

Sterculia tragacantha Lindl Leaf Extract Ameliorates STZ-Induced Diabetes, Oxidative Stress, Inflammation and Neuronal Impairment

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Background: *Sterculia tragacantha* is a medicinal plant commonly used in the western part of Nigeria, for managing diabetes mellitus. However, there is a dearth of scientific information on the antidiabetic and neuroprotective properties of the plant.

Methods: The in silico, in vitro and in vivo models were used to evaluate the antioxidants, antidiabetic, anti-inflammatory and neuroprotective potential of aqueous extract of *Sterculia tragacantha* leaf (AESTL) in streptozotocin (STZ)-induced diabetic rats. Thirty (30) male albino rats (155.34±6.33 g) were intraperitoneal injected with 40 mg/kg of freshly prepared streptozotocin and were divided into 5 groups (A-E) of 6 animals each. Groups A–D were treated with 0, 150 and 300 mg/kg of AESTL, and 200 mg/kg body weight of metformin respectively, while group E serve as the normal control.

Results: The results of in vitro analysis revealed dose-dependent antioxidant activities; ABTS (IC₅₀ = 63.03±2.57 µg/mL), DPPH (117.49±2.35 µg/mL), FRAP (15.19±0.98 mmol/100g), TAC (43.38±0.96 mg/100g), hypoglycaemic effect; α-amylase (IC₅₀ = 77.21±4.35 µg/mL) and α-glucosidase (IC₅₀ = 443.25±12.35), and anti-cholinesterase; AChE (IC₅₀ = 113.07±3.42 µg/mL) and BChE (IC₅₀ = 87.50±4.32 µg/mL) activities of AESTL. In vivo study revealed dose-dependent hypoglycemic effect and body weight improvement in rats treated with the AESTL. In addition, AESTL improved the antioxidant status and attenuated STZ-induced dysregulations of Na⁺-K⁺-ATPase, cholinesterases and neurotransmitters in the brain tissue of experimental rats. The results also demonstrated that AESTL could regulate anti-inflammatory response via inhibition of COX-2/NO signaling axis in the brain of diabetic rats. Molecular docking analysis revealed that epicatechin and procyanidin B2, the bioactive compounds from AESTL, docked well to the binding cavities of acetylcholinesterase, butyrylcholinesterase, α-amylase and α-glucosidase with binding affinities ranges between -8.0 and -11.4 kcal/mol, suggesting that these compounds are the bioactive component that could be responsible for the antidiabetic and neuroprotective activities of AESTL.

Conclusion: The results of the present study strongly suggested that the AESTL extract could be very useful for halting diabetes progression and its associated neuroinflammation complications.

Keywords: *Sterculia tragacantha*, cholinesterase, antidiabetic, neurotransmitters, oxidative stress markers

Introduction

Diabetes is a metabolic disorder characterized by inadequate insulin secretion or abnormality in the action of insulin or both.¹ The most common types of diabetes mellitus (DM) included Type 2 diabetes mellitus (T2DM), type 1 diabetes mellitus

(T1DM) and gestational diabetes mellitus (GDM). According to the current statistic, DM affects more than 463 million of the global population and is projected to be 700 million by 2045.² An increase in its prevalence and enormous social and economic significance together with the severity make DM a global challenge.³

Free radical generation has been implicated in the pathogenesis of DM. High generations of free radicals coupled with depleted levels of endogenous antioxidants result in the impairment of cellular organelles, excessive lipid peroxidation, destruction of pancreatic β -cells, oxidative stress and development of diabetic condition.⁴ The progression of diabetes complications is characterized by the development of certain pathological conditions including nephropathy, retinopathy, and neuropathy.⁵ Early and adequate management of these diseases are critical to the prevention of diabetes-induced pathological impairments of nerve tissues and other vital organs.⁶

The pathogenesis of diabetes-induced brain injury is complex and includes combination of oxidative stress, neuroinflammation, mitochondrial dysfunction, reduction of neurotrophic factors, vascular disease, apoptosis, acetylcholinesterase (AChE) activation, neurotransmitters' changes, and impairment of brain repair processes.⁷ Hyperglycemia generates excess NADH and overloads the electron transport chain, leading to the production of superoxide radicals and subsequent mitochondrial and cytosolic oxidative stress. Defects in metabolic and vascular pathways intersect with oxidative stress to induce inflammation, and onset and progression of neuronal injury.⁸ The association between DM and pathological changes in the central nervous system causes memory loss and affective retardation, which later increase the risk of vascular depletion of the brain.⁹ However, the currently available drugs for the treatment of these conditions merely alleviate the symptoms.¹⁰

Acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) are 2 major forms of cholinesterase implicated in the pathogenesis of neurological impairment, and inhibiting the activities of these enzymes has been accepted as an effective strategy for the treatment/management of neurodegenerative diseases.¹¹ Recently, studies showed that the activity of BChE is significantly higher in degenerative disease than in the plaques of age-related non-demented brains.¹¹ Most of the approved AChE inhibitors do not alter the activity of BChE, which is very critical when managing those degenerative diseases. Therefore, the use of antioxidants to counter the cellular oxidative stress

within the nervous system has been suggested as a potential therapeutic strategy for neurological disorders.¹⁰ The α -glucosidase and α -amylase are important enzymes of the gastrointestinal tract and function to digest complex carbohydrates, thus contributing to postprandial hyperglycemia in T2DM.¹² The inhibition of α -amylase and α -glucosidase activities have been proposed as therapeutic strategy for the control of hyperglycemia.⁶

In developing and some developed countries, medicinal plants are commonly used as source of therapeutic agent to meet the primary health care need, and have received significant scientific validation of their activities against several diseases.^{13–16} The use of herbal medicines is now common across the world due to their normoglycemic potentials with minimal side effects. These medicinal plants contain various phytochemicals with antioxidants and other biological activities for the treatment and management of many diseases.¹⁵ The WHO verdict also suggested the need to grow and access better pharmacological agents that will improve the antioxidant and normal glycemic status, ameliorate the destruction of beta cells, and restore the diabetes-associated complications.¹⁷ Hence, recent efforts have focused on plant phytochemicals as natural sources of effective AChE, BChE, α -amylase and α -glucosidase inhibitors with little or no side effects, which could serve as a dietary intervention in the management of diabetes-induced complications. One example of the curative plant used in the prevention and management of diabetes and its complications is *Sterculia tragacantha*.

S. tragacantha belongs to the family *Sterculiaceae*. It is commonly known as Uhobo and Abalo in the Eastern and Western regions of Nigeria, respectively, where it is widely used for the treatment of pain, diarrhoea, edema, gout, whitlow, diabetes, cold and infectious diseases.¹⁸ Pharmacologically, the plant has been reported for antioxidants, antinociceptive, anti-inflammatory,^{19–22} neuroprotective,²³ mosquitocidal,²⁴ antimicrobial²⁵ and several other activities.²⁶ However, there is a dearth of information on the hypoglycemic and neuroprotective properties of the plant. The present study demonstrated that aqueous extract of *Sterculia tragacantha* leaf (AESTL) exhibited in vitro antioxidant activities and inhibited the activities of AChE, BChE, α -amylase and α -glucosidase in a dose-dependent fashion. The extract exhibited hypoglycaemic effect in an animal model and attenuated diabetes-induced oxidative stress and neuronal impairment in streptozotocin-intoxicated rats.

Materials and Methods

Plant Collections and Extractions

S. tragacantha leaves were obtained from the Osogbo Central Market, Osun State, Nigeria. The leaves were authenticated at the Forestry Research Institute of Nigeria (FRIN), Ibadan, Oyo State, Nigeria, where the voucher number (FHI: 112856) was deposited. The leaves were air-dried at 25° C for two weeks and pulverized using a mechanical blender (Type VIII machine of Christy and Norris limited). Two hundred grams (200 g) of the powdered sample was extracted in 2.5 L distilled water with constant shaking for 48 h and then filtered. Thereafter, the resulting filtrate was lyophilized using freeze dryer (Massion, UK) and the dried extract (AESTL) was stored in air-tight container.

Analysis of Phytochemical Compositions

The total flavonoid content of the extract was estimated using a spectrophotometer based on the formation of flavonoid–aluminium complex that absorb maximally at 415 nm.²⁷ Total phenol was estimated using Folin–Ciocalteu reagent protocol,²⁸ while the total terpenoid content was estimated as described by Ghorai et al.²⁹

Analysis of in vitro Antioxidant Activities DPPH Radical Scavenging Activity

The DPPH radical scavenging activity of AESTL was determined based on the ability to scavenge the stable DPPH free radical according to the procedure described by Tsado et al.³⁰ Briefly, 0.5 mL of the DPPH (50 mg/mL) diluted in 4.5 mL of methanol) was mixed with 0.1 mL of AESTL (10–100mg/mL). The reaction mixture was incubated for 45 min in the dark. The decrease in absorbance was measured at 517 nm against a blank.

FRAP (Ferric Reducing-Antioxidant Power) Assay

The FRAP was assessed based on the reduction of a ferric tripyridyl triazine (TPTZ) complex to its ferrous, coloured form in the presence of antioxidant.³¹ Briefly, 3 mL of FRAP reagent was mixed with 100 µL of the extract and incubated for 30 min at 37°C; the reduction of Fe³⁺ complex to the ferrous form was monitored by measuring the change absorbance at 593 nm. The FRAP values were expressed as mmol of Fe²⁺ equivalents per kg of the extract.

Total Antioxidant Capacity (TAC)

The total antioxidant capacity of the extract was evaluated by the phosphomolybdenum method according to the

procedure described by Kasangana et al.³² A 0.3 mL of extract (1 mg/mL extract in methanol) was incubated with 2.7 mL of phosphomolybdenum reagent (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate) at 95°C for 90 min. The reaction was monitored using a spectrophotometer at 695 nm. The total antioxidant activity was expressed as the number of grams equivalent of ascorbic acid.

Analysis of in vitro Hypoglycemic Activities

α-Amylase Inhibition Assay

The α-amylase inhibitory activity of the extract was assayed following the protocol of Worthington.³³ An aliquot of AESTL (20–100 µL) and 500 µL of 0.02 M sodium phosphate buffer (pH 6.9 with 0.006 M NaCl) containing α-amylase (0.5 mg/mL) were incubated at 25 °C for 10 min. One (1%) of starch solution (pH 6.9 with 0.006 M NaCl) was added and the reaction mixtures was incubated at 25 °C for 10 min. The reaction was stopped by the addition of dinitrosalicylic acid colour reagent followed by incubation in a boiling water bath for 5 min. The absorbance was recorded at 540 nm. The α-amylase inhibitory activity was expressed as percentage inhibition and the concentration of extract causing 50% inhibition of enzyme's activity (IC₅₀) was calculated.

α-Glucosidase Inhibition Assay

The α-Glucosidase inhibitory activities of the extract was assayed following the protocol of Apostolidis et al.³⁴ Appropriate dilution of the extract and 100 µL of α-glucosidase solution (1.0 U/mL) in 0.1 M phosphate buffer (pH 6.9) were incubated for 10 min at 25 °C. A solution of 5 mM p-nitrophenyl-α-D-glucopyranoside (pH 6.9) was added to the reaction mixture and incubated for 5 min at 25 °C. The absorbance was recorded at 405 nm. The percentage α-glucosidase inhibition and IC₅₀ was calculated.

In vivo Antidiabetic Study

Experimental Design

Male albino rats (155.34±6.33 g) were obtained from Suramos Farm Ltd, Ikere-Ekiti, Ekiti-State, Nigeria. The rats were fed with standard pelletized feeds (Ladokun Feeds, Nigeria Ltd) and provided with water ad libitum. All animal experiments were conducted in adherent to the regulations by the Ethics Committee on Animals Use of Afe Babalola University (Protocol approval number: AB235B). Diabetes was induced in the experimental

animals after overnight fasting by a single intraperitoneal injection (i.p.) of 40 mg/kg of freshly prepared streptozotocin (STZ). Glucose (50%) was given to the animals to alleviate mortality rates. Fasting blood sugar (FBS) levels were measured using a glucometer (Finetest, Roche Diagnostics) after 48 h and rats having FBS levels more than 250 mg/dL were selected and used for the present study. Thirty (30) hyperglycemic (FBS >250 mg/dL) rats were divided into 5 groups (A–E) of 6 animals each. Groups A–D were treated with 0, 150 and 300 mg/kg body weight of AESTL, and 200 mg/kg body weight of metformin respectively, while group E serve as the normal control. The therapeutic doses used for this study was selected based on the pilot toxicity studies on *S. tragacantha* which indicated its safety profile at high doses of up to 3000 mg/kg bw in rats.^{20,35} The fasting blood sugar (FBS) and body weight were monitored throughout the study period. The percentage hypoglycaemic effect was calculated as follows: $\text{FBS} ((\text{Vehicle control} - \text{Treatment})/\text{Vehicle control}) \times 100$.

Sample Collection and Processing

On the 22nd day of the experiment, the animals were fasted overnight but had free access to water. Thereafter, the animals were anaesthetized using diethyl ether and were humanly sacrificed. The brain samples were harvested into plain bottles, and homogenized using 0.1 M phosphate buffer solution. The homogenates were further centrifuged for 10 minutes at 5000 rpm after which the resulting supernatants were processed immediately for biochemical analysis.

Analysis of Biochemical Parameters

Enzymatic and Non-Enzymatic Biomarkers of Oxidative Stress in the Brain Tissue

The activity of superoxide dismutase (SOD) in the brain homogenate was determined as described by Kakkar et al.³⁶ The catalase activity (CAT) was determined using the hydrogen peroxide (H₂O₂) reaction protocol as described by Sinha.³⁷ The activity of glutathione peroxidase (GPx) was determined by using the Ellman's reagent (19.8 mg, DTNB in 100 mL 0.1% sodium nitrate) protocols as described by Rotruck et al.,³⁸ while the reduced glutathione (GSH) level was determined by a modified colorimetric method.³⁹

Analysis of Lipid Peroxidation

The estimation of lipid peroxidation (LPO) in the brain homogenate was determined spectrophotometrically by

measuring the thiobarbituric acid reactive substances (TBARS) as described by Shagirtha et al.⁴⁰ Briefly, 0.5 mL of the brain homogenate sample was treated with 2 mL of TBA-trichloroacetic acid reagent (0.37% TBA, 0.25 N HCl, and TCA 15% in ratio 1:1:1). The reaction mixture was incubated in water bath for 15 min, then cooled and centrifuged for 10 min at room temperature. Change in absorbance against a reference blank was monitored at 535 nm.

Analysis of Inflammatory Biomarkers

The cyclooxygenase-2 activity (COX-2) in the brain tissue was estimated using the methods of Shimizu et al.⁴¹ Nitric oxide level was measured by calculating nitric oxide content in a reaction medium consisting of 0.4 mL of working reagent (weighed 2 g vanadium chloride in 400 mL 5% hydrochloric acid), 0.2 mL of 0.1% N-(1-naphthyl) ethylenediamine dihydrochloride then 0.2 mL of 2 g sulfanilamide in 5% of hydrochloric acid. The nitrite level, which has the same estimative of NO level, was measured using a spectrophotometer at 540 nm, after 60 minutes of incubation at 37 °C. The levels of nitrite and nitrate on the brain were computed in nanomole NO.⁴²

Analysis of Cholinesterase (ChEs) and Neurotransmitters

Acetylcholinesterase (AChE) activity was assessed in the brain using acetylthiocholine iodide as a substrate as described by Shagirtha et al.,⁴⁰ while the activities of butyrylcholinesterase (BChE) in the brain were analyzed following a method described by Jońca et al.⁴³ The levels of dopamine and serotonin were assayed based on the method of Pagel et al.,⁴⁴ while the levels of the epinephrine and non-epinephrine were determined using the ELISA kits (Aviva Systems Biology, Corp., USA) according to the manufacturer protocols.

Analysis of Na⁺-K⁺-ATPase Activity

The activity of Na⁺-K⁺-ATPase was estimated by a modified colorimetric method described previously.⁴⁵ In brief, 0.2 mL of brain tissue homogenate was added to the mixture containing 1 mL 184 mM Tris–HCl buffer (pH 7.5), 0.2 mL 50 mM MgSO₄, 0.2 mL 50 mM KCl, 0.2 mL 600 mM NaCl, 0.2 mL (1 mM) EDTA and 0.2 mL 10 mM ATP and incubated for 15 min at 37 °C. The reaction mixture was arrested by the addition of 1 mL ice-cold 10% TCA. The amount of Pi (inorganic phosphate) liberated was estimated in protein-free supernatant.

Molecular Docking Studies of Ligand Receptor Interaction

The bioactive compound was characterized, and two compounds (procyanidin B2 and epicatechin) were identified as the major bioactive components of the extract. The crystal structures of the receptors – AChE (PDB:1H23), BChE (PDB:5LKR), alpha-amylase, alpha-glucosidase (PDB:3WY4) – in PDB file format were obtained from the Protein Data Bank, while the three-dimensional (3D) structure of the ligands (procyanidin B2 and epicatechin) was obtained in mol2 format using the Avogadro molecular builder and visualization tool version 1.XX.⁴⁶ The mol2 were converted to PDB files using the PyMOL Molecular Graphics System, version 1.2r3pre. All PDB files were subsequently converted to PDBQT files using AutoDock Vina (version 0.8, Scripps Research Institute, La Jolla, CA, USA).⁴⁷ The receptors were prepared for docking by removing water molecules, adding polar hydrogen atoms and Kolman charges.^{48,49} Docking was conducted using AutoDock Vina as described in previous studies,^{48,50,51} and results were visualized using the PyMOL, Discovery studio visualizer version 19.1.0.18287 (BIOVIA, San Diego, CA, USA).⁵²

Statistical Analysis

All analyses were conducted in triplicate and analyzed using a GraphPad prism version 8.0. One-way analysis of variance (ANOVA) was used to compare the significant differences between treatment groups. Results were considered significant at * $p < 0.05$, ** $p < 0.01$ or *** $p < 0.001$ and are presented mean \pm standard error of the mean.

Results

In vitro Antioxidants and Anti-Hyperglycemic Effects of AESTL

We used different in vitro approaches to evaluate the antioxidants' effect of AESTL. The results revealed that AESTL contains total flavonoids, phenols and terpenes contents of 107.90 ± 1.04 , 12.12 ± 0.03 , and 31.29 ± 0.94 mg/100g, respectively (Table 1). The result revealed that both the standard drugs and AESTL scavenged the free radicals in a concentration-dependent manner (Figure 1). The extract demonstrated dose-dependent ABTS and DPPH radicals scavenging effects with IC_{50} of 63.03 ± 2.57 and 117.49 ± 2.35 μ g/mL, respectively. Furthermore, extract exhibited FRAP and TAC values of 15.19 ± 0.98 mmol/100g and 43.38 ± 0.96 mg/100g, respectively. However, as expected, the activities demonstrated

Table 1 Phytochemical Compositions and in vitro Antioxidants and Hypoglycemic Effects of Aqueous Extract of *Sterculia tragacantha* Leaf (AESTL)

Assays	AESTL	Standards
Flavonoids (mg/100g)	107.90 ± 1.04	136.36 ± 0.89
Phenolics (mg/100g)	12.12 ± 0.03	67.26 ± 2.34
Terpenoids (mg/100g)	31.29 ± 0.94	151.29 ± 1.90
FRAP (mmol/100g)	15.19 ± 0.98	217.49 ± 2.34
TAC (mg/100g)	43.38 ± 0.96	49.74 ± 0.23
ABTS (IC_{50})	63.03 ± 2.57	3.38 ± 0.05
DPPH (IC_{50})	117.49 ± 2.35	41.57 ± 1.03
AChE (IC_{50})	113.07 ± 3.42	67.59 ± 2.35
BChE (IC_{50})	87.50 ± 4.32	47.02 ± 2.34
α -Amylase (IC_{50})	77.21 ± 4.35	18.45 ± 0.34
α -Glucosidase (IC_{50})	443.25 ± 12.35	106.88 ± 3.24

by AESTL was lower than the standard drugs. We also evaluated the anti-hyperglycemic effects of the extract using the α -amylase and α -glucosidase assays, and found that AESTL exhibited dose-dependent inhibitions of α -amylase and α -glucosidase with IC_{50} values of 77.21 ± 4.35 and 443.25 ± 12.35 , respectively. Altogether, the results of the study strongly suggested the antioxidants and hypoglycemic effects of the plant extract and hence form the basis for the in vivo evaluation of its efficacy.

In vitro Cholinesterase Inhibitory Activities of AESTL

The AChE and BChE inhibitory potential of AESTL were estimated and the result revealed that AESTL significantly inhibited the activities of AChE (Figure 2A) and BChE (Figure 2B) in a dose-dependent manner, having IC_{50} values of 113.07 ± 3.42 and 87.50 ± 4.32 μ g/mL for AChE and BChE, respectively. However, the standard drug (nicotinic acid) demonstrated higher activities and hence lower IC_{50} against the enzymes.

AESTL Demonstrated Hypoglycemic Effects in Streptozotocin-Induced Diabetic Rats

Injection of streptozotocin caused significant elevation of the fasting blood sugar (Table 2) and loss of body weight (Table 3) of the experimental rats. Interestingly, treatment with AESTL caused dose dependent and progressive decreases in the FBS ($p < 0.0001$), having percentage hypoglycemic effects of 68.27 ± 2.34 and 71.92 ± 2.45 at 150 and 300 mg/kg BW, respectively. The in vivo hypoglycemic

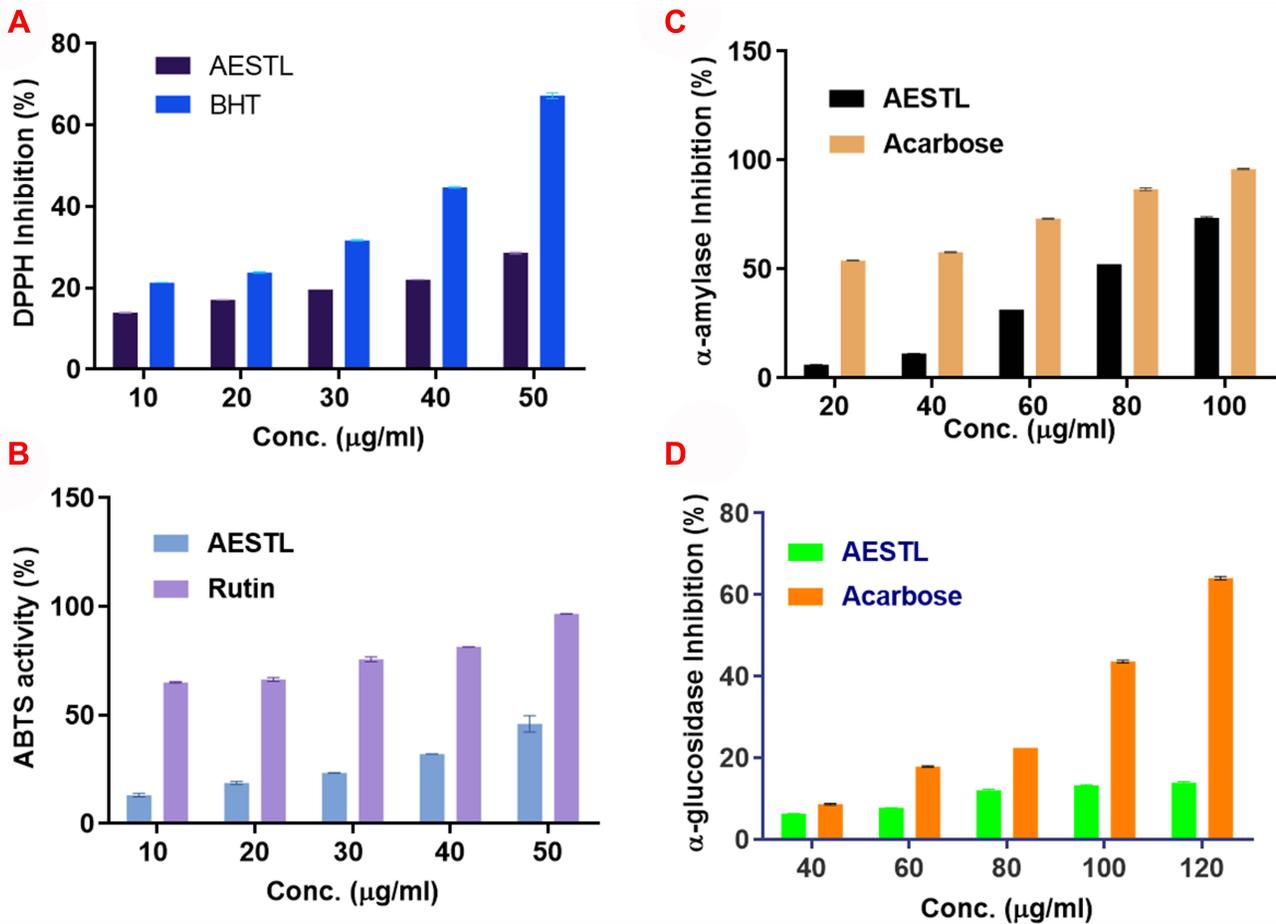


Figure 1 In vitro antioxidants and anti-hypoglycemic effects of AESTL. Bar graphs of dose vs inhibition showing the effect of AESTL and standard drugs on (A) DPPH, (B) ABTS, (C) α-amylase and, (D) α-glucosidase activities. Values are mean ± SEM (n = 6).

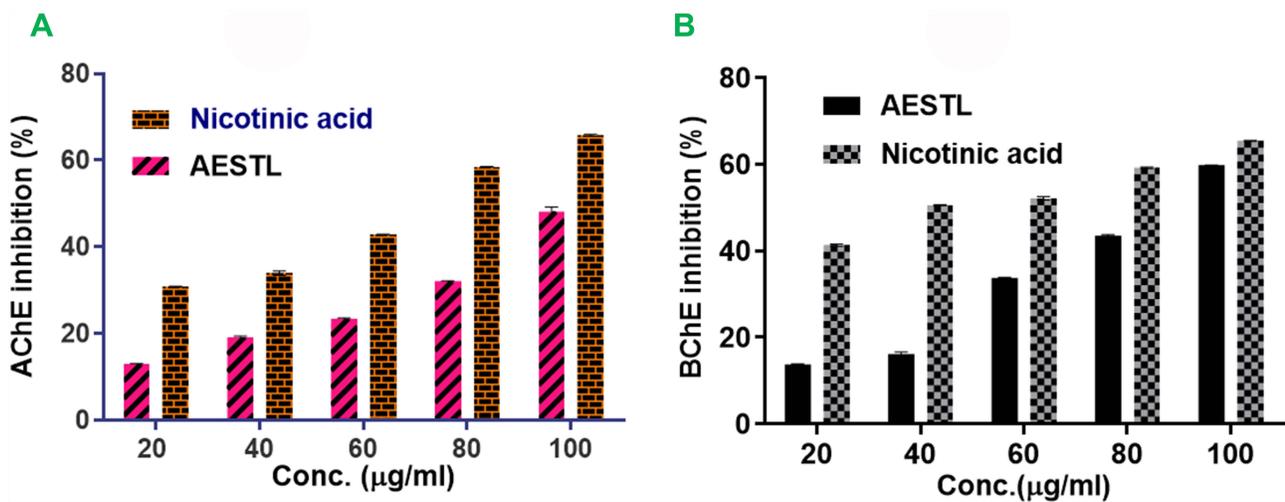


Figure 2 The cholinesterase inhibitory activities of aqueous extract of *Sterculia tragacantha* leaf (AESTL). Bar graphs of dose vs inhibition showing the effects of AESTL on the (A) acetylcholinesterase and (B) butyrylcholinesterase activities of AESTL. Values are mean ± SEM (n = 6).

Table 2 Hypoglycaemic Effect of the Aqueous Extract of *Sterculia tragacantha* Leaf (AESTL) in Streptozotocin-Induced Diabetic Rats

Groups	FBS (mg/dL)				Hypo Effect (%)
	Day 0	Day 2	Day 14	Day 21	
Normal Control	84.56 ± 4.07	85.26 ± 3.21***	83.18 ± 5.34***	85.08 ± 7.23***	–
Vehicle, 2.5 mL/kg	86.63 ± 3.80	289.54 ± 11.18	299.41 ± 11.34	310.09 ± 15.22	
AESTL, 150 mg/kg	88.22 ± 4.44	314.21 ± 13.22	196.77 ± 10.56**	98.39 ± 10.49***	
AESTL, 300 mg/kg	84.50 ± 3.22	298.24 ± 11.29	110.88 ± 9.67***	87.06 ± 4.76***	
Metformin, 200 mg/kg	85.33 ± 5.28	293.13 ± 10.32	156.45 ± 12.78**	84.99 ± 5.54***	

Notes: Data expressed as mean ± SD (n = 6). *p < 0.05, **p < 0.01, ***p < 0.05. Hypo effect (%); percentage hypoglycaemic effect = FBS ((Vehicle control - Treatment)/ Vehicle control) × 100.

activities of the plant extract is comparable with the standard drug, metformin (72.59±3.45%) (Table 2). Furthermore, treatment with the plant extract caused significant improvement of the body weight (p<0.001) when compared with the vehicle control (Table 3).

AESTL Attenuates the Dysregulation of Cholinesterase and Neurotransmitters in Brain Tissue of Streptozotocin-Induced Diabetic Rats

The effect of AESTL on the levels of cholinesterases (AChE and BChE) and neurotransmitters (dopamine, serotonin, epinephrine and norepinephrine) in the brain of STZ-induced diabetic rats is shown in Figure 3. Diabetic untreated rats demonstrated a significant increased levels of AChE and BChE (Figure 3A), dopamine and serotonin (Figure 3B), and epinephrine and norepinephrine (Figure 3C) when compared with the rats in the normal control group. However, treatment with AESTL significantly reversed the levels of AChE, BChE, dopamine, serotonins, epinephrine and norepinephrine (Figure 2B) towards normalization compared to untreated diabetic rats. Collectively, our results demonstrated that AESTL attenuated the dysregulation of cholinesterase and neurotransmitters in brain tissue of STZ-induced diabetic rats.

AESTL Regulates Na⁺-K⁺-ATPase Anti-Inflammatory Response via Inhibition of COX-2/NO Signaling Axis in Brain of Streptozotocin-Induced Diabetic Rats

The role of nitric oxide synthase and cyclooxygenase 2 in inflammation and pathology of neurodegenerative diseases has been well described. We evaluated the protective effect of AESTL on STZ-induced dysregulation of Na⁺-K⁺-ATPase and inflammation makers (Figure 4). Our results revealed that the activities of cyclooxygenase-2 (COX-2), (Figure 4A), Na⁺-K⁺-ATPase (Figure 4B) and the level of NO (Figure 4C) in the brain of STZ-induced diabetic untreated rats were significantly (p < 0.05) elevated when compared with the normal control. Treatment with AESTL significantly (p < 0.05) reduced the levels of these biomarkers when compared with the diabetic untreated rats.

AESTL Attenuates Oxidative Stress in Brain of Streptozotocin-Induced Diabetic Rats

Oxidative stress has been implicated in the pathogenesis of diabetes and neurodegenerative diseases. We evaluated the protective effect of AESTL in STZ-induced oxidative stress in brain tissue of diabetic rats. Our results revealed that AESTL demonstrated dose-dependent antioxidant activities by

Table 3 Effect of the Aqueous Extract of *Sterculia tragacantha* Leaf (AESTL) on Bodyweight Changes in Streptozotocin-Induced Diabetic Rats

	Day 0	Day 14	Day 21	Weight Changes (%)
Normal Control	150.89 ± 8.14	154.03 ± 7.10	165.98 ± 5.69	9.09 ± 2.4***
Vehicle, 2.5 mL/kg	160.89 ± 10.2	153.11 ± 10.1	140.10 ± 5.89	-14.84 ± 5.56
AESTL, 150 mg/kg	158.24 ± 6.80	159.19 ± 7.17	162.31 ± 6.16	2.51 ± 1.37**
AESTL, 300 mg/kg	150.34 ± 7.90	153.12 ± 8.14	156.19 ± 7.23	3.75 ± 1.50**
Metformin, 200 mg/kg	156.39 ± 7.56	157.59 ± 9.21	160.34 ± 9.31	2.46 ± 1.06**

Notes: Data expressed as mean ± SD (n = 6). * p < 0.05, ** p < 0.01, *** p < 0.05.

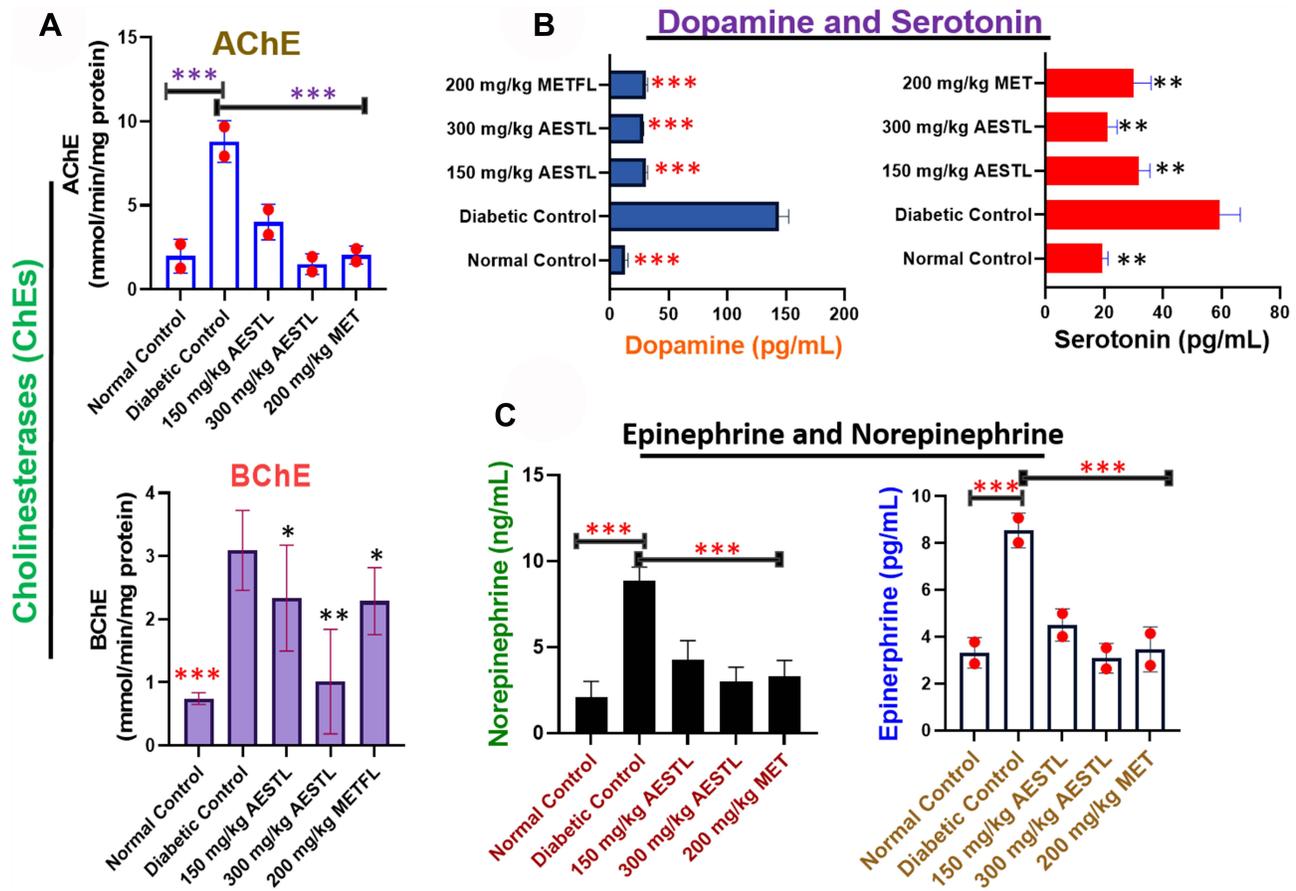


Figure 3 Aqueous extract of *Sterculia tragacantha* leaf (AESTL) attenuates the dysregulation of cholinesterase and neurotransmitters in brain tissue of streptozotocin-induced diabetic rats. Bar plots showing the effect of AESTL on the levels of (A) cholinesterases (AChE and BChE), (B) dopamine, serotonin, (C) epinephrine and norepinephrine in brain of streptozotocin-induced diabetic rats. Values are mean ± SEM (n = 6). *p<0.05, **p<0.01 or ***p<0.001. **Abbreviations:** AChE, acetylcholinesterase; BChE, butyrylcholinesterase.

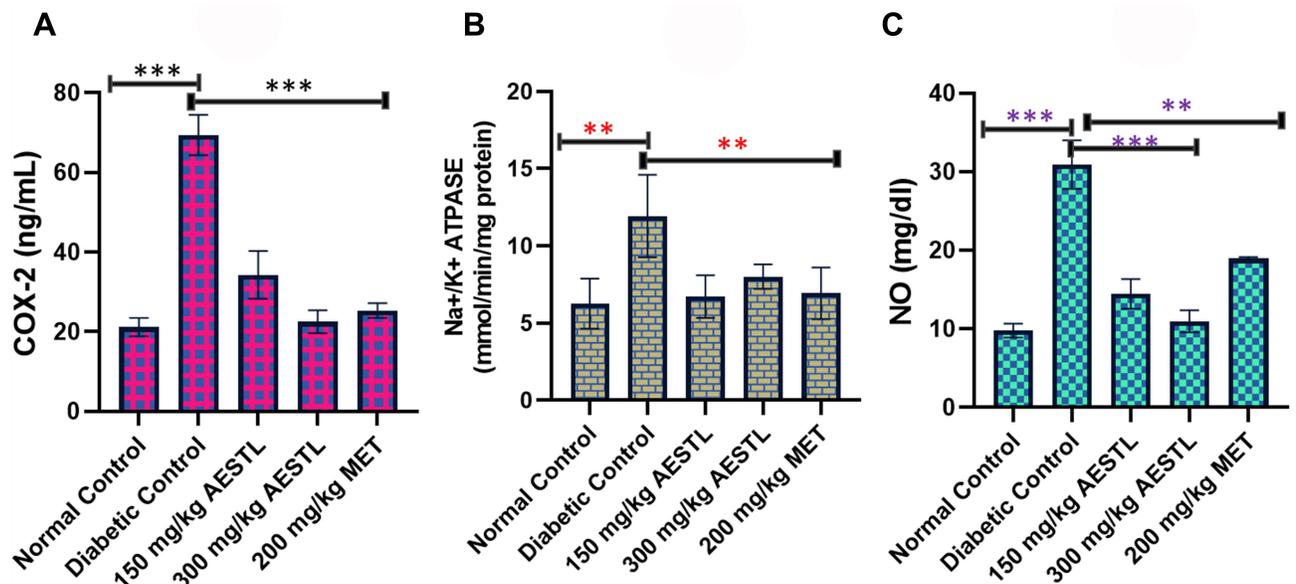


Figure 4 AESTL regulates Na⁺-K⁺-ATPase anti-inflammatory response via inhibition of COX-2/NO signaling axis in brain of streptozotocin-induced diabetic rats. Bar plots showing the effect of AESTL on the activities of cyclooxygenase-2 (COX-2), (A) Na⁺-K⁺-ATPase (B) and the level of NO (C) in brain of streptozotocin-induced diabetic. Values are mean ± SEM (n = 6). **p<0.01 or ***p<0.001.

significantly ($p < 0.05$) improving the activities of enzymatic antioxidants including the SOD (Figure 5A), GPx (Figure 5B) and CAT (Figure 5C), and level of non-enzymatic antioxidant (GSH) (Figure 5D), and lipid peroxidation (Figure 5E) in the brain of diabetic rats when compared with the diabetic untreated rats. Interestingly, the antioxidant activities demonstrated by AESTL at 300 mg/kg are significantly higher than the antioxidant activities demonstrated by the standard drug (metformin). Collectively, our results demonstrated that AESTL exhibited significant antioxidant activities in vitro as well as in vivo in the brain of STZ-intoxicated rats.

Epicatechin and Procyanidin B2, the Bioactive Compounds From AESTL, Have the Molecular Properties to Interact Efficiently with Cholinesterase, α -Amylase and α -Glucosidase

We evaluated the ligand–receptor interactions between two bioactive compounds from AESTL (epicatechin and procyanidin B2) and cholinesterases, α -amylase and α -glucosidase using the molecular docking studies. Interestingly, our results revealed that epicatechin and procyanidin B2 docked well to the binding cavities of acetylcholinesterase, butyrylcholinesterase, α -amylase and α -glucosidase with respective binding affinities ranges between -8.0 and -11.4 kcal/mol (Figures 6 and 7). Comparatively, procyanidin B2 demonstrated higher affinities for BChE, α -amylase and α -glucosidase than does the epicatechin. Both epicatechin and procyanidin B2 are bound to the binding pocket of the receptors by several conventional H-bonds, alkyl and multiple π -interactions. Furthermore, several van der Waals forces were found around the ligand backbones with the respective amino acid residues of receptors binding pockets. Compared to the epicatechin and procyanidin B2, the standard drugs acarbose and nicotinic acid demonstrated lower binding affinities to their respective receptors (Figures 6 and 7). Altogether, our molecular docking analysis revealed that both epicatechin and procyanidin B2 have molecular properties to interact efficiently with cholinesterase, α -amylase and α -glucosidase, suggesting that these compounds are the bioactive components that could be responsible for the biological activities of AESTL reported in this study.

Discussion

Medicinal plants are important therapeutic agents used in the management of several diseases including diabetes. Herein, *Sterculia tragacantha*, one of the commonly used herbal

remedies in the management of diabetes, was investigated for antidiabetic, antioxidants, anti-inflammatory and neuroprotective potential in streptozotocin-induced diabetic rats. In addition, we demonstrated that two bioactive compounds from the plant extract, epicatechin and procyanidin B2, has molecular properties to interact with molecular targets, suggesting that these compounds are the bioactive component that could be responsible for the antidiabetic and neuroprotective activities of AESTL.

Free radicals play an important etiologic role in the pathogenesis of metabolic and neurodegenerative diseases.⁵ Therefore, antioxidants serve as an important agent for the prevention of free radical-induced oxidative stress and tissue damage.¹⁵ The reducing power due to hydrogen donating ability has been attributed to the biological activities of medicinal plants.⁵ Interestingly, the results of the present study revealed that AESTL induced the scavenging of free radicals and improved the antioxidants status of glycemic and neuronal impaired rats, thus serving as a potential therapeutic agent in the management of oxidative stress and associated disorders.

Impaired cholinesterase activities and neuronal dysfunction are diabetes-mediated complications.⁷ The increased inhibition of BChE and AChE activities following treatment with AESTL extract could be attributed to the significant amounts of its phytochemical composition, particularly the flavonoids and phenols (Table 1), which have been reported to exhibit cholinesterase inhibition activities.⁵³ Lunke's et al⁵⁴ reported that serum cholinesterase activity is highly over expressed in diabetes and associated pathologies. Therefore, results of the present study strongly suggested that the AESTL extract could be very useful for halting diabetes progression and its associated neuronal complications. In line with the findings from the present study, previous studies had proved that pharmacological inhibition of acetylcholinesterase represents an important therapeutic strategy for the treatment of various metabolic and neurodegenerative diseases.^{11,55}

α -Amylase and α -glucosidase are important enzymes of the gastrointestinal tract and function to digest complex carbohydrates, thus contributing to postprandial hyperglycemia in type 2 diabetes.¹² The inhibition of α -amylase and α -glucosidase activities has been suggested as an effective strategy for the control of hyperglycemic conditions in type 2 diabetes mellitus⁶; hence, the search for pharmacologically active phytoconstituents that could inhibit the activities of these enzymes is of increasing

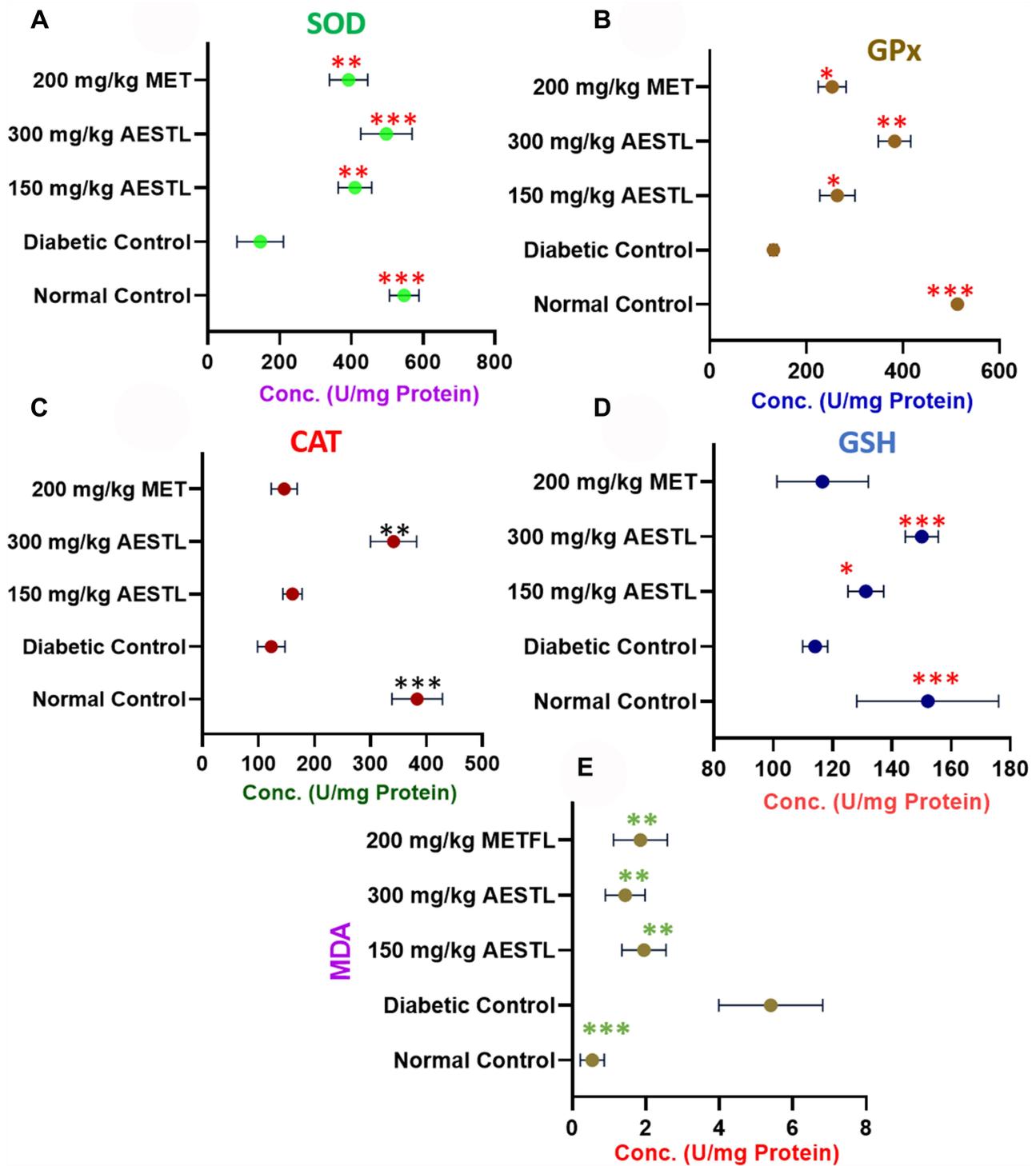


Figure 5 Aqueous extract of *Sterculia tragacantha* leaf (AESTL) attenuates oxidative stress in brain of streptozotocin-induced diabetic rats: Effect of the aqueous extract of *S. tragacantha* leaf extract (AESTL) on the level of enzymatic antioxidants including the (A) SOD, (B) GPx and (C) CAT, (D) level of non-enzymatic antioxidant (GSH) and (E) MDA in the brain of streptozotocin-induced diabetic rats. Values are mean \pm SEM (n = 6). * $p < 0.05$, ** $p < 0.01$ or *** $p < 0.001$.
Abbreviations: CAT, catalase; SOD, superoxide dismutase; GSH, reduced glutathione; GPx, glutathione peroxidase; MDA, malondialdehyde.

scientific interest.⁵⁶ Therefore, the inhibition of α -glucosidase and α -amylase by AESTL extract is an appraisable property. This would facilitate the retardation of

elevation of blood sugar and suppress postprandial hyperglycemia by decreasing the rate of blood sugar absorption.⁵⁶

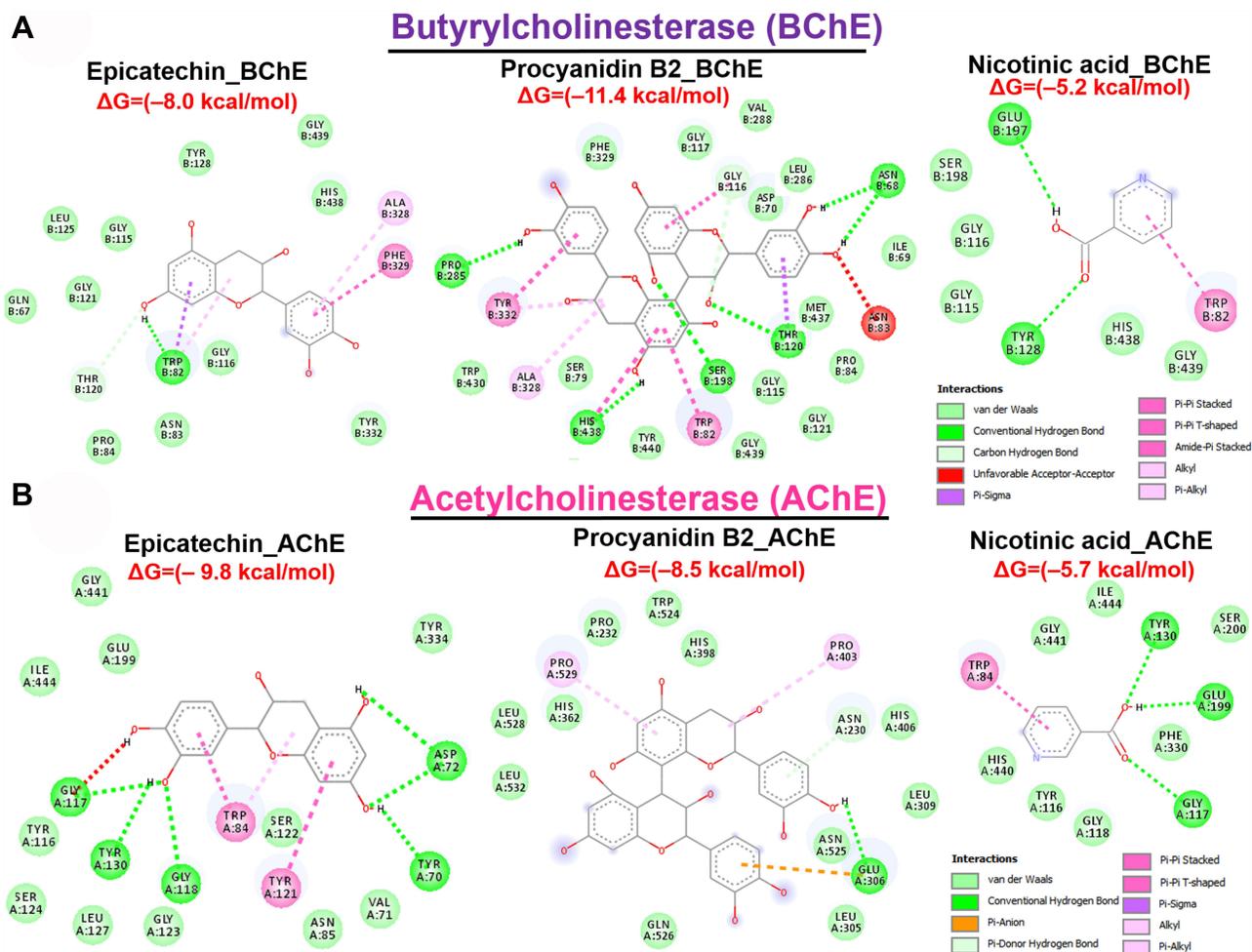


Figure 6 Molecular docking profile of epicatechin and procyanidin B2 with cholinesterase. Two-dimensional (2 D) representations of the interaction occurring between epicatechin and procyanidin B2 and (A) butyrylcholinesterase and (B) acetylcholinesterase.

Abbreviations: AChE, acetylcholinesterase; BChE, butyrylcholinesterase.

The increased blood glucose levels in diabetic untreated rats could be attributed to the oxidative status induced by the STZ treatment, which is in line with the results observed in previous studies.^{57,58} Similarly, lowered level of the bodyweight observed in untreated diabetic rats corroborates with the finding of Ajiboye et al.²³ This loss of bodyweight could be attributed to the increased catabolism of fats and proteins, leading to muscle reduction and weight loss. Importantly, our results revealed hypoglycaemia and improvement of body weight in AESTL-treated rats, suggesting a metabolic restoration and antidiabetic effect of the plant.

The in vivo inhibition of AChE and BChE in the brain tissue of rats treated with the AESTL corroborates with our in vitro findings and thus suggests that the plant has potential therapeutic importance for the management of neuronal impairment in diabetes mellitus. Norepinephrine (NE) and

epinephrine (E) are very similar neurotransmitters and hormones associated with metabolic and neurodegenerative disorders.⁵⁹ The high levels of NE and E in diabetic untreated rats could be attributed to their accumulation due to the inhibition of presynaptic release of NE and/or increased reuptake of released NE and E⁶⁰ in the brain tissue of STZ-induced diabetic rats. The decreased levels of NE and E following treatment with AESTL could be due to either decreased synthesis or an increased rate of degradation.

Dopamine and serotonin (5-HT) are important neurotransmitters that are widely distributed in the central nervous system and have been explored as a biomarker in metabolic and neurological disorders.⁶¹ Results of the present study revealed significantly high levels of dopamine and serotonin (5-HT) in brain tissue of STZ-intoxicated rats, which is in agreement with the result of the previous study.²³ The reversal effect of AESTL on the

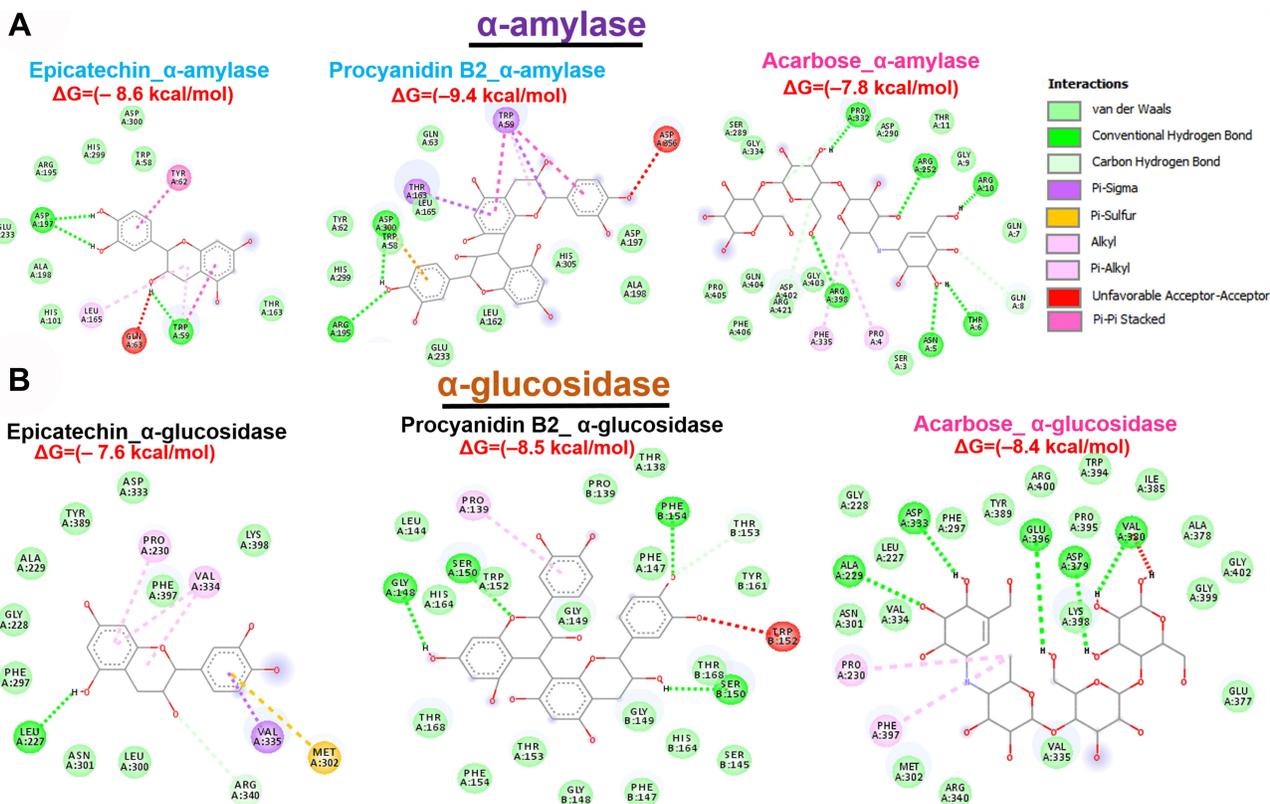


Figure 7 Molecular docking profile of epicatechin and procyanidin B2 with carbohydrate metabolizing enzymes. Two-dimensional (2 D) representations of the interaction occurring between epicatechin and procyanidin B2 and (A) α -amylase and (B) α -glucosidase.

tissue levels of neurotransmitters suggests the preservation of neuronal integrity, a finding which is in line with previous pharmacological evaluations of some natural products.^{23,62}

Hyperglycaemic-induced alteration of cyclooxygenase (COX) pathway activity with subsequent impaired production and function of prostaglandins (PGs) is one mechanism that is associated with the pathogenesis of diabetic neuropathy.⁶³ COX-2, the inducible COX isoform, is upregulated in a variety of pathophysiological conditions including diabetes. In addition, the upregulation of COX-2 has tissue-specific consequences and is associated with the activation of downstream inflammatory reactions.^{63,64} Alterations in $\text{Na}^+\text{-K}^+\text{-ATPase}$ activity also represent an important mechanism of diabetics-induced neurotoxicity.⁶⁵ In the same vein, nitric oxide, as a neurotransmitter, is reported to mediate synaptic activity, neural plasticity, and memory function.⁶⁶ Interestingly, we found that treatment with AESTL reverses the overexpression of $\text{Na}^+\text{-K}^+\text{-ATPase}$, COX-2 and NO to the baseline value, hence suggesting the anti-inflammatory and therapeutic role of AESTL in the maintenance of ion gradients across biological membranes and thus confer significant

protection to the brain by stabilizing the functional integrity of the membrane.⁶⁷

Antioxidant enzymes such as SOD, catalase, glutathione peroxidase as well as glutathione form the defense system, which usually protects the cell against oxidative damage. The depleted levels of these antioxidants in diabetic untreated rats could be attributed to the STZ-mediated generation of free radicals in the brain of diabetic rats, which results in oxidative damages to membrane lipid, protein and decreased levels of the endogenous antioxidants.⁶⁸ It is noteworthy that treatment with AESTL significantly reversed the depleted levels of GSH, SOD, catalase and GPx which is supported by earlier findings where natural antioxidants are used as a remedy in oxidative stressed rats.⁶⁹ Cytotoxic agents such as malondialdehyde become stable and easily diffused in secondary injuries caused by oxidative stress.⁷⁰ The increased LPO in STZ-intoxicated rats corroborates the research carried out by Oyinloye et al,⁷¹ where an elevated generation of reactive free radicals when exposed to toxic compounds resulted in increased LPO that eventually compromised cellular integrity of the neurons. However, the inhibition of LPO by AESTL further strengthened its antioxidants' capacity in STZ-intoxicated rats.

Although the present study is limited by the lack of histopathological examination of brain tissue, the biochemical analysis of the tissue homogenate revealed the ability of AESTL to attenuate diabetic-mediated oxidative stress, inflammation and neuronal impairment in STZ-induced diabetic rats.

Molecular docking simulation of the interaction between a receptor and ligand has been widely used in elucidating the binding affinities between a target and a drug candidate and for unveiling the behavior of a drug molecule within the binding cavities of the targets.^{72–74} Consequently, we conducted a molecular docking study to elucidate the potential druggability of cholinesterase α -amylase and α -glucosidase by two bioactive compounds from AESTL (epicatechin and procyanidin B2). Interestingly, our results revealed that epicatechin and procyanidin B2 docked well to the binding cavities of acetylcholinesterase, butyrylcholinesterase, α -amylase and α -glucosidase with respective binding affinities ranges between -8.0 and -11.4 kcal/mol (Figures 6 and 7). Comparatively, procyanidin B2 demonstrated higher affinities for BChE, α -amylase and α -glucosidase than does the epicatechin. Both epicatechin and procyanidin B2 are bound to the binding pocket of the receptors by several conventional H-bonds, alkyl and multiple π -interactions. Furthermore, several van der Waals forces were found around the ligand backbones with the respective amino acid residues of receptors binding pockets would create strong cohesive environments, that stabilizes the receptor–ligand complexes.^{75–77} Compared to epicatechin and procyanidin B2, the standard drugs acarbose and nicotinic acid demonstrated lower binding affinities to their respective receptors (Figures 6 and 7). Altogether, our molecular docking analysis revealed that both epicatechin and procyanidin B2 has molecular properties to interact efficiently with cholinesterase, α -amylase and α -glucosidase, suggesting that these compounds are the bioactive components that could be responsible for the biological activities of AESTL reported in this study. Another limitation of the present study, therefore, lies in the individual isolation and biological evaluation of these compounds against STZ-induced oxidative and neuronal impairment. This is however under investigation in our laboratory.

Conclusion

Conclusively, the results of the present study revealed that *Sterculia tragacantha* Lindl (AESTL) exhibited therapeutic efficacy against STZ-mediated oxidative stress, inflammation and neuronal impairment thus represents a source of bioactive agents for the management of diabetes and associated complications. Interestingly, epicatechin and

procyanidin B2, the bioactive compounds from AESTL, have the molecular properties to interact with high affinities to the binding cavity of acetylcholinesterase, butyrylcholinesterase, α -amylase and α -glucosidase, suggesting that these compounds are the bioactive components that could be responsible for the antidiabetic and neuroprotective activities of AESTL.

Institutional Review Board Statement

Institutional review board approval is not required for original research with animal studies.

Informed Consent Statement

This study did not require nor include human participants or tissues.

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Author Contributions

All authors contributed to data analysis, drafting or revising the article, have agreed on the journal to which the article was submitted, gave final approval of the version to be published, and agree to be accountable for all aspects of the work.

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Disclosure

The authors declare no conflicts of interest for this work.

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