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Full length Research Article

Effect of Standardized *Eucalyptus globulus* Leaf Extract on Brain Oxidative Stress and Aberrant Neurochemistry of Fructose-streptozotocin-induced Diabetic Rats.

***Akinmoladun, A.C., Jiddah-Kazeem, B.S., Bello, M. and Olaleye, M.T.**

Phytomedicine, Biochemical Pharmacology and Toxicology Laboratories, Department of Biochemistry, School of Life Sciences, The Federal University of Technology, Akure 340110, Nigeria

Summary: The neuro-pharmacological effect of *Eucalyptus globulus* ethanol leaf extract in fructose-streptozotocin-induced diabetic rats was evaluated in this study. The phytochemical analysis of the extract was carried out using HPLC-DAD. Diabetes was induced in rats with 10% fructose in drinking water and a single intraperitoneal injection of 40 mg/kg streptozotocin (STZ). Diabetic animals were orally treated with 100-400 mg/kg of the extract for 21 days with glibenclamide as the reference drug. Blood and brain tissue were processed for the determination of serum electrolyte levels, hematological indices, and biochemical estimations. Ergosterol, pinitol, catechin, quercetin, robinetinidol, and other polyphenols were identified in the extract. Diabetic animals showed decreased serum potassium and sodium ion levels and decreased hematocrit, hemoglobin, red blood cells, white blood cells and lymphocytes but increased neutrophils. The brains of animals in the untreated diabetic group with increased blood glucose level showed oxidative stress (increased level of MDA and myeloperoxidase but decreased level of reduced glutathione and superoxide dismutase) and disturbed neurochemistry (increased level of acetylcholinesterase and monoamine oxidase but decreased level of Na+K+ ATPase, tyrosine hydroxylase and dopamine). Administration of the *Eucalyptus globulus* leaf extract remarkably ameliorated the observed hyperglycemia, electrolyte, and hematological imbalances in animals. In addition, the administration of the extract attenuated the brain redox imbalance and neurochemical disturbances in the rats. These results show that *Eucalyptus globulus* leaves contain antioxidant and neurotransmitter-modulating phytochemicals with the potential to be developed as therapeutic agents for the management of diabetic cerebrovascular problems and related complications.

Keywords*: Type-2 diabetes; hematological disturbances; brain redox stress; neurotransmitter dysfunction; phytoextract*

**Authors for correspondence: acakinmoladun@futa.edu.ng , Tel: +23480344458930*

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INTRODUCTION

Diabetes mellitus is a group of metabolic disorders characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both. The disease burden related to diabetes is high and has continued to rise in every country, fuelled by the global rise in the prevalence of obesity and unhealthy lifestyles such as poor diets and physical inactivity. The two common types of diabetes mellitus are type 1 and type 2 diabetes mellitus. Type-2 diabetes represents over 90% of all cases of diabetes (Obafemi *et al*., 2017).

Diabetes mellitus (DM) and its complications pose a major threat to global health. The International Diabetes Federation (IDF) estimated in 2019 that 1 in 11 adults aged 20–79 years (463 million adults) had diabetes mellitus globally and this is projected to rise to 700 million by 2045, fthe largest increase is projected to come from Africa with an estimated increase of 143% (IDF, 2019). Diabetes is estimated to be associated with 11.3% of global deaths from all causes among people in this age group (IDF, 2019). DM and its complications impose an enormous health and economic burden upon individuals, families, societies and nations (Li *et al*., 2019).

Chronic hyperglycemia of diabetes is associated with long-term damage, dysfunction, and failure of different organs (Fasil *et al*., 2018). Hyperglycaemia is associated with several physiological changes, and the most profound effects are seen in the brain, where glucose is the major substrate for energy metabolism and both the local energy store and the supply of alternative sources are limited. Neurotransmitters have a role in maintaining glucose homeostasis (Güemes & Georgiou, 2018). Brain injury results from a derangement of several biochemical processes in the organ that are initiated when blood glucose concentration is altered. It is also known that the expression of some genes is involved in the pathophysiology of diabetic neurological dysfunction, but the action mechanisms are often obscure.

Although several drugs are available to control elevated blood glucose levels in diabetic patients, they still suffer from treatment complications like nephropathy,

neurological dysfunction, and retinopathy. Hence, the search for new treatment strategies is required to manage diabetes and mitigate these chronic widely spreading complications. Medicinal plants and plant-derived antioxidant agents have been demonstrated to have a promising therapeutic influence on various neurodegenerative disorders associated with diabetes and oxidative stress. *Eucalyptus globulus* (Fever tree) belongs to the family of Myrtaceae (Akin *et al*., 2012). It is the commonest eucalyptus (Damjanović-Vratnica *et al*., 2011), and grows in a wide range of conditions. It is used as a traditional treatment for diabetes and previous studies showed its anti-inflammatory, antioxidant and antidiabetic properties (Gray & Flatt, 1998). Therefore, this study was designed to investigate the neuropharmacological effect of ethanol leaf extract of *Eucalyptus globulus* in diabetes mellitus with a focus on its effect on brain redox status and disturbed neurochemistry.

MATERIALS AND METHODS

Chemicals and Reagents: Thiobarbituric acid (TBA), trichloroacetic acid (TCA), reduced glutathione (GSH), glutamic acid, adenosine triphosphate (ATP), 6,7-Dimethyl-5,6,7,8-tetrahydropterine (DMTHP), 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB), perchloric acid (PCA), benzylamine hydrochloride (BAHC), dopamine hydrochloride, reduced nicotinamide-dinucleotide(NADH), ethylenediaminetetraacetic acid (EDTA), 1-amino-2-naphthol-4-sulphonic acid (ANSA), 2,4-dinitrophenyl hydrazine (DNPH), acetylcholine iodide, epinephrine, tetramethylbenzidine (TMB) streptozotocin and ammonium molybdate were obtained from Sigma-Aldrich (St-Louis, MO, USA). Glibenclamide was obtained from HOVID Bhd. (Darul Ridzuan, Malaysia). All other chemicals and reagents were of analytical grade and obtained from standard sources.

Plant material and preparation of extract: The leaves of *Eucalyptus globulus* were collected from a fully grown tree at Oda (7◦12′40.4″N 5◦12′45.7″E) in Akure, Nigeria in August 2018. The leaf was authenticated at the Herbarium of the Federal University of Technology, Akure, Nigeria where a voucher sample (voucher number: 0265) was deposited.

The leaves were air-dried at room temperature and pulverized using an electric grinder. The technique of continuous hot extraction using a Soxhlet extractor was employed. The ethanol leaf extract was prepared by refluxing 200 g of the plant sample with 1000 ml of absolute ethanol for 12 h. The extract obtained was stored in a refrigerator for further use. The yield of the extract (EG) was 35.59 g. EG was standardized by HPLC-DAD fingerprinting.

Quantification of compounds in EG by HPLC-DAD fingerprinting: The extract (10 mg) was dissolved in aqueous acetonitrile (10 mg/20 ml) and mixed vigorously for 30 min. After mixing, the aqueous end was run off while the organic solvent end was collected into a 25 ml standard flask. The analysis was performed on a Shimadzu (NexeraMX) HPLC system fitted with a uBONDAPAK C18 column (length 100 mm, diameter 4.6 mm, and thickness 7 μm). The mobile phase consisted of a mixture of aqueous acetonitrile (acetonitrile/water, 80:20). The sample was injected at a volume of 5 μl and the flow rate was set at 0.08 ml/min for water and 5 ml/min for acetonitrile at a pressure of 15 mpa. Compounds were detected by a UV detector at 254 nm (Diode Array Detector, DAD). The retention times of the identified compounds of interest were measured using a standard solution at a concentration of 15.69 mg/g. The extract was injected into the high-performance liquid chromatographic machine to obtain a curve providing peak area and retention time in a chromatogram. Then the peak area of the sample is compared with that of the standard relative to the concentration of the standard to obtain the concentration of the sample and their concentration was calculated as shown below:

Concentration=

$$\frac{peak\ area\ of\ compound \times standard\ concentration}{peak\ area\ of\ standard}$$

Induction of type-2 diabetes mellitus: Healthy male Wistar rats at 10 weeks of age and weighing 140–200 g were used for these studies. Rats were fed chow (15% crude protein, 4% crude fat, 6% crude fiber, 1.1% calcium, 0.3% phosphorus, 0.7% lysine, 0.25% methionine) and water, ad libitum. The animals were acclimatized for 7 days before studies began. The experiments were approved by the animal research ethics committee of the Federal University of Technology, Akure, Nigeria (FUTA/ETH/21/05). Animal research was conducted following the internationally accepted principles for laboratory animal use and care as found in the European Community guidelines (EEC Directive of 1986; 86/609/EEC). Rats were provided with 10% fructose in drinking water for 2 weeks after which a single dose of 40 mg/kg STZ was administered by intraperitoneal injection (Obafemi *et al*., 2019). After 72 h of STZ injection, blood was collected from the tail vein for blood glucose level determination. Animals with fasting blood glucose levels > 250 mg/dl were considered diabetic and used for the experiment. Rats were randomly divided into ten groups with nine animals per group as follows: normal control, diabetic control, diabetic + 100 mg/kg EG, diabetic + 200 mg/kg EG, diabetic + 400 mg/kg EG, diabetic + 5 mg/kg glibenclamide, 100 mg/kg EG, 200 mg/kg EG and 400 mg/kg EG. The extract was dissolved in distilled water. A uniform suspension of the standard drug glibenclamide in distilled water was prepared. Both extract and reference drug were administered by oral gavage. The normal control and diabetic control groups received distilled water. Treatment lasted for 21 days after which the animals were fasted overnight before sacrifice. After sacrifice, three animals were randomly picked for histopathological evaluation and gene expression analysis.

Biochemical estimations: The brains of the sacrificed rats were excised and washed in ice-cold 1.15% (w/v) potassium chloride solution, blotted with filter paper, and weighed. They were then blended in 0.1 M, pH 7.4 phosphate-buffered saline (PBS) using a Teflon homogenizer, to prepare a 10% (w/v) homogenate which was centrifuged at 10,000 x g at 4 °C for 25 min to obtain the supernatant used for biochemical analyses. Blood was collected through cardiac puncture and stored in sample tubes. It was later

centrifuged and the serum was collected and used for biochemical estimations.

Estimation of glucose and electrolytes: Glucose, potassium ion, and sodium ion concentrations were estimated in the serum using assay kits obtained from Randox Laboratories Ltd (Crumlin, UK) according to instructions provided by the manufacturer.

Determination of hematological indices: Hematocrit was determined using high-speed centrifugation of blood-filled hematocrit tubes with a Zipocrit Hematocrit Centrifuge (ThermoFisher Scientific, Philadelphia, PA). All white blood cell (WBC) count estimates were performed by the same technician, at a location on the slide where the cells were one layer thick, adjacent to one another (membranes touching), evenly distributed, and showed no signs of morphological change. White blood cell estimates were made by using a 100X objective lens with immersion oil, counting the number of white blood cells in 10 fields, calculating the average, and then multiplying the number of cells by 2000. The absolute cell count for each type of cell was calculated by multiplying the percentage of the type of cell by the overall WBC estimate.

Assessment of brain redox indices: Reduced glutathione concentration was estimated using the method of Beutler (Beutler *et al*., 1963), and the assessment of lipid peroxidation was carried out using the method described by Varshney and Kale (Varshney & Kale, 1990). Superoxide dismutase activity was measured using the method of Misra and Fridovich (Misra & Fridovich, 1972) while Myeloperoxidase (MPO) activity, an indicator of polymorphonuclear leukocyte accumulation and oxidative stress was evaluated according to the method of Eiserich and others (Eiserich *et al*., 1998).

Assessment of markers of neurochemical disturbances: The effect of diabetes and EG treatment on brain Na+ K+ ATPase activity was evaluated as previously described (Svoboda & Mosinger, 1981). Acetylcholinesterase (AChE) activity in brain homogenate was measured as previously described (Ellman *et al*., 1961). The turnover of the striatal catecholamine, dopamine, was estimated by measuring the activity of tyrosine hydroxylase (Shiman *et al*., 1971), the level of dopamine (Guo *et al*., 2009), and the activity of MAO (Holt *et al*., 1997) using previously established methods.

Statistical analysis: Results were expressed as mean ± standard deviation (SD). All data were analyzed using one-way analysis of variance (ANOVA) followed by Tukey's test. Values of $p < 0.05$ were considered statistically significant. All the statistical analyses were performed using GraphPad Prism 6 software (GraphPad Software Inc., San Diego, CA, USA).

RESULTS

The phytoconstituents and their respective concentrations in the extract as revealed by HPLC-DAD fingerprinting in Table 1 show that ergosterol (10.55 ± 0.15 mg/g) and catechin (10.05 ± 0.10 mg/g) were the most abundant phytochemicals in the plant extract. Pinitol and quercetin were also detected.

Table 1:
HPLC-DAD quantified compounds of *Eucalyptus globulus* ethanol leaf extract

Compounds	Concentration (mg/g)
Catechin	10.05±0.10
Catechol	2.19±0.04
Catecholamine	2.71±0.03
Catechutannic acid	0.20±0.01
Quercetin	2.41±0.13
Ergosterol	10.55±0.15
Apigenin	0.70±0.02
Luteolin	0.29±0.01
Robinetinidol	0.24±0.08
Pinitol	0.18±0.001

Results are expressed as mean ± standard deviations of two determinations.

Table 2:
Effect of *Eucalyptus globulus* leaf extract on weight change and serum glucose of fructose/streptozotocin-induced diabetic rats

Group	Weight Gain/ Loss (g)	Glucose concentration (mg/dl)
Negative control	4.00±1.66	43.17±11.17
Diabetic control	-28.43±7.8$^{\#\#\#\#}$	258.30±2.28$^{\#\#\#\#}$
Diabetic + 100EG	11.8±2.21****	150.93±17.21****
Diabetic + 200EG	13.34±3.54****	133.05±4.99****
Diabetic + 400EG	16.86±5.66****	103.24±7.95****
Diabetic + GLI	-2.66±0.15****	94.92±9.39****
100EG	2.00±0.24****	42.74±6.81****
200EG	4.00±0.33****	38.42±5.02****
400EG	7.67±1.67****	34.75±7.81****

Results are expressed as Mean ± SD (n=6). $^{\#\#\#\#}P<0.0001$ vs negative control; $^{****}P<0.0001$ vs diabetic control. EG: ethanol leaf extract of Eucalyptus globulus , GLI: Glibenclamide, 100EG: 100 mg/kg *Eucalyptus globulus* ethanol leaf extract, 200EG: 200 mg/kg *Eucalyptus globulus* ethanol leaf extract, 400EG: 400 mg/kg *Eucalyptus globulus* ethanol leaf extract.

Table 3:
Effect of EG on serum sodium and potassium ion levels of diabetic rats

GROUP	K^+ (mEq/l)	Na^+ (mEq/l)
Negative control	6.05±0.15	121.54±0.64
Diabetic control	1.89±0.14$^{\#\#\#\#}$	80.12±2.35$^{\#\#\#\#}$
Diabetic + 100EG	3.53±0.12****	93.43±0.64****
Diabetic + 200EG	3.75±0.20****	96.43±0.43****
Diabetic + 400EG	4.22±0.38****	102.10±0.64****
Diabetic + GLI	4.55±0.25****	100.82±0.21****
100EG	6.02±0.09****	120.86±0.21****
200EG	6.00±0.06****	117.08±0.43****
400EG	6.10±0.08****	123.35±0.43****

Results are expressed as Mean ± SD (n=6). $^{\#\#\#\#}P<0.0001$ versus negative control; $^{****}P<0.0001$ versus diabetic control; EG: ethanol leaf extract of Eucalyptus globulus , GLI: Glibenclamide, 100EG: 100 mg/kg Eucalyptus globulus ethanol leaf extract, 200EG: 200 mg/kg Eucalyptus globulus ethanol leaf extract, 400EG: 400 mg/kg Eucalyptus globulus ethanol leaf extract, K^+: potassium ion and Na^+: sodium ion.

Table 4:
Effect of *Eucalyptus globulus* on the hematological indices of fructose/streptozotocin-induced diabetic rats

GROUP	PCV %	HB g/dl	RBC1x0^9/L	WBC x 10^9/L	Neu %	Lym %
Negative control	42.33 ± 2.12	14.43±0.56	4.47±0.35	6700 ± 494	22.33 ± 0.71	74.67 ± 2.83
Diabetic control	27.00 ± 1.41$^{\#\#\#\#}$	11.4±0.21$^{\#\#\#\#}$	2.63±0.09$^{\#\#\#\#}$	4233 ± 251$^{\#\#\#\#}$	35.33 ± 1.41$^{\#\#\#\#}$	62.33 ± 2.82$^{\#\#\#\#}$
Diabetic + 100EG	35.67±0.70****	12.87±0.07***	3.59±0.32**	4433 ± 70	28.33 ± 0.71****	69.33 ± 1.15****
Diabetic + 200EG	37.00±0.70****	13.56±0.07****	3.72±0.21***	4933 ± 115	26.00 ± 1.41****	71.33 ± 1.52****
Diabetic + 400EG	38.67±2.12****	13.96±0.28****	4.11±0.24****	5700 ± 353****	25.33 ± 2.82****	72.67 ± 2.12****
Diabetic + GLI	34.00±0.00****	12.03±0.35****	3.47±0.08**	5900 ± 251****	22.67 ± 2.82****	73.00 ± 2.12****
100EG	35.67±0.70****	12.87±0.07***	3.59±0.32**	6733 ± 494****	21.67 ± 0.71****	76.33 ± 1.52****
200EG	42.00±2.12****	14.83±0.29****	4.33±0.15****	6766 ± 636****	21.33 ± 0.71****	75.33 ± 1.41****
400EG	43.67±2.12****	14.9±0.07****	4.56±0.21****	6833 ± 353****	20.67 ± 1.41****	75.67 ± 1.15****

Results are expressed as Mean ± SD (n=6). $^{\#\#\#\#}$P<0.0001 versus negative control; **P<0.01, ***P<0.001, ****P<0.0001 versus diabetic control; EG: ethanol leaf extract of *Eucalyptus globulus* , GLI: Glibenclamide, 100EG: 100 mg/kg *Eucalyptus globulus* ethanol leaf extract, 200EG: 200 mg/kg *Eucalyptus globulus* ethanol leaf extract, 400EG: 400 mg/kg *Eucalyptus globulus* ethanol leaf extract, PCV: packed count volume, HB: hemoglobin, RBC: red blood cell, WBC: white blood cell count, NEU: neutrophils and LYM: lymphocytes.

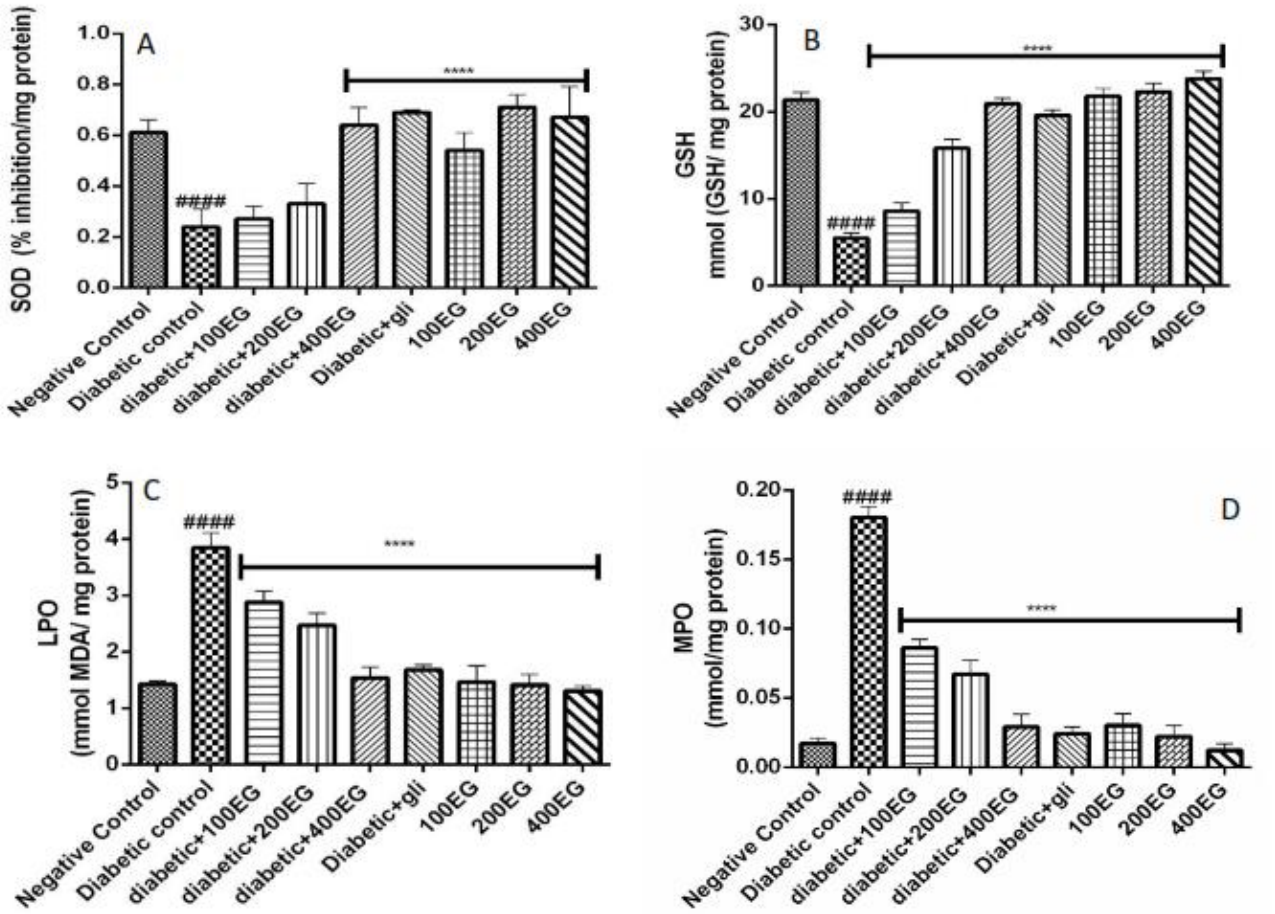

Figure 1:
Effect of *Eucalyptus globulus* on redox stress in the brain tissue of fructose/streptozotocin-induced diabetic rats. (A) superoxide dismutase activity, (B) reduced glutathione concentration, (C) extent of lipid peroxidation and (D) myeloperoxidase activity. Each bar is expressed as mean ± standard deviation (n=6). ####P<0.0001 vs. negative control; ****P<0.0001 vs. diabetic control; GLI: Glibenclamide, 100EG: 100 mg/kg *Eucalyptus globulus* ethanol leaf extract, 200EG: 200 mg/kg *Eucalyptus globulus* ethanol leaf extract, 400EG: 400 mg/kg *Eucalyptus globulus* ethanol leaf extract, SOD: superoxide dismutase, GSH: reduced glutathione, LPO: lipid peroxidation, MDA: malondialdehyde, MPO: myeloperoxidase.

The induction of diabetes caused a significant ($p < 0.0001$) decrease in the weight of the animals while EG treatment resulted in a significant ($p < 0.0001$) gain in weight. EG at 400 mg/kg produced a 12% weight gain but treatment with glibenclamide did not correct the loss in weight. EG administration to diabetic rats significantly ($p < 0.0001$) ameliorated the induced hyperglycemia (Table 2). The groups administered glibenclamide had the least serum glucose level at the end of the experiment which was not significantly different from the glucose level of animals administered 400 mg/kg EG.

Hyponatremia and hypokalemia were observed in the untreated diabetic group as shown in Table 3. Treatment with the extract and glibenclamide ameliorated the electrolyte disturbances. The effect of the ethanolic extract of E. globulus on the hematological parameters of normal

and diabetic rats is presented in Table 4. Untreated diabetic rats showed a significant decrease ($p<0.0001$) in packed cell volume, red blood cells, white blood cells, hemoglobin, and lymphocytes but increased neutrophils when compared with the normal control group. Administration of ethanolic extract of E. globulus ameliorated these changes in hematological indices.

Figure 1 shows that induction of diabetes led to brain redox imbalance as revealed by the decreased SOD activity (Figure 1A) and GSH level (Figure 1B), and the increased lipid peroxidation (Figure 1C) and myeloperoxidase activity (Figure 1D). This redox imbalance was ablated by treatment with EG. The 400 mg/kg extract-treated group showed the highest anti-oxidative stress activity.

As shown in Figure 2, altered brain neurochemistry was reflected in decreased Na+K+ ATPase activity (Figure 2A), increased acetylcholinesterase activity (Figure 2B), decreased tyrosine hydroxylase activity (Figure 2C), decreased dopamine level (Figure 2D), and increased monoamine oxidase activity (Figure 2E) in the brain of diabetic control animals compared with the normal control. These alterations were ameliorated in diabetic animals administered EG with the 400 mg/kg dose presenting the best activity.

DISCUSSION

Poor control of hyperglycemia is associated with the development and progression of complications such as brain dysfunction in diabetic patients. Progressive cognitive deterioration, low intelligent quotient, neurodegeneration, dementia, and brain atrophy are frequent consequences of diabetes mellitus (Hamed, 2017). Mechanisms of brain injury in diabetes mellitus include inflammation, increased acetylcholinesterase (AChE) activity, defective neurotrophic factors, neurotransmitter changes, and other vascular risk factors. Decreased level of acetylcholine was reported to contribute to cognitive deficits observed in diabetic rats (Maciel *et al*., 2016). Results from the present study support these observations.

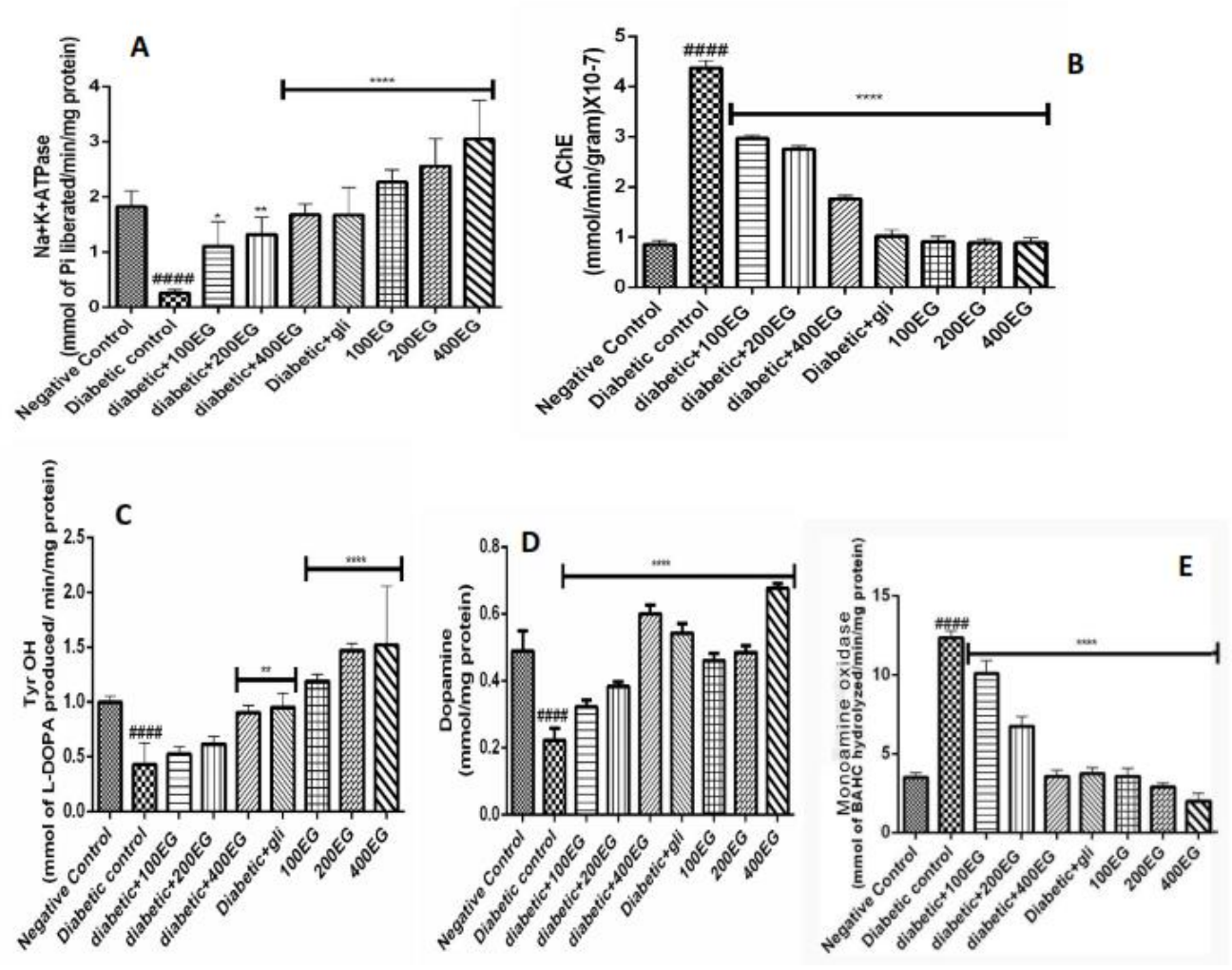

Figure 2:
Effect of *Eucalyptus globulus* on altered brain chemistry of fructose/streptozotocin-induced diabetic rats. (A) Na^+K^+ATPase activity, (B) acetylcholinesterase activity, (C) tyrosine hydroxylase activity, (D) dopamine level, and (E) monoamine oxidase activity. Each bar is expressed as mean ± standard deviation (n=6). $^{####}P<0.0001$ vs negative control; $^{****}P<0.0001$ vs. diabetic control; GLI: Glibenclamide, 100EG: 100 mg/kg *Eucalyptus globulus* ethanol leaf extract, 200EG: 200 mg/kg *Eucalyptus globulus* ethanol leaf extract, 400EG: 400 mg/kg *Eucalyptus globulus* ethanol leaf extract, Na^+P^+ATPase: sodium-potassium adenosine triphosphatase, AChE: acetylcholinesterase, Tyr OH: Tyrosine hydroxylase.

Some of the detected phytoconstituents in the extract have been shown to have antidiabetic potential. Ergosterol may be a potential hypoglycemic agent for the treatment of type-2 diabetes mellitus with the probable mechanism of stimulating GLUT4 translocation and expression. Catechin, a flavonoid polyphenol has been reported to possess antidiabetic property (Mrabti *et al*., 2018). The neuroprotective effect of catechin has also been documented (Cheruku *et al*., 2018; Jiang *et al*., 2017). Pinitol and quercetin have been reported to possess antidiabetic effects. D-Pinitol showed an antihyperlipidemic effect in STZ-induced type-2 diabetes mellitus (Geethan & Prince, 2008) and prevented lipid peroxidation by increasing cellular antioxidant levels (Rengarajan *et al*., 2015) while quercetin was reported to lower serum glucose at a dose as low as 10 mg/kg (Bule *et al*., 2019). The presence of Pinitol, Ergosterol, and Catechin in the E. globulus leaf extract could have contributed to the observed antidiabetic and neuroprotective effects.

EG administration to diabetic rats ameliorated the induced hyperglycemia. Of note is the observation that the effectiveness of the extract at 400 mg/kg was comparable to that of glibenclamide, the reference standard drug. Also, the improvement of weight abnormalities of diabetic rats by EG supports its antidiabetic activity. Weight changes may accompany diabetes mellitus as observed in the present study. This may result from aberrations in lipid and protein catabolism.

Treatment with the extract and glibenclamide ameliorated the electrolyte disturbances. Electrolyte disturbances are features of diabetes and are also associated with brain dysfunction. Electrolytes are involved in controlling fluid levels, acid-base balance (pH), nerve conduction, blood clotting, and muscle contraction. Potassium, sodium, and calcium are all important for proper electrolyte balance (Khanduker *et al*., 2017). In this study, the induction of diabetes caused an abnormally low level of potassium ions and sodium ions in the serum of diabetic rats. Hyponatremia is the most common electrolyte abnormality in clinical practice and is associated with increased morbidity and mortality (Waikar *et al*., 2009). Imbalance in sodium level is associated with an increased probability of adverse outcomes such as cognitive impairment, osteoporosis, and fracture (Podestà *et al*., 2015). Hypokalemia is associated with impaired insulin secretion and decreased peripheral glucose utilization resulting in carbohydrate intolerance and hyperglycemia (Khanduker *et al*., 2017). Amelioration of these conditions by EG treatment indicates its antidiabetic and neuroprotective effects.

Untreated diabetic rats showed alterations in hematological indices which were ameliorated by the administration of EG. White blood cell and platelet abnormalities are common among people with diabetes. The reduction in the total white blood cell count of the diabetic control group indicated a weak immunity against infection and diseases. Neutrophils participate in both protective and detrimental responses to a diverse array of inflammations and infections (Witter *et al*., 2016). Chronic inflammation in adipose tissue is considered a crucial risk factor for the development of insulin resistance and type-2 diabetes in obese individuals (Zatterale *et al*., 2020). An increase in the neutrophil count of the diabetic control group in this study is an indication of inflammation which was ameliorated by treatment with EG. Anaemia is a common phenomenon in diabetes and the main factor contributing to its prevalence in diabetes is the inability of the kidney to increase erythropoietin secretion in response to decreased hemoglobin (Obafemi *et al*., 2017). The occurrence of anemia in diabetic conditions has been established (Barbieri *et al*., 2015) and this is in agreement with the report of this study. Anemia or low hematocrit has been associated with impaired brain function, neurological injury, and increased mortality, indicating that the brain is vulnerable to anemia-induced injury (Hare, 2004). The increase in packed cell volume, red blood cell count, and hemoglobin level in all the EG-treated groups suggests the neuroprotective effect of EG in diabetic rats.

The induction of diabetes led to brain redox imbalance which was ablated by treatment with EG. The 400 mg/kg extract-treated group showed a protective effect. Myeloperoxidase (MPO) is a mammalian pro-oxidant and pro-inflammatory enzyme mainly released by activated neutrophils that have been implicated in the progression of diabetes (Adedara*et al*., 2019a). The observed decrease in the activity of MPO in the brain tissue after treatment with EG along with the observed decrease in neutrophil counts in all EG treated groups supports the anti-inflammatory and antioxidative activities of the extract.

The altered brain neurochemistry of diabetic control animals was ameliorated in diabetic animals administered EG. Altered function of the electrogenic transmembrane ATPase, Na+K+ ATPase, is common in brain dysfunctions (Ojo *et al*., 2019; Pop-Busui *et al*., 2017). Sodium pump dysfunction may lead to neuronal depolarization, release of excess neurotransmitters (such as glutamate), and neuronal excitotoxicity. The reduction in the activity of Na+K+ ATPase observed in untreated diabetic rats and the increase in the activity of the enzymes in EG-treated animals support the neuroprotective potential of the extract. Furthermore, disturbed neurotransmitter metabolism in diabetic conditions and its various consequences on neurological functions have been documented (Parashar *et al*., 2018; Prabhakar *et al*., 2015). Dopamine functions as a neurotransmitter with regulatory functions in movement, memory, pleasurable reward, behavior and cognition, and attention. The direct precursor of dopamine, L-DOPA, is generated via the action of tyrosine hydroxylase that catalyzes the conversion of L-Tyrosine to L-DOPA. Monoamine oxidase on the other hand is involved in the catabolism of dopamine. Acetylcholinesterase is a neurotransmitter metabolizing enzyme. It catalyzes the breakdown of acetylcholine during the process of neurotransmission. The observed increase in the activity of acetylcholinesterase in diabetic rats in this present study corroborates earlier findings (Adedara *et al*., 2019a, 2019b; Rajput & Sarkar, 2017). Both dopaminergic and acetylcholinergic neurotransmission critically modulate synaptic transmission, plasticity, and motor coordination (Ojo *et al*., 2019). Treatment with EG reduced the activity of acetylcholinesterase thereby making more acetylcholine available in the synapses for neurotransmission. EG treatment also increased the production of dopamine via optimization of tyrosine hydroxylase activity with simultaneous suppression of monoamine oxidase activity. The attenuation of diabetes-induced neurotransmitter

dysregulation by EG treatment further shows its beneficial property in diabetic brain dysfunction.

In conclusion, this study has revealed the neuropharmacological property of *Eucalyptus globulus* in a model of type-2 diabetes. Results from this research revealed the antidiabetic property of the extract and its neuroprotective property against diabetes-induced brain redox stress and altered neurochemistry. The plant is therefore of potential therapeutic relevance in managing diabetic complications, especially diabetic brain injury. Further studies on the neuroprotective effect of *Eucalyptus globulus* phytoconstituents are recommended to give insights into the roles and contributions of the compounds identified in the extracts.

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