedepo and Femurewa [42].

### 2.6. In vivo antidiabetic studies

Forty male Wistar rats (115.78 ± 4.89 g) were obtained from the animal holding facility of the FUTMinna. Rats were fed a standard pellet diet, given ad libitum access to clean water, and were kept under standard laboratory conditions (12-h dark/light cycle). Regulations of the Ethics Committee of FUTMinna on the use of experimental animal were strictly adhered to. Diabetes was induced by an intraperitoneal injection of 40 mg/kg body weight (BW) of streptozotocin (STZ), and a 5% glucose solution which was administered 24 h after the STZ injection. Animals with fasting blood sugar (FBS) of 250 mg/dL [43] were considered diabetic and were divided into four treatment groups ($n = 6$). Group 2 received normal saline, group 3 received 100 mg/kg BW metformin, while groups 4 and 5 received 150 and 300 mg/kg BW of PAme, respectively. All treatments were administered daily for 21 days via oral gavage.

### 2.7. Post-treatment sample collection and preparation

At the end of treatment, animals were weighed, anaesthetized with diethyl ether, and sacrificed. Blood was collected via the jugular vein/cardiac puncture and allowed to clot. Clotted blood was then centrifuged at 3000 rpm for 15 min, and the serum was decanted and properly preserved in a refrigerator for biochemical analyses [44,45]. Whole blood was collected in EDTA-containing bottles for the hematological analysis. The excised organs were blotted to remove blood stains, weighed, and homogenized in a 0.25 M cold sucrose solution [46,47]. The mixture was centrifuged at 4000 rpm for 10 min. The supernatant was collected for immediate analysis.

### 2.8. Analysis of full blood counts

Methods described by Dacie and Lewis [48] were used for the analysis of hematological indices, including erythrocyte indices (hemoglobin [HGB], packed cell volume [PCV], red blood cells [RBCs], mean-corpuscular hemoglobin [MCH], mean cell volume [MCV], and mean corpuscular hemoglobin concentration [MCHC]; leucocyte indices (white blood cells [WBCs] and its differentials; and thrombocytic indices (platelets [PLTs]). These were estimated using an automated hematologic analyzer (Sysmex, KX-21, Japan).

### 2.9. Analysis of serum biochemical parameters

Standard experimental protocols were used for the analysis of serum biochemical parameters: alanine transaminase (ALT) [49], aspartate transaminase (AST) [50], alkaline phosphatase (ALP) [51], total protein [52], bilirubin [53], albumin [54], creatinine [55], and urea [56]. Serum lipid profile levels including high-density lipoprotein (HDL)-cholesterol[57], triglycerides (TGs) [58], and total cholesterol (TC), were measured by colorimetric methods while low-density lipoprotein (LDL)-cholesterol (mg/dl) was computed as [TC − (HDL + very low density lipoprotein [VLDL])] [59]. Serum electrolyte concentrations were analyzed according to a method described by Tietz [60].

### 2.10. Analysis of insulin levels and monoamine oxidase (MAO) activity

Serum and pancreatic insulin levels (μIU/mL) were assayed using insulin ELISA kits (catalog no: IN374S; Calbiotech, El Cajon, CA and an ELISA reader (Sunrise, Tecan, Austria) following the manual's instructions. MAO activity was analyzed using an MAO assay kit. The conversion of 4-dimethylambenzylamine to p-dimethylaminobenzaldehyde by MAO was monitored at a 355-nm wavelength.

### 2.11. Analysis of antioxidant parameters

The method described by Misra [48] was used to estimate activities of superoxide dismutase (SOD). Briefly, to 0.2 mL of a sample, 2.5 mL of 0.05 mol/L of carbonate buffer (pH 10.2) was added. The reaction was initiated by the addition of freshly prepared 0.3 mmol/L epinephrine. The absorbance was read at 480 nm, and changes in the absorbance were recorded every 30 s for 150 s to estimate SOD activity. Catalase (CAT) activity was estimated as described by Sinha [61]. To 0.1 mL of

serum or tissue supernatant, 1 mL of 0.01 M phosphate buffer at pH 7.0 and 0.4 mL of a 0.2 M $H_2O_2$ solution were added. The resulting solution was gently mixed, and the reaction was terminated by the addition of 2 mL dichromate acetic acid reagent. Reduced glutathione (GSH) levels were determined by a modified colorimetric protocol [62], while the lipid peroxidation biomarker, malondialdehyde (MDA), was assayed by thiobarbituric acid-reactive substance (TBARS) estimation [63].

### 2.12. Analysis of inflammatory biomarkers (cyclooxygenase-2 [COX-2]/ nitric oxide [NOx]/nuclear factor [NF]-κB)

A rat PTGS2 (prostaglandin endoperoxide synthase 2)/COX-2 ELISA kit (catalog no: E-EL-R0792) was used for the analysis of COX-2 activities. NF-κB activities were estimated using a rat NFKB-p105 (p105 subunit) ELISA kit (catalog no: E-EL-R0673) which was based on color development when rat NFKB-p105 is conjugated with a rat NFKB-p105-specific biotinylated detection antibody and avidin-horseradish peroxidase (HRP) conjugate. The ability of NOx to reduce nitrate to nitrite was used to estimate NOx levels as described by Miranda et al. [64].

### 2.13. Analysis of cholinesterase (CHE) and neurotransmitters (serotonin and dopamine)

Activities of acetylcholinesterase (ACHE) and butyrylcholinesterase (BChE) were determined according to the method of Ellman et al. [65]. A reaction mixture containing phosphate buffer (0.1 M, pH 8.0), DTNB (10 mM), 50 μL cytosol, and 150 mM of acetylthiocholine iodide (for the ACHE assay) or 150 mM of butyrylthiocholine iodide (for the BChE assay) was incubated, and changes in the absorbance were monitored at 412 nm for 3 min. A serotonin assay was conducted using an ST/5-HT (serotonin/5-hydroxytryptamine) ELISA kit (catalog no: E-EL-0033, Elabscience, USA), while the dopamine assay was conducted using a dopamine ELISA kit (catalog no: E-EL-0046) according to the manufacturer's protocols.

### 2.14. Data analysis

All analyses were conducted using GraphPad prism 9 software. Results are expressed as the mean ± standard error of the mean (SEM) of replicate determinations. Data were analyzed using a one-way analysis of variance (ANOVA) and Student's *t*-test, and differences were considered statistically significant at a level of at least $p < 0.05$: * $p < 0.05$, * * $p < 0.01$, * ** $p < 0.001$, * ** * $p < 0.0001$.

## 3. Results and discussion

### 3.1. In vitro studies revealed the antioxidant, anti-inflammatory and hypoglycemic potentials of the PAme

The maintenance of normal blood glucose levels is key to controlling DM and its complications. The in vitro hypoglycemic activities of the PAme revealed dose-dependent increases in glucose uptake by yeast cells (Fig. 1) and inhibition of α-amylase (50% inhibitory concentration ($IC_{50}$) = **217.19** μg/mL). α-Amylase and α-glucosidase are essential for the metabolism of poly/oligosaccharides into monomers, thus increasing postprandial blood glucose levels which can induce the non-enzymatic glycosylation of various proteins, resulting in the development of chronic hyperglycemia-induced complications [66]. Therefore, inhibition of these enzymes is vital to controlling hyperglycemia, and several conventional inhibitors have been developed [67,68]. However, limitations of those conventional α-amylase and α-glucosidase inhibitors call for alternative inhibitors. The inhibitory effects of PAme extract on α-Amylase activities suggested it potential to slow down digestion of carbohydrate and absorption of glucose, thereby reducing postprandial glucose transport and attenuation of hyperglycemia. Accordingly, there was increased glucose uptake by yeast cells indicating the extract's potential mobilization of glucose into cells. This is in line with a previous hypoglycemic study [77].

Antioxidant activity is an important property of plant extracts and products [69]. Three different models of in vitro antioxidant assays were performed to investigate the potential of the PAme. Our analysis revealed that the PAme produced increased DPPH inhibition ($IC_{50}$

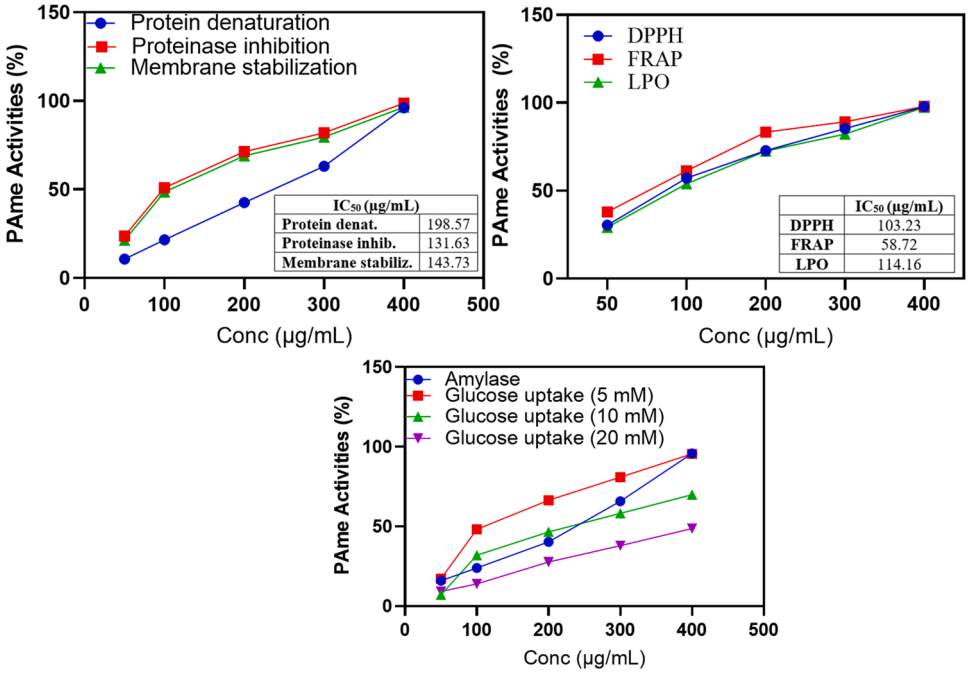


**Fig. 1. :** *In vitro* hypoglycemic, antioxidant, and anti-inflammatory activities of the *Pulmeria alba* methanolic extract. Values are presented as mean ± standard error of mean (SEM) of three replicates. DPPH, 2,2-diphyl-1-picrylhydrazyl; FRAP, ferric-reducing activity of plasma; LPO, lipid peroxidase; $IC_{50}$, 50% inhibitory concentration.

=103.23 μg/mL). DPPH is an unstable synthetic free radical that can be converted into a stable and harmless hydrazine derivative by accepting an electron or a hydrogen atom from an antioxidant molecule [70]. Thus, the DPPH scavenging effects of PAme suggest its ability to serve as an electron donor, thus alleviating the DPPH radical and preventing oxidative injury [71].

The reducing power assay indicated an ability to donate an electron and reduce oxidized intermediates of the lipid peroxidation (LPO) process [72]. The extract also demonstrated ferric-reducing activity of plasma (FRAP) ($IC_{50}$ =58.72 μg/mL) which suggests conversion of the $Fe^{3+}$ complex to $Fe^{2+}$. Inflammation triggers secondary damage via free radical-induced LPO [73]. Our analysis revealed dose-dependent increases in the inhibition of LPO ($IC_{50}$ =114.16 μg/mL) by the PAme, thus confirming its potential antioxidant effect.

Lysosomal membrane stabilization is vital to avoiding inflammatory events by preventing the release of lysosomal constituents of activated neutrophils, such as proteases and bacterial enzymes, which cause tissue inflammation and damage upon extracellular release [74]. Our data revealed that the PAme demonstrated HRBC membrane stabilization potential ($IC_{50}$ =143.73 μg/mL). Furthermore, our analysis revealed that the extract not only stabilized lysosomal membranes but also exhibited significant proteinase inhibitory activities (($IC_{50}$ =131.63 μg/mL) which further supported the anti-inflammatory properties of the extract [75]. Our analysis also revealed dose-dependent increases in inhibition of protein denaturation ($IC_{50}$ =198.57 μg/mL) by the PAme, thus confirming its potential anti-inflammatory effect.

### 3.2. PAme reversed STZ-induced hyperglycemia and attenuated insulin deficiency

Our preliminary safety analysis revealed that PAme exhibited high safety profile with MTD of 2800 mg/kg bw, and no death or deterioration in health were recorded throughout the study period (Table S1). An in vivo analysis was carried out to further investigate the antidiabetic effects of the PAme. Upon the establishment of diabetes by STZ induction, there was evident weight loss in the untreated groups which could be attributed to protein mobilization from muscles as well as utilization of stored fat as energy sources [7], whereas the PAme-treated groups exhibited significant attenuation of high blood glucose in a dose-dependent fashion (Fig. 2**a, b**) and at the same time improved the BW of rats (Fig. 2**c**). Thus, making glucose available for utilization by cells could further induce lipogenesis for building body fat. Reductions in weight as a result of diabetic conditions are well documented in experimental and clinical settings [76,77]. The improvement in animals body weight and attenuation of the FBS levels after treatment with PAme provides scientific support for the uses of the plant extract in traditional management of DM and its complications [78]. Flavonoid and phenolic compounds have been previously attributed to enhance glucose uptake [79], scavenge free radicals [80], and mediate pancreatic insulin production and secretion [81,82], therefore the therapeutic

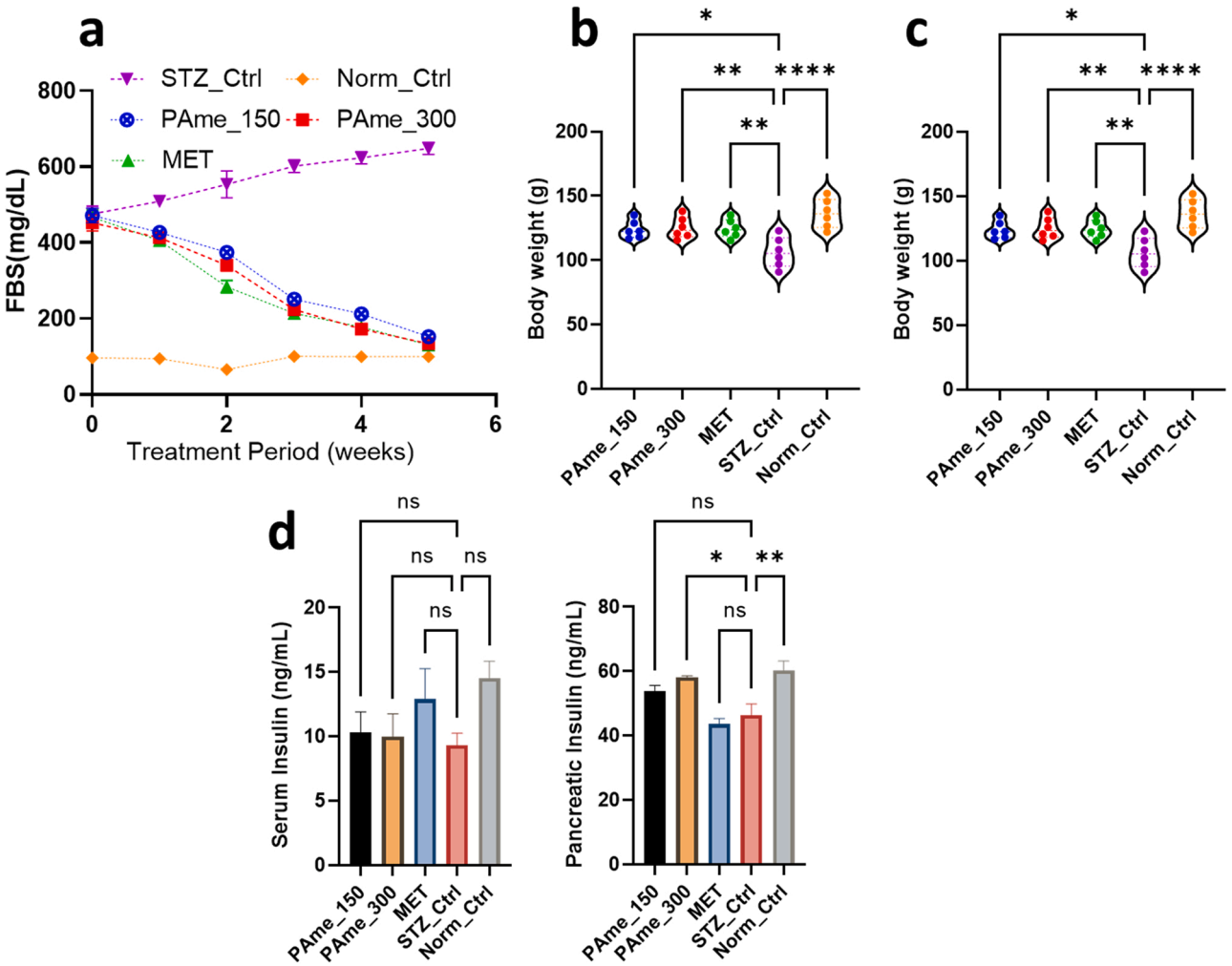


**Fig. 2. :** *Pulmeria alba* methanolic extract (PAme) reversed STZ-induced hyperglycemia and attenuated insulin deficiency. Fasting blood sugar (FBS). (A) Line graph of FBS vs. time (B) Violin plot of mean FBS across the treatment groups. (C) Violin plots showing mean body weight vs. experimental groups. (D) Bar graph of serum and pancreatic insulin levels in rats with STZ-induced diabetes treated with the PAme. ns, non-significant; * $p < 0.05$, * * $p < 0.01$, * ** $p < 0.001$, * ** * $p < 0.0001$.

efficacy demonstrated by PAam could be attributed to its high phenol and flavonoid contents. Insulin is produced by β-cells of the islet of Langerhans, and it regulates the homeostasis of glucose by stimulating glucose uptake by muscles and adipose cells, while decreasing hepatic glucose production via glycogenolysis and gluconeogenesis [83]. STZ administration compromises the integrity of β-cells of the islet of Langerhans, leading to impaired insulin secretion and activities [84]. This is consistent with the decreases in pancreatic insulin levels observed in STZ-treated control rats. Interestingly, the hypoglycemic effect of the PAme at 300 mg/kg BW was concomitantly associated with a significant ($p < 0.05$) increase in pancreatic insulin secretion compared to rats with STZ-induced diabetes (Fig. 2**D**). The ability of the extract to increase insulin levels of the diabetic rats could be a reflection to its potential for preventing loss of β-cells or the ability to enhance insulin secretion by residual β-cells [85].

### *3.3. PAme attenuated neuronal oxidative stress and inflammation and neuro-cognitive-associated markers*

Oxidative stress and inflammation are widely recognized as intimately associated pathological mechanisms of diabetes-induced complications [7,8]. Since both redox signaling and oxidative stress lead to the release of proinflammatory mediators, the process of "ROS-induced inflammation" has been implicated in the progression of several chronic diseases including diabetes [86].

Antioxidant enzymes, including SOD, CAT, and GSH, protect tissue cells from oxidative damage by degrading free radicals ($H_2O_2$ and superoxide anions) and preventing MDA production, while GSH combats xenobiotics [87,88]. In this study, levels of enzymatic (SOD and CAT) but not non-enzymatic antioxidants (GSH) were significantly reduced in the brains of STZ-treated control rats compared to normal control rats. Interestingly, results obtained from the treatment group implied that the PAme significantly attenuated SOD, CAT, and GSH activities, and improved the antioxidant status of rats with STZ-induced diabetes, suggesting that the PAme possesses antioxidative properties capable of protecting tissues of rats with STZ-induced diabetes from oxidative stress. An increased MDA concentration (Fig. 3**A**) was observed in STZ-treated control rats compared to normal control rats. During diabetes, oxidative stress arises from deficient antioxidant defense mechanisms and increased production of free radicals, LPO, and the formation of MDA, which lead to membrane and cellular damage [89].

Proinflammatory markers such as COX, NF-κB, and NOx are activated during tissue damage or elevated glucose [90]. In agreement with previous studies, increased levels of inflammatory markers (COX2, NF-κB and NOx; Fig. 3**B**) as well as increased AChE activities (Fig. 3**C**) were observed in STZ-treated control rats compared to normal control

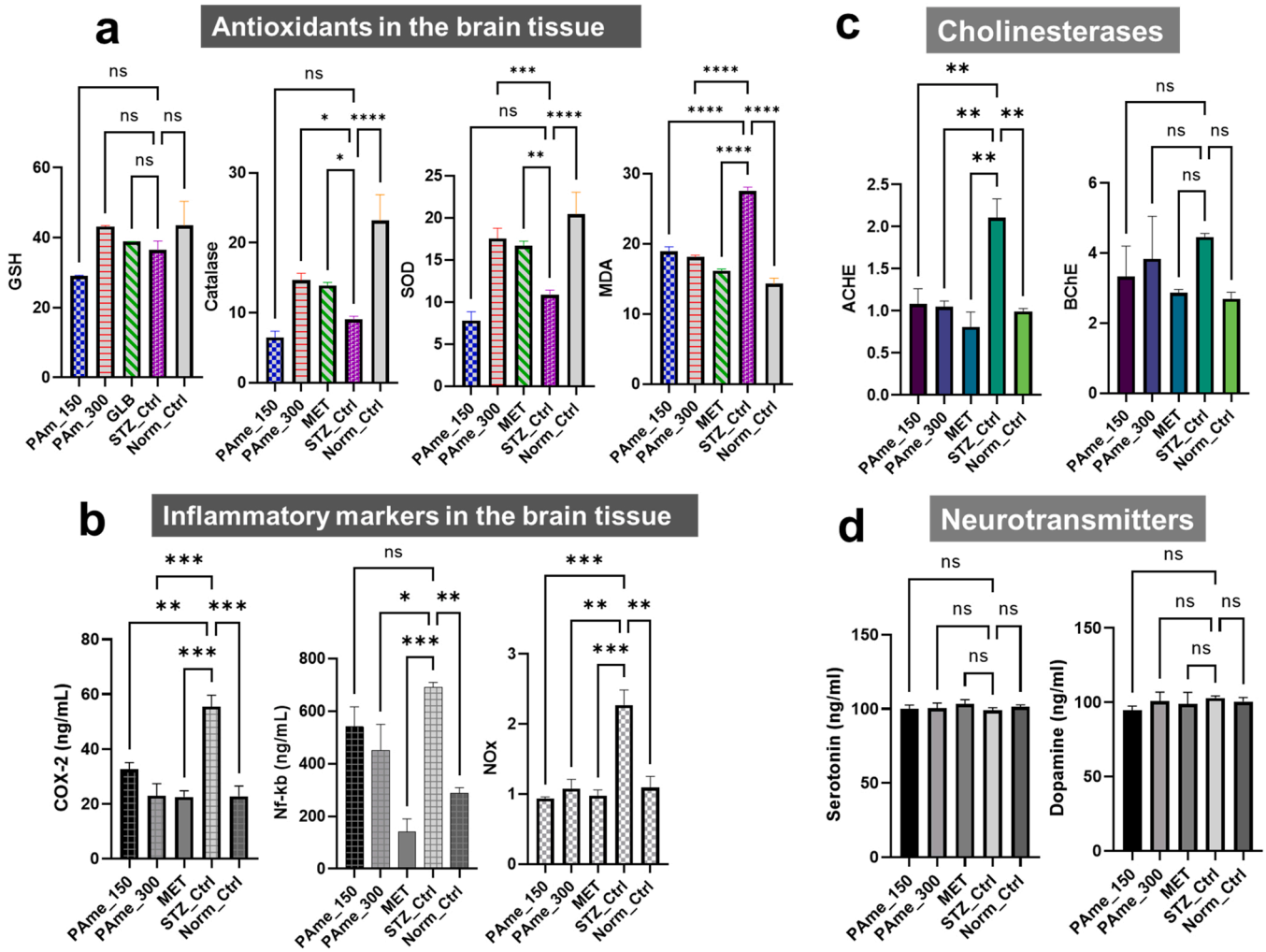


**Fig. 3. : The** *Pulmeria alba* methanolic extract (PAme) attenuates neuronal oxidative stress and inflammation, and neuro-cognitive associated markers. (A) Bar graph of (A) antioxidant, (B) cholinesterase, (C) inflammatory marker, and (C) neurotransmitter activities levels in rats with STZ-induced diabetes treated with the PAme. Levels of superoxide dismutase (SOD), catalase (CAT), reduced glutathione (GSH), malondialdehyde (MDA), acetylcholinesterase (AChE), butyrylcholinesterase (BChE), nuclear factor (NF)-κB, cyclooxygenase (COX)– 2, and nitrogen oxide (NOx), metformin (MET). Bars are the mean ± SEM of five determinations. ns, non-significant; * $p < 0.05$, * * $p < 0.01$, * ** $p < 0.001$, * ** * $p < 0.0001$.

rats. Insulin in the brain protects neurons and improves the production of the important neurotransmitter, acetylcholine (ACh), which is necessary for neurological development [91]. Furthermore, diabetes also induces the upregulation of AChE activity, leading to increased ACh degradation and consequently leading to cognitive defects and other brain abnormalities [92]. Therefore, an insulin deficiency and dysregulation of glucose levels in the brain induce negative impacts on the brain's structure and function [93], and have been implicated in the increased incidence of cognitive/neuronal dysfunction, a diabetes complication known as diabetes encephalopathy [94]. Interestingly, an oral dosage of the PAme at 300 mg/kg BW produced a significant reversal effect ($p \leq 0.01$) on antioxidant, inflammatory, and AChE activities in treated rats. At a dose of 150 mg/kg BW, the extract demonstrated no significant attenuation effects on antioxidant enzymes or NF-κB activities.

Dopamine and serotonin are endogenous monoamine neurotransmitters known to modulate cognitive function, mood, motivation, sexual arousal, satisfaction, etc. in the central nervous system [95,96]. The levels of neurotransmitters analyzed (serotonin and dopamine) were not significantly ($p > 0.05$) altered by STZ administration compared to normal control rats (Fig. 3**D**). Collectively, these findings suggest that the PAme not only regulates insulin secretion and blood glucose levels, but also alleviates cognitive deformation and other complications associated with diabetes [97].

### *3.4. The PAme attenuated STZ-induced dyslipidemia and alterations in biochemical markers of hepatorenal dysfunction*

Analyzing these biochemical markers is a useful strategy for evaluating the functionality of organs in a diabetic condition and for treatment follow-up. STZ administration caused significant alterations in serum levels of AST, ALT, ALP, TP, albumin (Fig. 4**a**), urea, and creatine (Fig. 4**c**), but produced no alterations in levels of serum electrolytes including sodium, potassium, and bicarbonate (Fig. 4**b**) compared to levels in normal control rats. Diabetes is frequently associated with abnormal glucose and glycogen metabolism by the liver and kidneys leading to diabetic nephropathies and hepatocellular injury [98]. When the functional integrity of the liver and kidneys is compromised, levels and activities of the tissues' functional index markers, such as ALP, ALT, AST, blood urea nitrogen (BUN), albumin, urea, creatinine, and electrolytes becomes elevated in the serum due to their unregulated in-flow from the organs [99–101]. Hence, the significant attenuation of biochemical alterations in the serum of rats treated with 300 mg/kg BW of the PAme suggested restoration of the functional integrity of the liver and kidneys. Diabetic dyslipidemia, characterized by high levels of cholesterol, TGs, and LDL, contributes to the increased production of

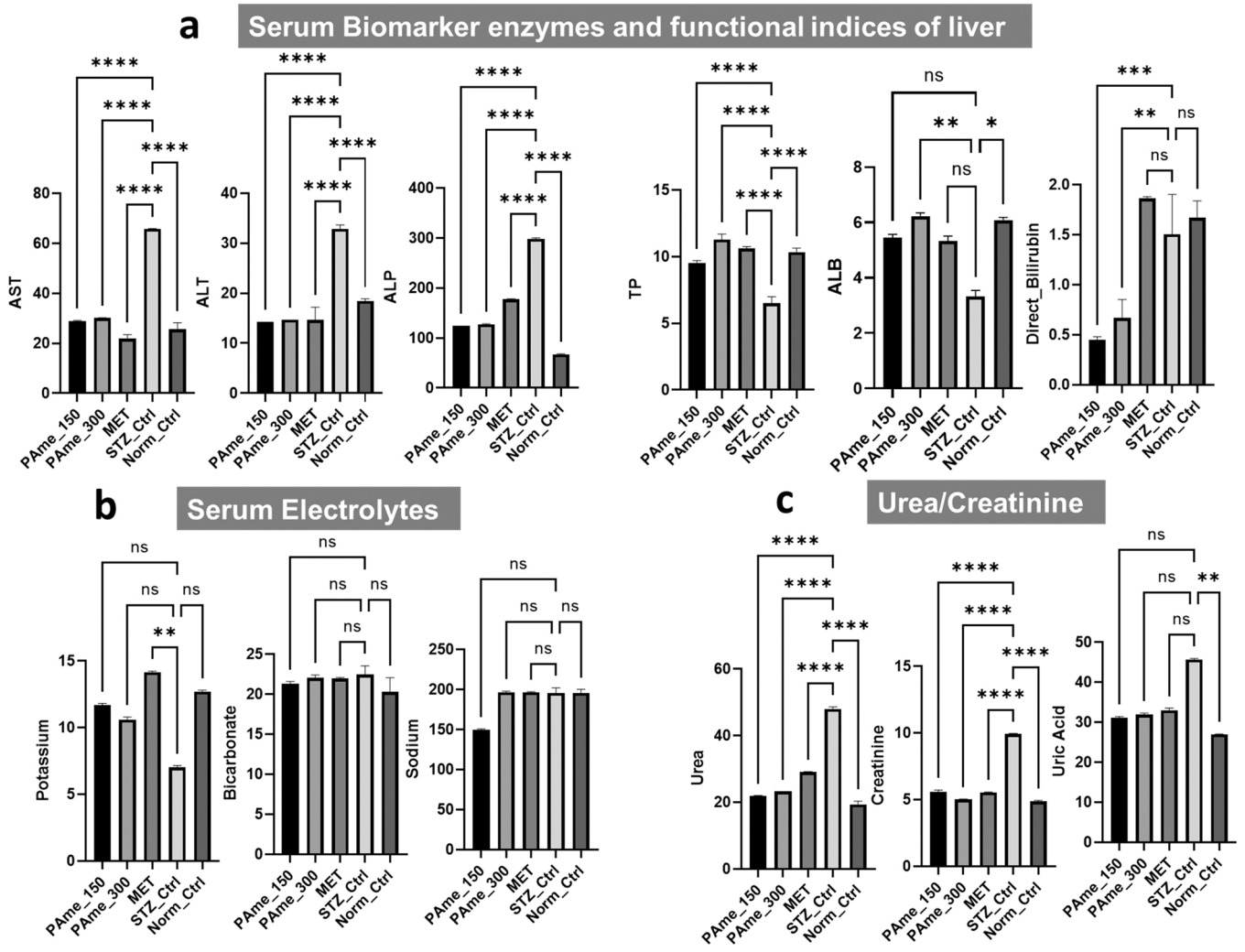


**Fig. 4. :** The *Pulmeria alba* methanolic extract (PAme) attenuated STZ-induced alterations in biochemical markers of hepatorenal dysfunction. (A) Serum biomarker enzyme and functional indices of the liver and (B) serum electrolytes, and (C) urea/creatinine levels in rats with STZ-induced diabetes treated with the PAme. Aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), and albumin (ALB), metformin (MET). Bars are the mean ± SEM of five determinations. ns, non-significant; * $p < 0.05$, * * $p < 0.01$, * ** $p < 0.001$, * ** * $p < 0.0001$.

ROS, activates cascades of inflammatory events, induces IR, and accelerates vascular diseases in diabetic patients [102]. Furthermore, STZ-treated control rats exhibited a dyslipidemic status as evidenced by elevated levels of cholesterol and LDL-C, and decreased HDL-C compared to the normal controls. PAme treatment at 300 mg/kg BW restored these parameters. However, at 150 mg/kg BW, the extract could not attenuate the elevated level of TGs (Fig. 5**).**

Among the hematological indices analyzed, only HGB and RBC counts were significantly ($p < 0.05$) reduced in STZ-treated control rats compared to the normal controls. These alterations were reversed only in the group of rats treated with 300 mg/kg BW of the PAme **(**Fig. 6**),** suggesting that the extract prevented erythrocytic damage or enhanced the production of RBCs in rats with STZ-induced diabetes.

### *3.5. PAme attenuated histopathological distortion of the brain and pancreas in STZ-induced diabetic rats*

A photomicrograph of pancreatic tissue sections of the control rats show normal architecture of pancreatic cells, with prominent Islets of Langerhans and β cells. Contrarily, the diabetic control rats show a severe pancreatic degeneration and destruction of β cells. However, the rats treated with PAM extract showed significant improvement in histological architecture of the pancreas and demonstrated a near-normal histological appearance of the pancreas **(**Fig. 7**).** Histological evaluation of the brain in the normal rats shows normal brain tissue composed of preserved neuronal bodies surrounded by fibrillary glial matrix. The hippocampus shows preserved molecular and granular layers in all its regions. There are no features of degenerative changes or significant inflammation. However, the diabetic control rats show obvious signs of brain tissue distortion, compared with the normal rats. The degree of injury and were significantly alleviated after administering PAme at 150, and 300 mg/kg bw. This finding demonstrated an important attenuative effect of PAme on brain tissue damage in diabetes mellitus **(**Figs 8 and 9**).**

### *3.6. LC-MS characterization identified the PAme as being enriched in apigetrin*

The LC-MS characterization of the PAme was carried out to identify its bioactive compounds (Fig. 6). The peaks generated from LCMS was analyzed using mzmine software for mass detection. The parameter set includes a mass-to-charge tolerance of 0.02 Da or 5.0 ppm, Rt tolerance of 0.01 min and a maximum charge of 2. By comparing fragments of the peaks with those on online database, including PubChem, mass bank, and Kegg, compounds were tentatively identified [103]. LC-MS identified apigetrin (retention time (RT): 21,227 s, **30.48%,** *m/z*: 433.15) as the major bioactive compound in the PAme. Three other compounds, including vanillic acid 4-β-D-glucoside (RT: 17,511 s, 21.11%, *m/z*: 331.1), N-propyl-16,16-dmethyl-5z,8z,14z-docosatetraenoyla amine (RT: 20,202 s, **18.16%,** *m/z*: 403.1), and cinnamic acid (RT: 16,273 s, **17.86%,** *m/z*: 147.15), were found in appreciable amounts. Other compounds identified in minute amounts included protoplumericin a, stigmast-7-enol, iridoid, and protocatechuic acid 4-glucoside (Table 1). Some of the compounds identified have been previously reported in other species of the genus Pulmeria [104,105]. The identification of these major compounds is consistent with the activities demonstrated by the extract; for instance, cinnamic acid was reported to exert antidiabetic activity by stimulating insulin secretion and improving glucose tolerance [106] and diabetes-associated complications [107]. Apigetrin was reported to inhibit neuro-inflammation [108], and ameliorate STZ-induced pancreatic β-cell damage via attenuating endoplasmic reticular stress [109]. Altogether, the present study provides preclinical evidence of the therapeutic potential of the apigetrin-enriched PAme for treating oxidative stress and neuro-inflammation associated with diabetes.

### *3.7. In silico simulation of ligand-receptor interactions reveals the potential of apigetrin for targeting AChE/COX-2/NOX/NF-κB*

These bioactive compounds are hence adduced to the displayed effect which provides further insights into the plant's potential to be developed as a drug candidate against diabetes. Consequently, we provide insights into the potential of apigetrin for targeting AChE/COX-2/

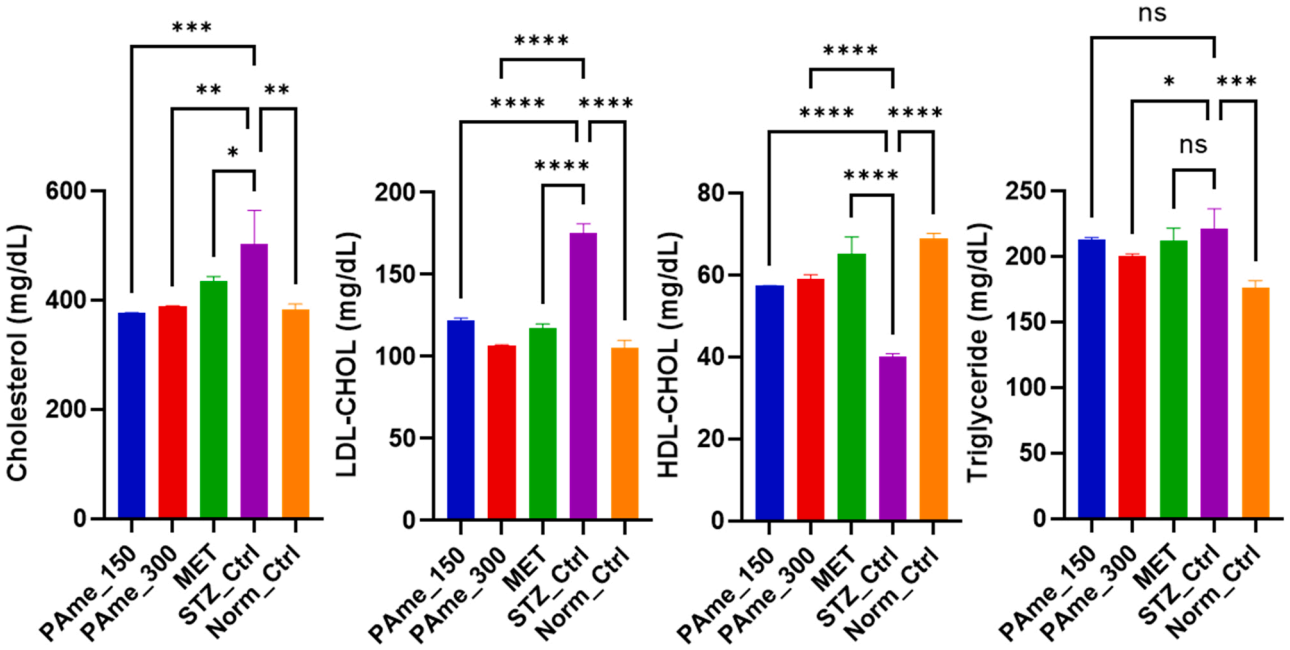


**Fig. 5. :** The *Pulmeria alba* methanolic extract (PAme) attenuated STZ-induced dyslipidemia. Low-density lipoprotein (LDL)-cholesterol, high-density lipoprotein (HDL)-cholesterol, metformin (MET). Bars are the mean ± SEM of five determinations. ns, non-significant; * $p < 0.05$, * * $p < 0.01$, * ** $p < 0.001$, * *** * $p < 0.0001$.

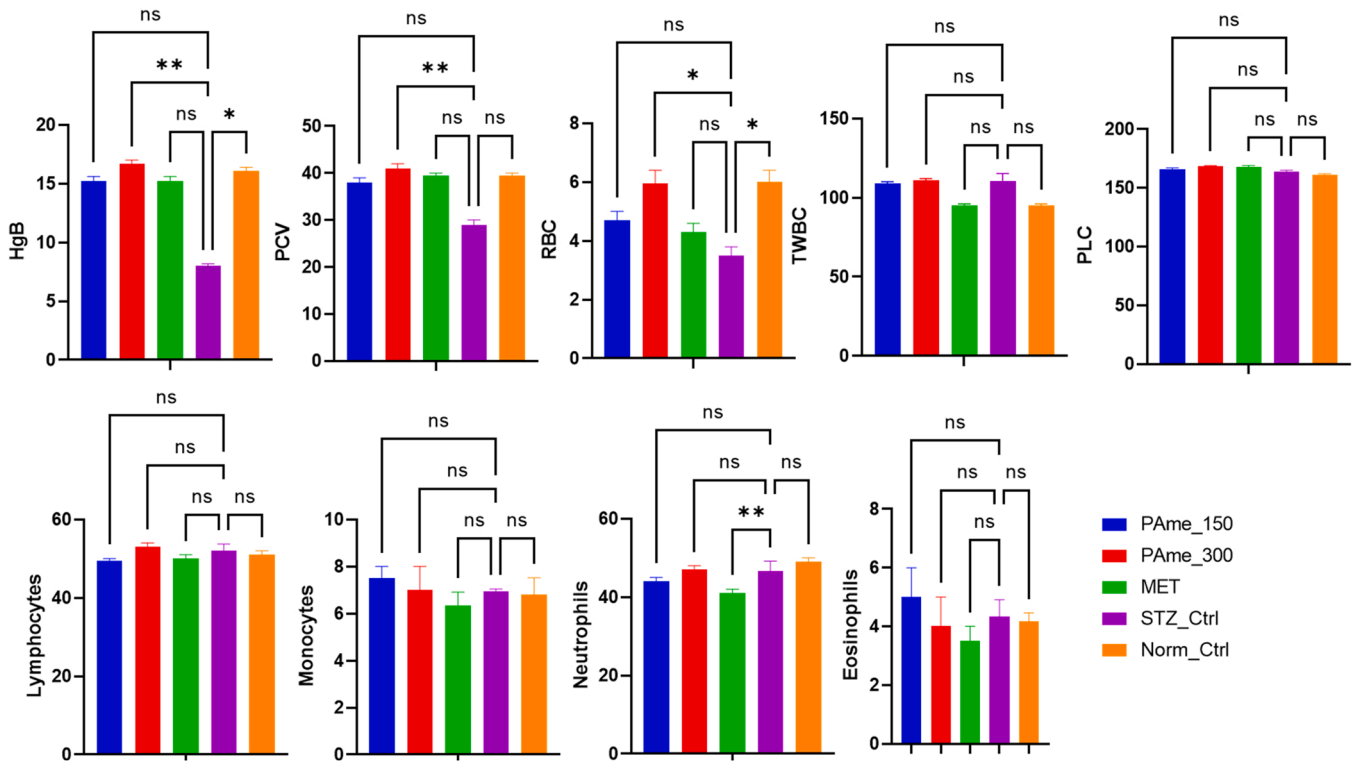


**Fig. 6. :** The *Pulmeria alba* methanolic extract (PAme) attenuated STZ-induced hematological alterations in hemoglobin (HGB), packed cell volume (PCV), mean cell hemoglobin (MCH), red blood cells (RBCs), the total white blood count (TWBC), neutrophils (N), platelets (P), eosinophils (E), and monocytes (M), metformin (MET). Bars are the mean ± SEM of five determinations. ns, non-significant; * $p < 0.05$, * * $p < 0.01$, * ** $p < 0.001$, * ** * $p < 0.0001$.

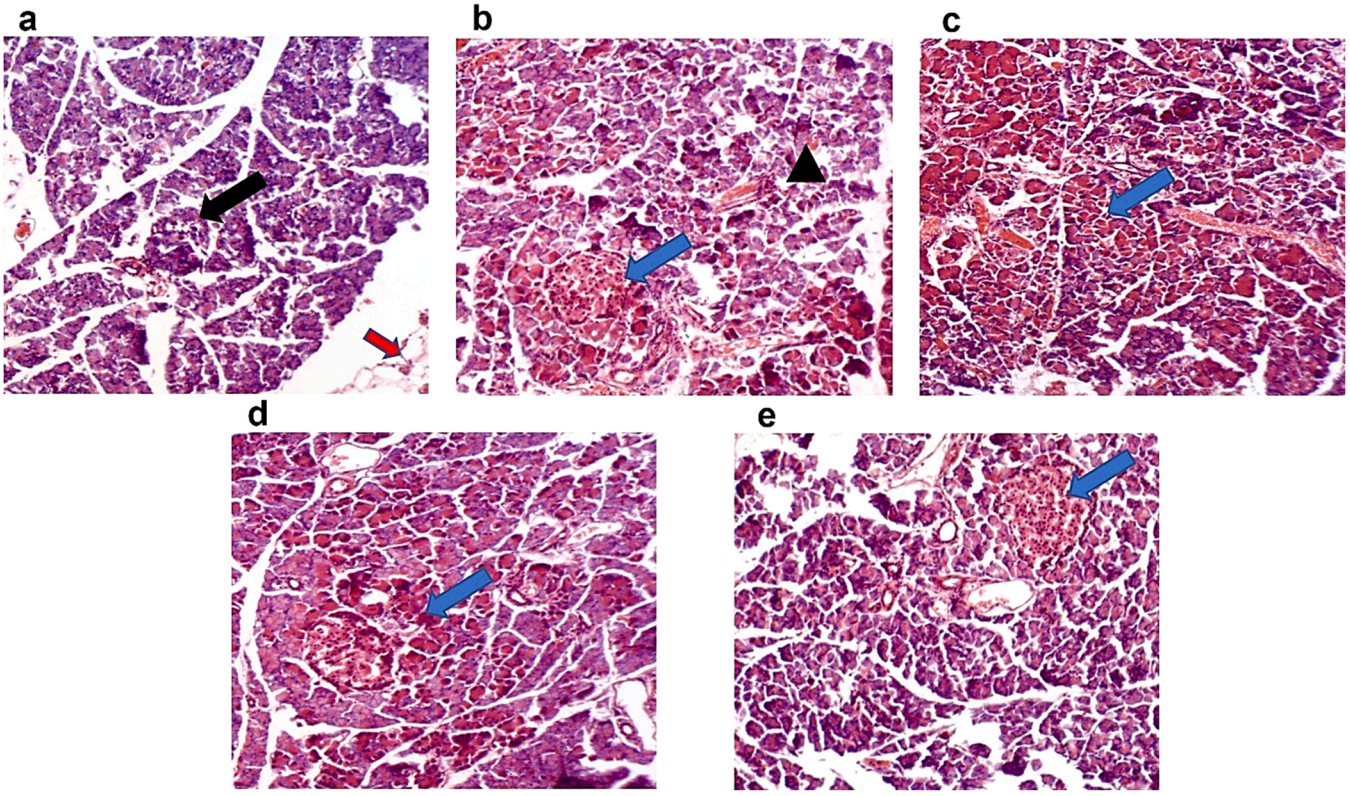


**Fig. 7. :** Effects of PAM on histopathology of the pancreas in streptozotocin induced diabetic rats: (A) Negative, (B) Normal, (C) metformin, (D) PAM_150 mg/kg, (E) PAM_300 mg/kg. Blue arrow; islets β-cells, black arrow; β cells degeneration, red arrow; pancreatic fat. Black arrowhead; interlobular septum.

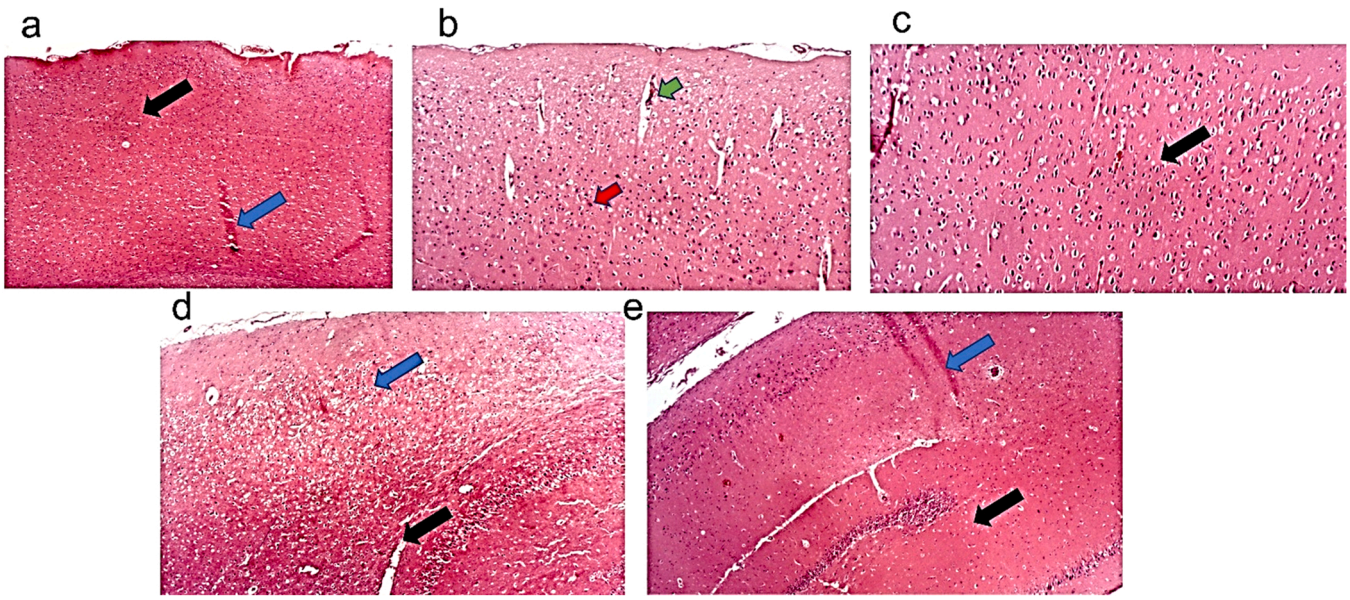


**Fig. 8. :** Effects of PAM on histopathology of the brain in streptozotocin induced diabetic rats: (A) Normal, (B) Negative, (C) metformin, (D) PAM_150 mg/kg, (E) PAM_300 mg/kg. Blue arrow; granular cell layer, black arrow; molecular layer, red arrow; inflammatory cells, green arrow; PCE pericellular edema. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

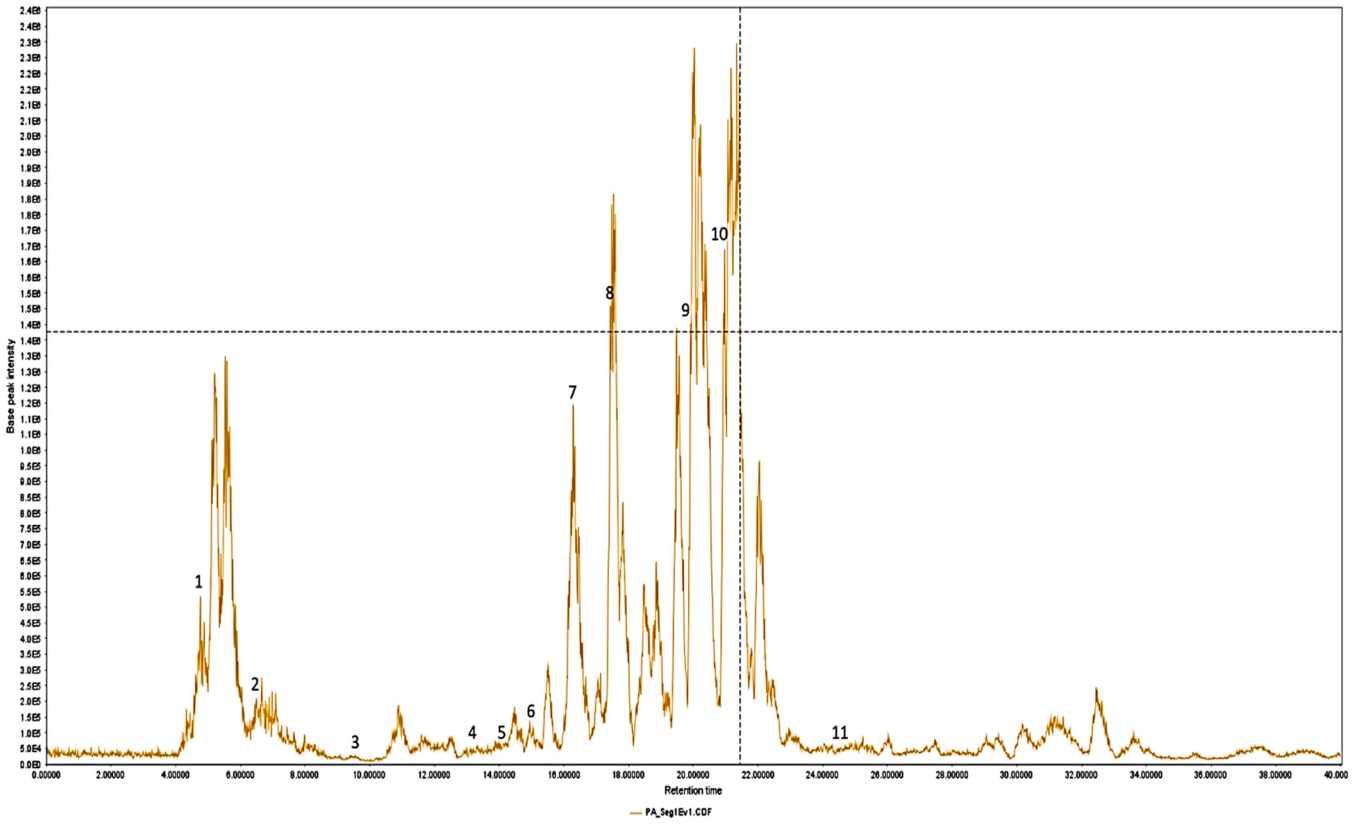


**Fig. 9. :** LC-MS chromatogram of the crude methanolic extract of *Pulmeria alba.*

NOX/NF-κB in diabetes based on an *in silico* molecular docking analysis (Figs. 10–13**)**. Molecular docking is a simulation of binding affinities and interactions between a drug candidate and a protein target [110,111]. Our molecular docking analysis revealed that apigetrin docked well to the binding cavities of AChE/COX-2/NOX/NF-κB. In particular, apigetrin bound most efficiently to AChE with the strongest binding affinity of − 10.40 kcal/mol. High binding affinities of − 9.50 kcal/mol (BChE_Apigetrin), − 10.0 kcal/mol (COX-2_Apigetrin), and − 10.0 kcal/mol (NOx_Apigetrin) complexes were observed. The complexes were established by several hydrogen bonds, pi-interactions, hydrophobic contacts, and van der Waals forces.

**Table 1**
LC-MS-based identification of compounds from the methanolic extract of *Pulmeria alba*.

| Peak# | Retention time (s) | Area | Area % | Height | Base Peak *m/z* | Compound name | Fragments |
|---|---|---|---|---|---|---|---|
| 1 | 4445 | 2830,431 | 3.192512377 | 534,909 | 104.3 | Valeric acid | 104, 116, 131 |
| 2 | 6533 | 1097,538 | 1.237939964 | 180,082 | 303.1 | Benzoaric acid | 302, 303 |
| 3 | 9103 | 55,357 | 0.062438515 | 35,871 | 778.7 | Protoplumericin A | 778 |
| 4 | 13,316 | 112393 | 0.126770815 | 45122 | 414.2 | Stigmast-7-enol | 414 |
| 5 | 14,034 | 35,631 | 0.040189077 | 67,062 | 457.25 | Iridoid | 457 |
| 6 | 15,543 | 508,516 | 0.573567638 | 140,846 | 317.1 | Protocatechuic acid 4-glucoside | 317 |
| 7 | 16,273 | 158,30,681 | 17.85581243 | 1258,971 | 147.15 | Cinnamic acid | 147, 148 |
| 8 | 17,511 | 18,717,138 | 21.1115179 | 1581,121 | 331.1 | Vanillic acid 4-β-D-glucoside | 331 |
| 9 | 20,202 | 16,101,524 | 18.16130288 | 1485,742 | 403.1 | N-propyl-16,16-dmethyl-5z,8z,14z-docosatetraenoyla amine | 345, 403 |
| 10 | 21,227 | 27,019,375 | 30.47581415 | 1651,011 | 433.15 | Apigetrin | 117, 434 |
| 11 | 24,672 | 349,835 | 0.394587456 | 37,388 | 617.5 | Plumieride (*E*)-P-coumarate | 617 |

*m/z*, molecular weight.

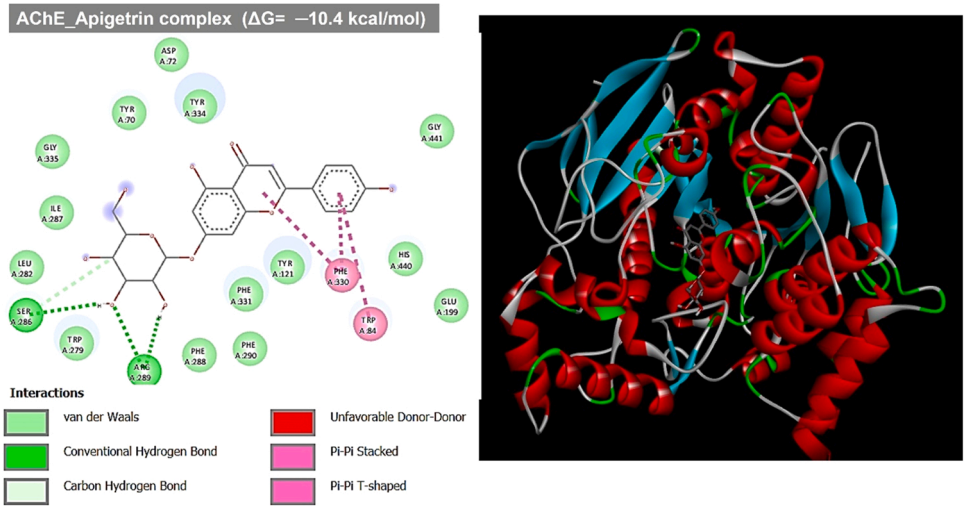


**Fig. 10.** Two- (2D) and three-dimensional (3D) structures of apigetrin in complex with acetyl-cholinesterase.

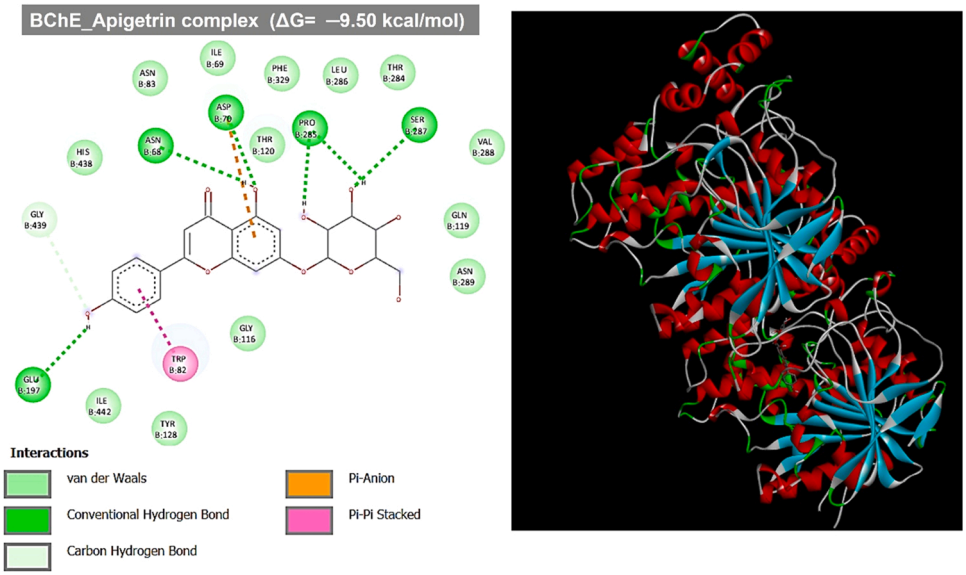


**Fig. 11.** Two- (2D) and three-dimensional (3D) structures of apigetrin in complex with butyl-cholinesterase.

## 4. Conclusions

We provide preclinical evidence of the therapeutic potential of the PAme against oxidative stress and inflammatory events associated with diabetes. Our findings also revealed that the PAme is a rich source of useful antioxidant compounds, such as apigetrin and cinnamic acid. The bioactive compounds are hence adduced to the displayed effect which provides further insights into the plant's potential for development as a drug candidate against diabetes. Consequently, we provide insights into the potential of apigetrin for targeting AChE/COX-2/NOX/NF-κB in

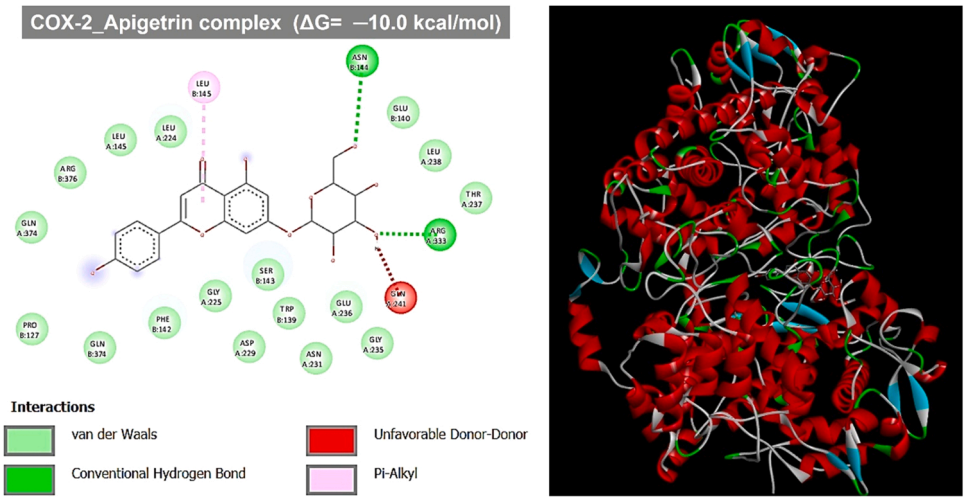


**Fig. 12.** Two- (2D) and three-dimensional (3D) structures of apigetrin in complex with cycloxygenase-2.

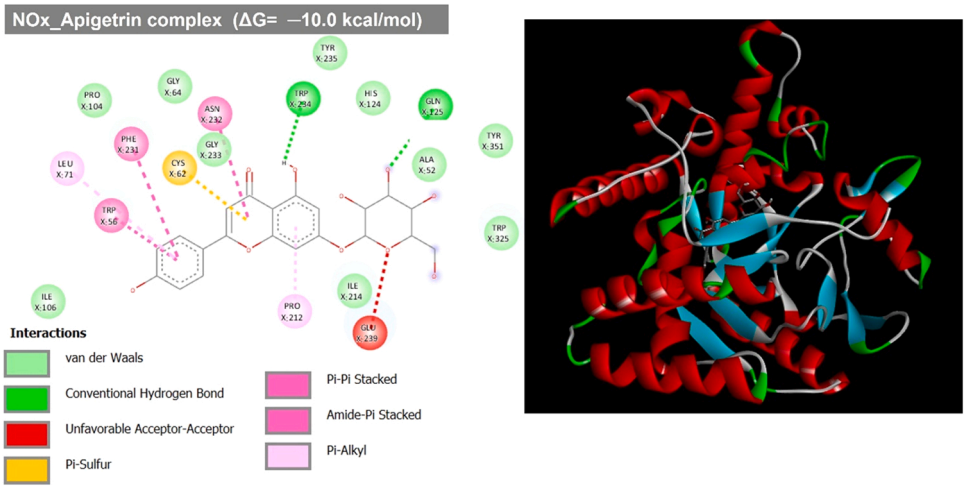


**Fig. 13.** Two- (2D) and three-dimensional (3D) structures of apigetrin in complex with nitric oxide synthase.

diabetes.

## Author contributions

All authors read and approved the final version of the manuscript.

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## CRediT authorship contribution statement

**Tawakaltu Abdulrasheed-Adeleke:** Conceptualization, Methodology, Data analysis, Software, Investigation, visualization, Writing – original draft preparation, **Bashir Lawal:** Conceptualization, Methodology, Data analysis, Software, Investigation, Visualization, Writing – original draft preparation, **Yu-Cheng Kuo:** Conceptualization, Methodology, Data analysis, Software, Investigation, Writing – original draft preparation, **Amos Sunday Onikanni:** Conceptualization, Methodology, Data analysis, Software, Investigation, Writing – original draft preparation, **Eyuwa Ignatius Agwupuye:** Conceptualization, Funding, Methodology, Data analysis, Software, Investigation, Writing – original draft preparation, **Amarachi Mary Eni:** Data curation, Investigation, Software, Validation, Writing – reviewing and editing, **Okwukwe Faith Ekoh:** Data analysis, Software, Writing – reviewing and editing, Supervision, Funding, **Yunusa Olatunji Ibrahim:** Conceptualization, Methodology, Data analysis, Software, Investigation, Writing – original draft preparation, **Gaber El-Saber Batiha:** Conceptualization, Supervision, Writing – reviewing and editing, Software, Funding, **Hsu-Shan Huang:** Conceptualization, Methodology, Data analysis, Software, Funding, Writing – original draft preparation, **Halimat Yusuf Lukman:** Conceptualization, Methodology, Data analysis, Software, Investigation, Writing – original draft preparation, **Femi Olawale:** Conceptualization, Funding, Methodology, Data analysis, Software, Investigation, Writing – original draft preparation, **Maliha Abdullah Saleh Al Ghamdi:** Data curation, Investigation, Software, Validation, Writing – reviewing and editing, **Sarah S. Aggad:** Conceptualization, Methodology, Data analysis, Software, Investigation, Writing – original draft preparation, **Abdulrahman A. Alsayegh:** Conceptualization, Funding, Methodology, Data analysis, Software, Investigation, Writing – original draft

preparation, **Alexander T.H Wu:** Data curation, Investigation, Software, Validation, Supervision, Funding, Writing – reviewing and editing. **Nada H. Aljarba**: Conceptualization, Methodology, Data analysis, Software, Investigation.

## Conflict of interest statement

The authors declare no conflict of interest.

## Data availability statement

All data can be made available upon reasonable request.

## Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.biopha.2023.114582.

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