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Neuroprotective effect of *Lannea egregia* Alkaloid-rich leaf extracts in streptozotocin-induced diabetic rats

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ABSTRACT

Background: Studies suggest that medicinal plant extracts can help reduce the neuron degeneration associated with diabetes. In this study, the neuroprotective effect of the alkaloid-rich extract from the leaves of *Lannea egregia* was assessed in rats with diabetes induced by streptozotocin (STZ).
Methods: Lannea egregia alkaloid-rich analysis was carried out via a known procedure. The rats were randomly assigned into five treatment groups (n = 8): normal control, diabetic-induced rats (45 mg/kg STZ), and diabetic rats treated with low doses of *Lannea egregia* leaf alkaloid-rich extract (50 mg/kg b.w, LEL) and high (100 mg/kg b.w, LEH) (300 mg/kg and 150 mg/kg), and metformin (200 mg/kg). On 22nd day of the experiment, animals were sacrificed, and their blood and brains were collected for neuro-biomarker analysis.
Results: Diabetic-induced rats that received metformin, LEL and LEH exhibited considerably reduced levels of dopamine, serotonin, norepinephrine, NO, MDA, and AChE, BChE activities when compared to untreated diabetic animals. Additionally, rats with diabetes that received treatment with metformin, LEL and LEH displayed a noticeable increase in ENTPDase, Na/K ATPase, GST, CAT, GPx, and SOD activities when compared to the untreated diabetic rats. Histological examination revealed improved brain architecture in the treated groups in contrast to those in diabetic-induced rats.
Conclusion: The alkaloid-rich extracts of *Lannea egregia* might be effective in normalizing brain damage caused by complications of diabetes mellitus.

1. Background

Diabetes mellitus (DM) is one of the oldest human disorders. The difference between type 1 and type 2 DM was distinctly established in 1936. Type 2 diabetes, the most common form, is marked by hyperglycemia, impaired insulin secretion and insulin resistance [13]. Diabetes mellitus is a metabolic condition of chronic hyperglycemia characterized by abnormalities in the metabolism of carbohydrates, proteins, and fats caused by absolute or relative insulin insufficiency along with organ system failure [6]. According to a prevalence estimate provided by the International Diabetes Federation (IDF), over 400 million people globally are estimated to have diabetes mellitus. By the year 2040, this number is projected to increase to 640 million, with nearly half of these individuals remaining undiagnosed. A sizable portion of this sum comes from the growing economies of Asia and Africa (Papatheodurou *et al.,* 2018; [43]). With the increasing prevalence of this disease, DM complications are also becoming a greater burden. There are now reports of high incidences of kidney illness,

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cerebrovascular disease, heart issues, and amputations caused by DM in communities that were not previously recognized to have these difficult health issues [43]. Patients with type 1 or type 2 diabetes frequently experience diabetes complications, which are a major cause of morbidity and mortality. The long-term consequences of diabetes include peripheral artery disease (PAD), diabetic foot syndrome, nephropathy, neuropathy, retinopathy, and cardiovascular disease (Papatheodurou *et al.,* 2018).

Diabetes has been linked to cognitive dysfunction since it was first recognized as a contributor to Alzheimer's disease, vascular dementia, and other kinds of dementia in 1922 [28]. While maintaining a stable blood sugar level is necessary for cerebral metabolism and neuronal activity, prolonged low or high glucose levels as well as rapid glucose fluctuations can cause neuronal injury. Diabetes can also exacerbate brain damage by increasing the production of reactive oxygen species (ROS) and decreasing antioxidant enzyme activities [24]. When hyperglycemia occurs, antioxidant levels decrease, and free radical generation increases. These outcomes result in modifications in the cell's redox potential and the consequent activation of redox-sensitive genes, which contribute to tissue damage in diabetes mellitus [28]. Although insulin is well known for its role in controlling the metabolism of glucose in tissues, this hormone also has a significant impact on a variety of mental processes, including cognition, memory, and synaptic plasticity, via intricate insulin/insulin receptor (IR) communication networks. Therefore, it is plausible that aberrant insulin signaling in diabetes patients could impact mitochondrial function and cause an increase in oxidative stress, which plays a role in the etiology of neurological illnesses and causes neurodegeneration [32].

The use of STZ to induce diabetes to delineate the complications associated with diabetes and the ameliorative effect of new compounds and registered drugs is an interesting area of research as a result of the increased global rise in diabetes. Proper functioning of brain cells requires high levels of glucose; however, the cells are stressed in the case of uncontrolled hyperglycemia of the brain, referred to as glucose neurotoxicity (Ebokaiwe *et al.,* 2020). The cognitive dysfunction associated with diabetes is characterized by significant changes in the synaptic plasticity of the hippocampal region of the brain, which is essential for memory formation in animals. This further reduced locomotion frequency, decreased limb grip, dysfunctional short-term memory, depression, and impaired coordination of the nervous and muscular junctions. These findings explain the role of impaired central insulin signaling in diabetes-induced brain injury (Garcia-serrano and Duarte, 2020).

Lannea egregia Engl. K. Krause is an Anacardiaceae family tropical woody perennial plant with alternate leaves that grows in savanna areas. The plant is approximately 13 m tall spanning Guinea, Ghana, and Nigeria, the grassy plains of the West African zones contains approximately 40 different species of Lannea [29]. *L. egregia* has been used in traditional African medicine to treat a variety of human illnesses. In Ghana, Guinea, Nigeria, Burkina Faso, and the Central African Republic, the plant is typically used to treat cancer, hernia, hemorrhoids, ulcers, sores, leprosy, gastric pains, diarrhea, edema, paralysis, epilepsy, rheumatism, gonorrhea, respiratory tract diseases, madness, and postnatal problems [22,33]. *L. egregia* is also known as "ekudan" (Yoruba in Nigeria), "sambituliga" (Ivory Coast), and "tiuko" (Guinea). This plant is endowed with different phytochemicals, including alkaloids. Alkaloids have been reported to have high lipid solubility and the ability to cross the blood-brain barrier (BBB) [45].

Streptozotocin (STZ) is a chemical commonly used to induce diabetes in laboratory animals by reducing nicotinamide adenine dinucleotide in pancreatic beta-cells, leading to the destruction of beta-cells and subsequent insulin deficiency [16]. This chemical is produced by the bacterium *Streptomyces achromogenes* and has been found to have a wide range of antibacterial activities. However, due to its severe beta cell toxicity, STZ is also utilized to treat pancreatic islet metastatic cancer [1]. It is noteworthy that the use of STZ to induce diabetes in laboratory animals is common and is used to mimic diabetes mellitus in humans. Furthermore, STZ treatment of rats has been found to cause inflammation, oxidative stress, and other pathologies resembling Alzheimer's disease (AD). These pathologies lead to the loss of neuronal cells, cognitive dysfunctions, and learning deficits. It is important to note that these findings suggest that STZ-induced diabetes in laboratory animals can also serve as a model for studying the pathophysiology of AD and other neurodegenerative diseases [10]. Hence, this study investigated the neuroprotective effect of *L. egregia* alkaloid-rich extract in streptozotocin-induced diabetic rats.

2. Methods

2.1. Identification of the sources of plant materials

L. egregia leaves were purchased from a farmland in Ile-Igbon, along Iwo in Ibadan, Oyo State, Nigeria. The Forestry Research Institute of Nigeria (FRIN), Ibadan, Oyo State, Nigeria, identified and verified the leaf of *L. egregia*. The institution gave FHI: 113156 as the voucher number.

2.2. Chemicals, reagents and enzyme kits

The reagents used in the study were of analytical grade, and all chemicals were purchased from Sigma—Aldrich, Chemie Gesellschaft mit beschränkter Haftung (Steinheim, Germany). The Randox Laboratory, 55 Diamond Road, Crumlin, County Antrim, BT29 4QY, UK, provided the biochemical parameter kits.

2.3. Alkaloid extract preparation from Lannea egregia leaf

To achieve a constant weight, *Lannea egregia* leaves were dried at 25°C over a period of 14 days. It was then pulverized through the use of an electric blender to create a fine powder.

Thereafter, 100 g of the sample was soaked in n-hexane daily. Fifty grams of this solution was added to a mixture of ethanol and acetic acid (500 mL) at 9:1 v/v. For total extraction, the sample was left in an orbital shaker for 24 hours. Subsequently, the solution was filtered, and the filtrate concentrated via a rotary evaporator. Finally, the filtrate was treated with conc. ammonium hydroxide (NH_4OH) to enhance the separation according to Adefegha *et al.* (2017).

2.4. Animals and induction of diabetes model

Male Wistar rats (40) with a mean weight between 100 and 130 g were acquired from the Animal facility of Ekiti State University, Ado-Ekiti, Ekiti State, Nigeria. The rats were placed on rat pellet chow with an ambient temperature of 22±20°C and a regular light/dark rhythm as well as 20 % w/v fructose [36]. The rats were intraperitoneally injected with STZ (dissolved in sodium citrate buffer (pH 4.5) at a dose of 45 mg/kg b.w. It is noteworthy that before the induction of diabetes mellitus, the rats were fasted overnight, and their fasting blood glucose levels were assessed using an Accu Check Glucometer. Hence, the fasting blood glucose level of each animal was determined 72 hours post induction, and those with values $\geq$ 250 mg/dL [4] were considered to diabetic and were used in our study. The animals were then divided as follows (n = 8):

Group A: Uninduced rats (normal control);

Group B: Untreated Diabetic rats (diabetic control);

Group C: Treated diabetic rats given 50 mg/kg b.w alkaloid-rich extract of *Lannea egregia* leaf which is considered a low dose (LEL)

Group D: Treated diabetic rats given 100 mg/kg b.w alkaloid-rich extract of *Lannea egregia* leaf which is considered a high dose (LEH)

Group E: Treated diabetic rats given 200 mg/kg metformin (MET).

The dose used in this study was selected based on previously published work by Ajiboye et al. [3] and preliminary acute toxicity study.

2.5. Animal sacrificing

The treatments were administered for 20 days, and the animals were fasted overnight (7.00 pm to 8.00 am) on the 21st day of the experiment; thus, the animals were euthanized by cervical dislocation. The brain of each animal was excised, cleaned of blood, homogenized in potassium phosphate buffer. The homogenized tissue was then spun at 4000 rpm for 15 minutes and finally stored in a freezer (-40°C) for biochemical analysis.

2.6. Biochemical parameters studied

2.6.1. Determination of neurotransmitter levels

The level of several neurotransmitters (dopamine, serotonin, and norepinephrine) in the brain homogenates was measured via commercial ELISA kits (Cusabio; Houston, TX, USA) [38]. The neurotransmitters such as serotonin, dopamine and norepinephrine were examined in this study.

2.6.2. Determination of cholinesterase activity

The activities of acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) activities in the homogenized brain were determined by the protocol of Ellman et al. [14]. 'Briefly, 50 mL of brain homogenate was mixed with 50 mL of 2-nitrobenzene acid and 175 mL of 0.1 mol/l phosphate-buffered saline (pH 8.0), and incubated at 25°C for 20 minutes. Then, 25 mL each of acetylthiocholine iodide and butyrylthiocholine iodide were added. Absorbance was measured at 412 nm using a microplate reader.

2.6.3. Determination of nitric oxide levels

The protocol described by Montgomery and Dymock [26] was used in this assay. Briefly, 0.1 mL of brain tissue supernatant was added to both sample and blank test tubes. Thus, 0.1 mL of reagent R1 (sodium nitrite) of standard or block standard was added to the respective test tube. Hence, 0.1 mL of the reagent R2 (sulfanilamide) was added to all the test tubes. Each test tube was mixed thoroughly and left to stand for 5 minutes. Thereafter, 0.1 mL of reagent 3 (N – (1 – naphthyl) - ethylenediamine (NEDA)) was added to both the sample and standard test tubes. The solution was mixed thoroughly, and the absorbance was read spectrophotometrically at 540 nm.

2.6.4. Determination of Na^+/K^+ ATPase activity

This was described by Bewaji *et al.* (1985). ATPase activity was spectrophotometrically assayed in a medium composed of 120 mM KCl, 30 mM HEPES (pH 7.4), 3 mM $MgCl_2$, 0.2 mM $CaCl_2$, 0.1 mM EGTA, and approximately 100–200 μg of erythrocyte membrane protein, in a total volume of 0.8 mL. The reaction was initiated by the addition of ATP and terminated by the addition of 0.2 mL of a 5 % solution of sodium dodecyl sulfate. Inorganic phosphate liberated was quantified using a modified Fiske-Subbarow method (1925). Ascorbic acid served as the reducing agent, and the resulting blue color was measured at 820 nm after 30 minutes using a spectrophotometer. Control experiments without the enzyme were performed to account for non-enzymatic ATP hydrolysis. Each data point represents the average of three independent assays, with each experiment repeated two to four times.

2.6.5. Determination of Ectonucleoside triphosphate diphosphohydroase (ENTPDase) activity

This was determined according to the procedure reported by Akomolafe et al. [5]. Briefly, 20 μL of brain tissue supernatant was incubated with 200 μL of reaction buffer (containing 1.5 mM CaCl2, 5 mM KCl, 0.1 mM EDTA, 10 mM glucose, 225 mM sucrose, and 45 mM Tris-HCl) at 37°C for 10 minutes. After adding 20 μL of 50 mM ATP, the mixture was shaken at 37°C for 20 minutes. The reaction was stopped with 200 μL of 10 % TCA, then treated with 200 μL of 1.25 % ammonium molybdate and 9 % ascorbic acid, and chilled on ice for 10 minutes. Absorbance was measured at 600 nm.

2.6.6. Determination of Na^+/K^+-ATPase activity

For the test, 400 μL of 200 mM NaCl/40 mM KCl/60 mM Tris (pH 7.4) was added to a test tube, followed by 20 μL each of 80 mM MgCl2·6H2O, 20 mM EGTA, and diluted tissue supernatant and 240 μL of distilled water. After mixing and incubating at 37°C for 5 minutes, 100 μL of 8 mM ATP was added, and the mixture was incubated at 37°C for 30 minutes. Then, 200 μL of 5 % SDS and 2000 μL of reagent C were added, and the mixture was left at room temperature for 30 minutes for color development. A blank was prepared using 20 μL of distilled water instead of tissue supernatant. Absorbance was measured at 820 nm against the blank (Ronner *et al.* 1977).

2.6.7. Determination of antioxidant and oxidative stress biomarkers

The glutathione-S-transferase (GST), catalase (CAT), glutathione peroxidase (GPx), superoxide dismutase (SOD), and malondialdehyde (MDA) levels were determined by following the procedure described using their respective Randox commercial enzyme kits.

2.6.8. Brain histology examination

The brain histology examination was performed according to Sultana et al. [41]. Briefly, brain tissue was preserved in 10 % formalin and embedded in paraffin wax, with 5 μm sections stained with hematoxylin and eosin for histopathology. Tissue blocks were fixed in 2.5 % glutaraldehyde buffered with 0.1 M phosphate overnight at 4°C, then washed and osmicated with 1 % osmium tetroxide for 2 hours. Following washing and dehydration through graded ethanol solutions, the specimens were dried using a critical-point drying apparatus (Polaron SC 7620 Quorum Technologies Sussex United Kingdom). The most damaged area of each tissue block was selected and photographed.

2.7. Statistical analysis

The results are presented as eight replicates with standard deviation (SD) and were analyzed using GraphPad Prism 8. One-way ANOVA followed by the Tukey post hoc test was employed to determine differences with significant at $p < 0.05$.

3. Results

3.1. Preliminary acute toxicology study

As presented in Table 1, both rats that were administered 500 and 1000 mg/kg body weight did not show any sign, induce death or symptoms within 6 hours of administration, suggesting that alkaloid-rich extract of *Lannea egregia* is not toxic at concentrations $\leq$ 1000 mg/kg. All rats displayed normal behavior throughout the study and survived until the end of the 14-day experiment period. During the entire observation period, they did not present any significant clinical alteration. Alkaloid-rich extract of *Lannea egregia* shows that the extract was safe up to the dose of 1000 mg/kg.

Table 1
Acute oral toxicity studies of alkaloid-rich extract of *Lannea egregia*.

Dose (mg/kg)	Number of rats	Mortality	Adverse effect	Post treatment observations (2 weeks)
50	2	0	Nil	Nil
100	2	0	Nil	Nil
500	2	0	Nil	Nil
1000	2	0	Nil	Nil

3.2. Effect of the alkaloid-rich leaf extracts of Lannea egregia on neurotransmitter levels in STZ-induced diabetic rats

The effect of treatment with the alkaloid-rich extract of *Lannea egregia* on the concentrations of neurotransmitters is presented in Fig. 1. This study revealed that the untreated diabetic control group had significantly ($p<0.05$) greater dopamine, norepinephrine, and serotonin levels than the treated groups (LEL, LEH and metformin-treated groups) and normal control group. However, when the animals were treated with LEL and LEH, the neurotransmitter levels (dopamine, norepinephrine and serotonin) were significantly ($p<0.05$) different from those in the untreated diabetic control groups.

Each value is the mean of eight readings ± SD. # $p < 0.05$ vs. NC, * $p < 0.05$ vs. DC.**Legend**: NC: normal control, DC: diabetic control, LEL: diabetic rats treated with a low dose (50 mg/kg body weight) of alkaloid-rich extract of *Lannea egregia,* LEH: diabetic rats treated with a high dose (100 mg/kg body weight) of alkaloid-rich extract of *Lannea egregia*, and MET: diabetic rats given 200 mg/kg metformin

3.3. Effect of Lannea egregia leaf alkaloid-rich extracts on cholinesterase activity in streptozotocin-induced diabetic rats

The effect of treatment with the alkaloid-rich leaf extract of *Lannea egregia* on cholinesterase activity is presented in Fig. 2. In this study, the diabetic control group had greater activities of acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) than the normal control, LEL, LEH and metformin-treated groups. However, in the animals treated with LEL and LEH, the activities of AChE and BChE were significantly ($p < 0.05$) lower than those in the untreated diabetic group.

3.4. Effect of the alkaloid-rich leaf extracts of Lannea egregia on brain nitric oxide levelsin STZ-induced diabetic rats

The effect of treatment with the alkaloid-rich extract of *Lannea egregia* on the level of nitric oxide is presented in Fig. 3. The diabetic control group had a significantly ($p<0.05$) greater level of nitric oxide than the other groups. However, LEL-, LEH- and MET-treated diabetic rats significantly ($p<0.05$) had reduced levels of nitric oxide compared with those in the untreated diabetic group. However, there was no significant ($p>0.05$) difference in the levels of nitric oxide in the LEL-, LEH- or MET- treated group compared with those in the normal control group.

3.5. Effect of the alkaloid-rich leaf extract of Lannea egregia on brain ATPase activityin STZ-induced diabetic rats

The effect of the treatment with an alkaloid-rich leaf extract of *Lannea egregia* on the activities of ATPase, E-NTPDase and Na^+/K^+ ATPase are presented in Fig. 4. The results suggested that the diabetic control group had lower ATPase, E-NTPDase, and Na^+/K^+ ATPase activity than the normal control, LEL, LEH and metformin-treated groups. However, compared with diabetic control rats, LEL-, LEH-, amd MET-treated diabetic rats exhibited a significant ($p<0.05$) increases in Na^+/K^+ ATPase and E-NTPDase activities.

3.6. Effect of the alkaloid-rich leaf extract of Lannea egregia on the oxidative stress biomarkers in the brain of STZ-induced diabetic rats

The effects of treatment with the alkaloid-rich extract of *Lannea egregia* on the specific activities of superoxide dismutase (SOD), glutathione S-transferase (GST), catalase (CAT), and glutathione peroxidase (GPx), and the concentration of malondialdehyde (MDA) are presented in Fig. 5. A significantly ($p<0.05$) greater concentration of MDA was detected in the untreated diabetic group than in the normal control, LEL-, LEH- and metformin-treated groups. However, LEL-, LEH- and metformin-treated diabetic rats exhibited a significant ($p<0.05$) decrease in the MDA concentration compared with that in the diabetic control group. Additionally, at the end of the experimental period, diabetic animals treated with LEL, LEH and metformin exhibited significant ($p<0.05$) increases in the activities of antioxidant enzymes SOD, CAT, GST and GPx compared to those in the untreated diabetic group.

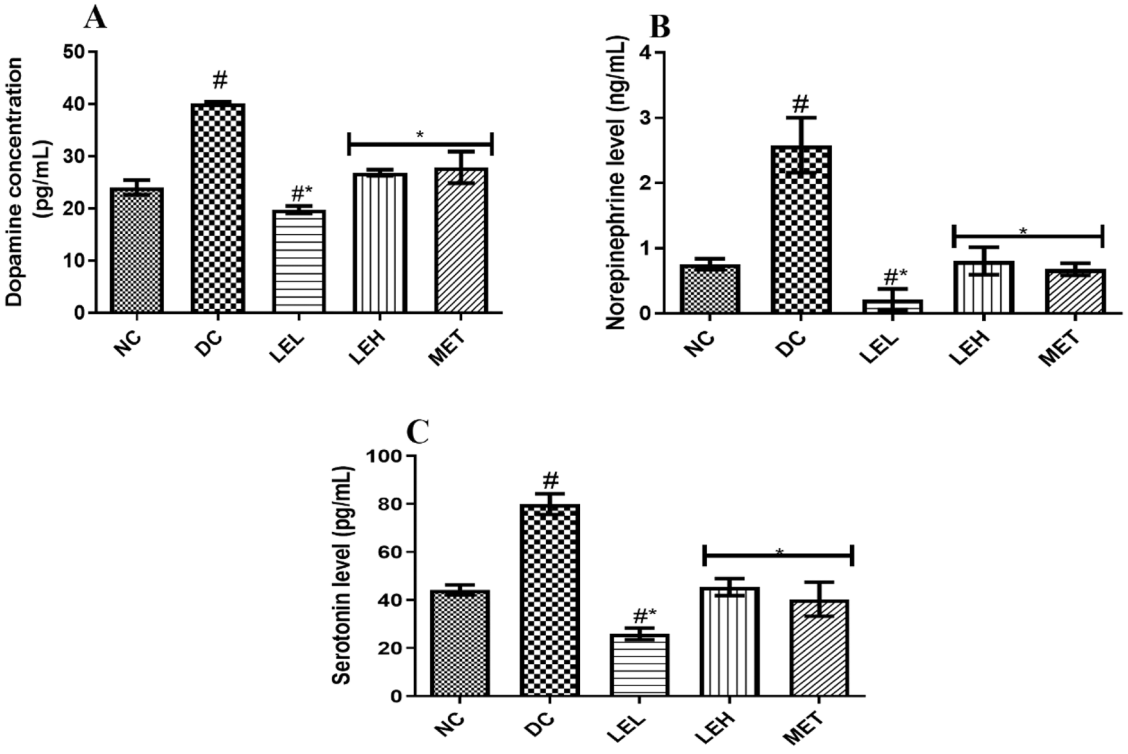

Fig. 1. Neurotransmitter levels in the brains of STZ-induced diabetic rats treated with alkaloid-rich extracts from *Lannea egregia* leaves (a) dopamine, (b) norepinephrine, and (c) serotonin levels.

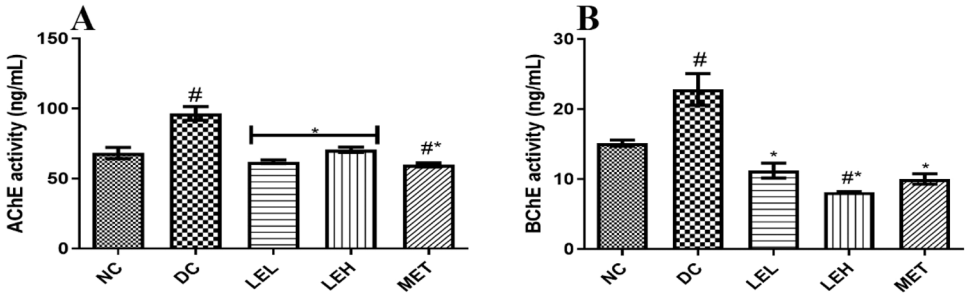

Fig. 2. Cholinesterase activities in STZ-induced diabetic rats after treatment with the alkaloid-rich extracts from *Lannea egregia* leaves (a) acetylcholinesterase and (b) butyrylcholinesyerase activities Data expressed as mean ± SD (n = 8). # $p < 0.05$ vs. NC, * $p < 0.05$ vs. DC.**Legend**: NC: normal control, DC: diabetic control, LEL: diabetic rats treated with a low dose (50 mg/kg body weight) of alkaloid-rich extract of *Lannea egregia,* LEH: diabetic rats treated with a high dose (100 mg/kg body weight) of alkaloid-rich extract of *Lannea egregia*, and MET: diabetic rats given 200 mg/kg metformin, AChE: acetylcholinesterase, and BChE: butyrylcholinesterase.

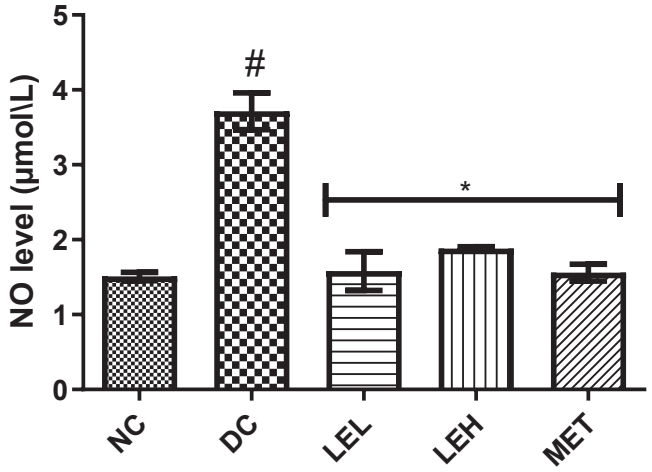

Fig. 3. Brain nitric oxide levels in STZ-induced diabetic rats treated with alkaloid-rich extracts from *Lannea egregia* leaves.Each value is the mean of eight readings ± SD. # $p < 0.05$ vs. NC, * $p < 0.05$ vs. DC.**Legend**: NC: normal control, DC: diabetic control, LEL: diabetic rats treated with a low dose (50 mg/kg body weight) of alkaloid-rich extract of *Lannea egregia,* LEH: diabetic rats treated with a high dose (100 mg/kg body weight) of alkaloid-rich extract of *Lannea egregia*, and MET: diabetic rats given 200 mg/kg metformin, and NO: nitric oxide.

Additionally, the antioxidant activities of the diabetic rats treated with LEL, LEH and metformin compared favorably well with the normal control.

3.7. Effect of alkaloid-rich leaf extracts of Lannea egregia on brain tissue histology in STZ-induced diabetic rats

As illustrated in Fig. 6, the blood vessels in the control group appeared normal, as indicated by the yellow arrows. Surrounding the blood vessels partly is an artifact of fixation in a space without cells or parenchyma. In the untreated diabetic group, STZ induced edema and neuron cell shrinkage, demyelination and axonal degradation. After treatment with LEL and LEH, there was still edema in diabetic rats placed on LEL; however, this edema was absent in LEH-treated diabetic rats.

4. Discussion

Neurotransmitters are natural substances that transmit signals between neurons, muscles, or gland cells at chemical synapses, like neuromuscular junctions [31]. Studies have shown that STZ-induced diabetes raises neurotransmitter levels [15,19]. This abnormal increase is attributed to neuropathic pain, hyperexcitability, and elevated production of reactive oxygen species in diabetic individuals, leading to neuronal degeneration [19,31]. Hussein et al. [20] and Okesola et al. [31] established a relationship between neurotransmitter concentrations and oxidative status, stating that elevated levels of neurotransmitters (serotonin, dopamine and norepinephrine) positively correlate with increased oxidative stress markers (MDA) and negatively correlate with antioxidant enzymes (SOD). The results of this study support this established relationship.

Acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) are crucial hydrolase enzymes (esterases) essential for cognitive functions. Persistent hyperglycemia, especially in individuals with type II diabetes mellitus, can lead to memory loss (Ebokaiwe *et al.,* 2020; [31]). Acetylcholinesterase hydrolyzes acetylcholine (Mushtaq *et al.,* 2014; Ebokaiwe *et al.,* 2020); butyrylcholinesterase, on the other hand, hydrolyzes acetylcholine as well as other choline esters and constitutes approximately 10 % of cholinesterase activity in the temporal cortex [11,44]. The activities of both AChE and BuChE were found to be greater in diabetic patients than in normal controls. This suggests that abnormal plasma levels of AChE and BuChE may serve as markers to predict the development of type 2 diabetes mellitus (Mushtaq *et al.,* 2014). Increased BChE activity is found in both type 1 and 2 forms of the disease in humans [19,44]. Memory loss, cognitive dysfunction, and neurodegenerative diseases may be present in diabetic control rats, as suggested by the increased activities of both AChE and BChE in the present study [18,31]. A decrease in the hydrolysis of acetylcholine, suggested by the inhibited AChE and BChE activity in rats with diabetes administered different concentrations of the alkaloid-rich leaf extract of Lannea egregia, may indicate amelioration of neuronal damage and is associated with the correction of memory loss. This outcome is in agreement with those of Sriraksa et al. [40] and Okesola et al. [31].

Nitric oxide (NO) is an unstable gaseous free radical that performs different functions under physiological and pathological conditions [12]. NO is secreted by the endothelium and has been shown to have anti-inflammatory and proinflammatory functions depending on its concentration [17]. Alterations in bioavailability have been reported in patients with hypertension, atherosclerosis, stroke, diabetes mellitus and its complications. At low concentrations, NO is a mediator of insulin secretion, while at high concentrations, it has cytotoxic effects on beta cells by inhibiting insulin secretion and inducing lipid peroxidation [17,7]. The elevated levels of NO reported in this study support the claims of elevated NO levels in individuals with type 2 diabetes mellitus, which is associated with endothelial dysfunction and beta cell dysfunction and may also be linked to high levels of glucose in individuals with diabetes [2,7]. Notably, the administration of different concentrations of the alkaloid-rich leaf extract of *Lannea egregia* restored the levels of NO, which may be indicative of the anti-inflammatory, antioxidative and insulin-enhancing effects of the plant extract.

Sodium-potassium adenosine triphosphatase (Na^+/K^+-ATPase), also

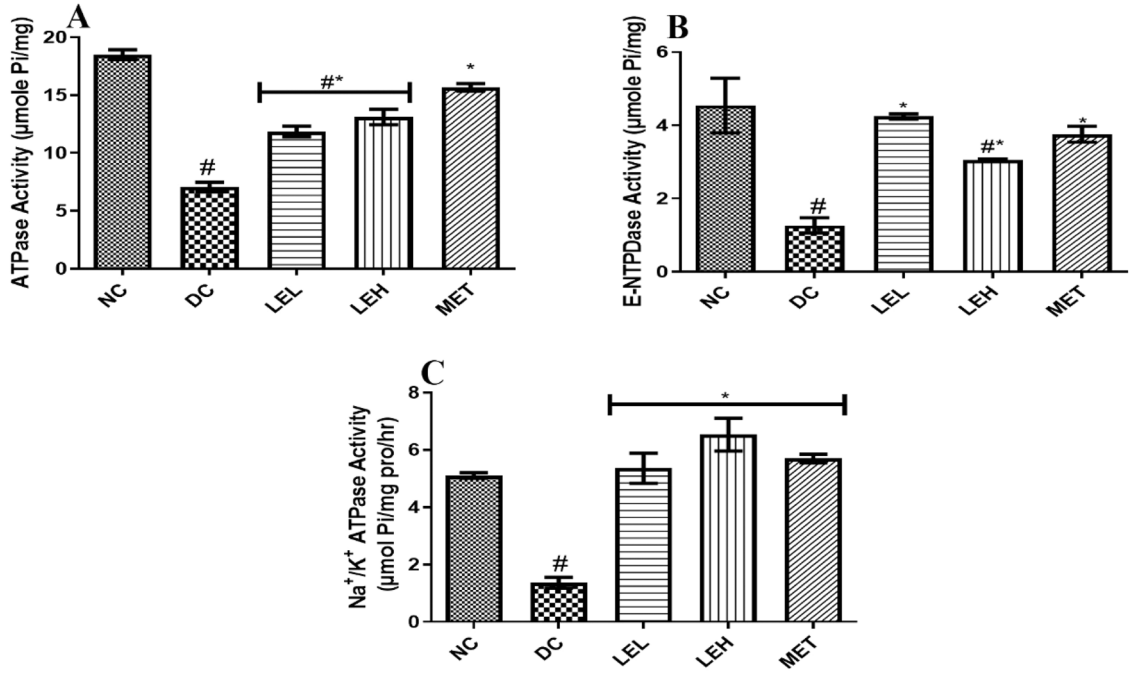

Fig. 4. Some brain ATPase activities of STZ-induced diabetic rats treated with alkaloid-rich extracts from *Lannea egregia* Leaf (a) ATPAse activity, (b) E-NTPDase, and (c) Na^+/K^+ ATPase activities.Each value is the mean of eight readings ± SD. # $p < 0.05$ vs. NC, * $p < 0.05$ vs. DC.**Legend**: NC: normal control, DC: diabetic control, LEL: diabetic rats treated with a low dose (50 mg/kg body weight) of alkaloid-rich extract of *Lannea egregia,* LEH: diabetic rats treated with a high dose (100 mg/kg body weight) of alkaloid-rich extract of *Lannea egregia*, and MET: diabetic rats given 200 mg/kg metformin, and E-NTPDase: Ecto-nucleoside triphosphate diphosphohydrolase.

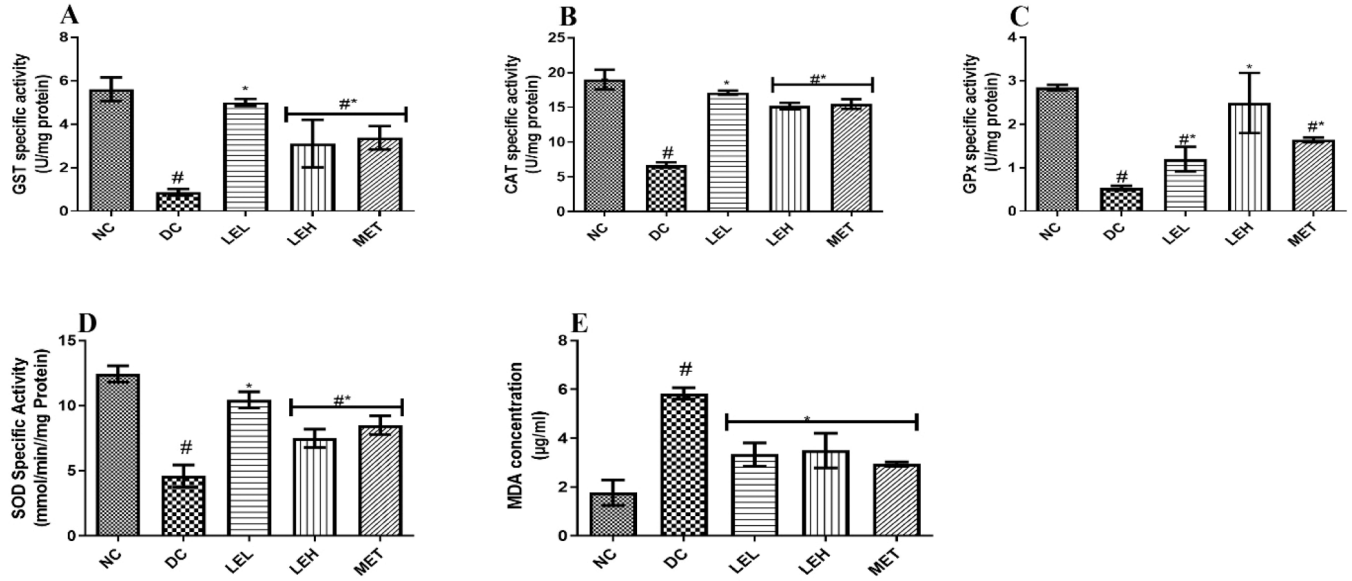

Fig. 5. Oxidative stress biomarker levels in the brains of STZ-induced diabetic rats treated with alkaloid-rich extracts from *Lannea egregia* leaf.Each value is the mean of eight readings ± SD. # $p < 0.05$ vs. NC, * $p < 0.05$ vs. DC.**Legend**: NC: normal control, DC: diabetic control, LEL: diabetic rats treated with a low dose (50 mg/kg body weight) of alkaloid-rich extract of *Lannea egregia,* LEH: diabetic rats treated with a high dose (100 mg/kg body weight) of alkaloid-rich extract of *Lannea egregia*, and MET: diabetic rats given 200 mg/kg metformin, GST: glutathione-S-transferase, CAT: catalase, GPx: glutathione peroxidase, SOD: superoxide dismutase, and *MDA:* malondialdehyde.

known as the sodium-potassium pump, is an electrogenic transmembrane enzyme in animal cell membranes that maintains resting potential and regulates cellular volume. It also regulates the reactive oxygen species, MAPK pathway, and intracellular calcium metabolism, highlighting its role in oxidative stress (Iman-Fulani *et al.,* 2016). Na^+/K^+-ATPase uses the energy from ATP hydrolysis to transport three Na^+ ions out of the cell and two K^+ ions into the cell, creating an electrochemical gradient essential for secondary active transport of nutrients and metabolites, as well as the excitability of neurons and muscle cells [9]. Diabetes significantly impacts the metabolism of various tissues, and since Na^+/K^+-ATPase is essential for maintaining membrane potential, and transport, a change in its activity in diabetic condition has an effect on these tissues. Dysfunction of Na^+/K^+-ATPase activity in the brain is linked to electrophysiological abnormalities,

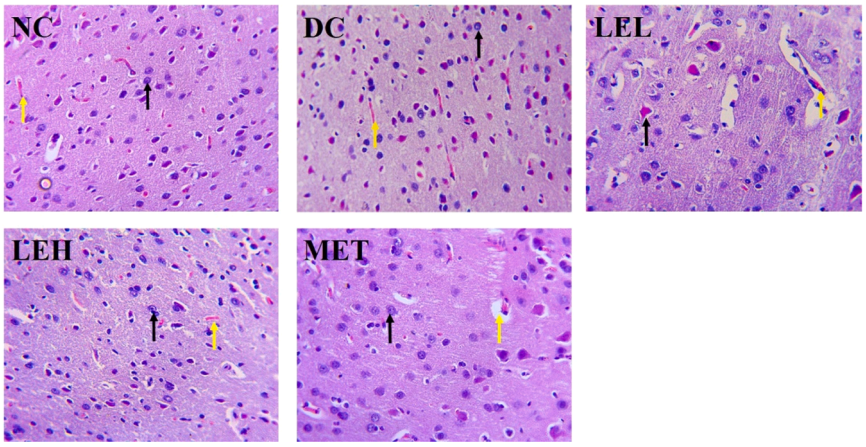

Fig. 6. Brain histological examination of STZ-induced diabetic rats treated with alkaloid-rich extracts from *Lannea egregia* leaves. H&E staining, magnification: x800. **Legend**: NC: normal control, DC: diabetic control, LEL: diabetic rats treated with a low dose (50 mg/kg body weight) of alkaloid-rich extract of *Lannea egregia,* LEH: diabetic rats treated with a high dose (100 mg/kg body weight) of alkaloid-rich extract of *Lannea egregia*, and MET: diabetic rats given 200 mg/kg metformin; black arrow points to neuronal cells while yellow arrow points blood vessel.

resulting in impaired excitation, reduced cerebral reactivity, inadequate nutrient delivery, and decreased cognitive function (Iman-Fulani *et al.,* 2016). Na+/K+-ATPase activity is decreased in the brains of STZ-induced diabetic animals (Iman-Fulani *et al.,* 2016; [35]). The results obtained in this study indicate that Na^{+}/K^{+}-ATPase activity in the treated diabetic animals was greater than that in the animals in the diabetic group, which might be due to reduced insulin levels and reduced glycolysis, which leads to a decrease in ATP levels and enzyme activity. This result is in agreement with the documentation of Iman-Fulani *et al.* (2016), Kherd et al. [23], Safiriyu et al. [35], and Buxbaum [9].

Ectonucleoside triphosphate diphosphohydrolases (ENTPDases) are a group of glycosylated extracellular hydrolases that hydrolyze extracellular adenosine di- and triphosphates to adenosine monophosphate. ENTPDases are activated by millimolar concentrations of either Ca^{2+} or $Mg^{2+,}$ and when these cations are absent, these enzymes show no activity. Schetinger et al., [37]. The communication processes triggered by extracellular adenine nucleotides are regulated by ectonucleotidases, such as ecto nucleoside triphosphate diphosphohydrolase (E-NTPDase) and ecto-5-nucleotidase. Recently, the role of ENTPDase in pathological conditions, such as ischemia, epilepsy, diabetes and cancer, has been investigated [39]. ENTPDase is crucial for regulating adenine nucleotide levels and controlling the duration and extent of receptor activation. In brain injury, there is a massive release of adenine nucleotides, which can accumulate and elevate intracellular calcium levels, thereby increasing cellular damage [39]. Different concentrations of the alkaloid-rich leaf extract of *Lannea egregia* prevented the decrease in ENTPDase in the brain of diabetic animals (Figure 4.4), which is in agreement with the findings of Maciel et al. [25]. This may be due to the antioxidant capacities of the plant extract, as it has been proposed that alterations in enzyme levels could be caused by oxidative stress [39].

Activation of molecular mechanisms associated with oxidative modifications in brain tissues is facilitated by STZ-induced diabetes, which provokes oxidative damage and disrupts the balance between antioxidant enzymes [34]. According to Tiwari et al. [42], hyperglycemia generates reactive oxygen species, which facilitate cellular damage and ultimately result in complications associated with diabetes mellitus. The peroxidation of lipids, which is a result of oxidative stress, produces highly reactive aldehydes, such as malondialdehyde (MDA), acrolein, 4-hydroxynonenal (HNE) and 4-oxononenal (ONE). Decrease in the enzymatic antioxidant defense mechanism suggests an increase in lipid peroxidation, which, in turn, indicates that peroxidative injury may be involved in the development of diabetic complications in patients. This explains the results of this study, where the diabetic rats had higher MDA levels than did the treated groups, and the antioxidant capacity of the alkaloid-rich extract of *Lannea egregia* was demonstrated by reducing the MDA levels in the treatment groups.

In addition to an increase in lipid peroxidation, uncontrolled hyperglycemia also alters the levels of antioxidant enzymes. Antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) and glutathione s-transferase (GST) play important roles in preventing damage caused by oxidative stress in cells (Alkreaty and Ahmad, 2020; [21,30]). As the main regulator of hydrogen peroxide metabolism, catalase plays a crucial role in mitigating oxidative stress-induced complications, including diabetes and cardiovascular diseases [42]. CAT neutralizes hydrogen peroxide in peroxisomes and GPx performs similar task in the cytoplasm (Sadi *et al.,* 2019). SOD facilitates the conversion of superoxide anions into hydrogen peroxide and molecular oxygen ([42]; Sadi *et al.,* 2019). The high flux of glucose through the activated polyol pathway in the diabetic brain may consume NADPH, which results in decreased levels and activities of GPX and GST [21]. The decreased activities of these antioxidants enzymes/molecule in diabetic conditions might result in decreased protection against free radical-induced damage [30]. In this study, the antioxidant potential of the alkaloid-rich extract of *Lannea egregia was* further demonstrated by restoring the antioxidant enzyme levels. This finding also suggested that the plant extract has neuroprotective effects and potential against insulin resistance caused by increased reactive oxygen species levels [30,8]. Furthermore, the results of this study were in agreement with those of Ibrahim and Rizk. [21], Sadi and Konat. [34] and Ogunyinka et al. [30].

Moreover, diabetes mellitus is known to reduce brain glucose transporters and disrupt glucose transport and metabolism, potentially heightening the risk of brain damage [27]. Treatment with the alkaloid-rich leaf extract of *Lannea egregia* was able to restore and reverse the damage to brain tissues of diabetic rats, as revealed by the study's histological findings. This may be due to the beneficial effects of the extracts on oxidative stress indicators and neurotransmitter levels.

5. Conclusion

This study demonstrated that alkaloid-rich leaf extracts of normalizes the levels of neurotransmitters, nitric oxide, oxidative stress and antioxidant biomarkers. The extracts normalized cholinesterase and enhanced the activities of ATPase, ENTPDase, and Na^{+}/K^{+} ATPase. This was supported by the results of the histological examination. Hence, *Lannea egregia* alkaloid-rich extract has a neuroprotective effect on STZ-induced diabetic rats.

Abbreviations

AchE: Acetylcholinesterase
AD: Alzheimer's disease
ATPase: adenosine triphosphatase
BchE: Butyrylcholinesterase
CAT: Catalase
DM: Diabetes mellitus
DNA: Deoxyribonucleic acid
LEH: *Lannea egregia* high dose
LEL: *Lannea egregia* low dose
ENTPDase: Ecto nucleoside triphosphate diphosphohydrolase
GPx: Glutathione peroxidase
MDA: Malondialdehyde
MET: Metformin
NC: Normal control
NO: Nitric oxide
PAD: peripheral artery disease
SOD: Superoxide dismutase
GSH: Reduced glutathione
ROS: Reactive oxygen species
STZ: Streptozotocin

Ethics approval

This experiment was approved by the FUOYE Faculty of Science Ethical Committee (FUOYEFSC 201122-REC2022/008).

Consent to participate

Not applicable

Consent for publication

All the authors agreed to this publication.

Authors' contributions

BOA, BEE, AWS, AOO, KK, SGB, BEO, OAO, TOJ designed, conducted the experiment, analyzed the results, draft and approved the manuscript for submission.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper. The authors declare that they no known financial interests that could have appeared to influence the work reported in this paper

Data availability

Data will be made available on request.

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