

HISTOMORPHOLOGIC AND HISTOMORPHOMETRIC EFFECTS OF ETHANOL LEAF EXTRACT OF *Telfairia occidentalis* ON THE HIPPOCAMPUS OF STREPTOZOTOCIN-INDUCED DIABETIC WISTAR RATS

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ABSTRACT

Background: *Diabetes mellitus is a multifaceted condition that is known to disrupt normal glucose metabolism, resulting in chronic hyperglycemia and glycosuria. This disruption not only affects glucose metabolism but also leads to increased oxidative stress, which damages multiple organs, contributing to approximately 80% of global morbidity and mortality.*

Aim: *To evaluate the histological effect of ethanol leaf extract of *Telfairia occidentalis* (ELETO) on the hippocampus of streptozotocin-induced diabetic Wistar rats.*

Materials and Method: *Forty-two adult male Wistar rats weighing between 160-180 g were used for this study, divided into six groups of seven rats (n=7), fed on standard laboratory rat chow and constituted High Fat Diet for four weeks and given free access to clean water. Diabetes was induced in overnight fasted Wistar rats with five days of low-dose intraperitoneal injection of 35 mg/kg of Streptozotocin. Group A (normal control) rats were given single dose of citrate buffer solution and fed on standard laboratory rat chow throughout the experiment, while groups B, C, D and F were fed on constituted High Fat Diet (HFD) for four weeks and given Streptozotocin (35 mg/kg) intraperitoneally, for 5 days. Group E (Prophylactic group) rats were fed on constituted High Fat Diet for four weeks and given ELETO (300 mg/kg) orally for two weeks before the administration of Streptozotocin (35 mg/kg), intraperitoneally. Three days post diabetic-induction, ELETO and Insulin administration commenced. Group B was the STZ Negative Control, Group C (low dose) received 300 mg/kg of ELETO for two weeks, Group D (High Dose) received 600 mg/kg of ELETO for two weeks, and Group F rats were given 5 IU/kg of insulin intraperitoneally for two weeks. On the 13th day post induction, blood glucose levels were accessed using a Fine test glucometer. Blood samples were obtained via cardiac puncture on the day of sacrifice, centrifuged at 3000 rpm for 30 minutes and the serum obtained was used for biochemical analysis. Routine histological tissue sections were made to access the histoarchitectural and histomorphometric effects of diabetes on the hippocampus of experimental animals. Data were analyzed using differential and inferential statistics. Results were expressed as Means \pm S.E.M, and $p < 0.05$ was considered statistically significant.*

Results: *The result showed that STZ intraperitoneal administration caused distortion of hippocampal histoarchitecture in the experimental animals. However, treatment with intervening agent showed a dose-dependent improvement in the ultra-structural derangement observed in the experimental animals. It was also observed that the experimental dose showed significant cognitive improvement when compared to negative control. Administration of the intervening agent showed a dose-dependent significant decrease in blood glucose levels and a substantial increase in body weight of treated animals when compared to the untreated diabetic group. *T. occidentalis* extract caused reversal of the damage done and near normal hippocampal histoarchitecture was restored.*

Conclusion: *This study concluded that T. occidentalis extract has antidiabetic potential as it was able to prevent diabetes in the prophylactic group and ameliorate hippocampal derangement in the curative groups.*

Keywords: *Telfairia occidentalis, Streptozotocin, Diabetes mellitus, Blood Glucose, High Fat Diet*

INTRODUCTION

Diabetes mellitus, a group of metabolic diseases, is characterized by elevated blood sugar level resulting from defect in insulin secretion, insulin action, or both. (American Diabetes Association, 2024). This disruption affects not only glucose metabolism but also leads to increase oxidative stress, which damages multiples organs, contributing to approximately 80% of global morbidity and mortality (Galicia-Garcia *et al.*, 2020; Waiz *et al.*, 2024).

Diabetes cases as of 2022 according to IDF's estimates was over 800 million worldwide, and if no effective preventive measures are taken, that number is expected to rise to a billion by 2045 (International Diabetes Federation, 2022). The prevalence of diabetes mellitus has steadily increased worldwide over the last four decades, and as a serious public health issue, it has enormous financial and social ramifications (Arayombo and Adewole, 2022; Ahmad *et al.*, 2022).

The complications of diabetes are numerous, affecting multiple organs, including the brain, (American Diabetes Association, 2020). The hippocampus has a vital role in learning and memory processing, making it vulnerable to diabetes-related damage as increase blood sugar level can lead to oxidative stress, inflammation, and neuronal death, resulting in impaired cognitive function, memory loss, and increased risk of neurodegenerative disease (Biessel & Despa, 2018; Aderinto *et al.*, 2023).

Currently, insulin and oral hypoglycemic (antidiabetic) medications such as sulfonylureas, biguanides, glinides, and Ozempic drugs such as semaglutide are available for the treatment of diabetes mellitus (Khardori, R., 2024). However, these contemporary drugs have numerous adverse effects that might result in kidney disease, hepatotoxicity, heart disease, etc., and they do not sustain proper glucose homeostasis for an extended length of time, (Deshmukh and Jain, 2015). Due to the increased rate of non-communicable diseases and high cost of healthcare, medicinal plants usage has been linked to their accessibility, affordability, and cultural acceptability (WHO, 2023). In addition to other anti-diabetic drugs, medicinal plants have a significant impact on lowering blood glucose levels (Raks *et al.*, 2018).

Numerous plants are abundant sources of antioxidants, including flavonoids, phenolic compounds like tannins and lignin, and vitamins A, C, and E (Ammar *et al.*, 2017). Due to their minimal to nonexistent adverse effects, medicinal plants are now favored for DM treatment (Jacob *et al.*, 2024; Waris *et al.*, 2024).

Telfairia occidentalis Hook f., or fluted pumpkin, is a tropical vine that is native to West Africa and employed both for culinary and medicinal purposes (Aworunse *et al.*, 2018; Akpasi *et al.*, 2023). It is a diocious, perennial vine shrub that is partially drought-tolerant and occurs mostly in its cultivated form in various parts of Africa (Aworunse *et al.*, 2018) *T. occidentalis* grows at 20m height, leave division of 3-5 leaflets, the fruits are pale green weighting between 3 and 10 kg and the seeds are 3-5 cm wide (Imosemi, 2018; Cabi, 2019). In Nigeria, *T. occidentalis* is known as "ugu" in both Yoruba and Igbo languages and "kabewa" in Hausa (Imosemi, 2018).

Researches indicate that *Telfairia occidentalis* contains useful quantities of minerals and phytochemicals like flavonoids, alkaloids, tannins, saponins, phenolics, phosphorus, calcium, zinc, iron and copper (Ammar et al., 2017; Jimoh, 2017; Imosemi, 2018). Its nephroprotective, hepatoprotective, anti-inflammatory, anticancer, antihypertensive, neuroprotective and antioxidant activities (Maduka et al., 2017; Jimoh, 2017; Airaodion et al., 2019; Udoudoh et al., 2020; Eru et al., 2021; Kingsley et al., 2022) have been reported. The hypoglycaemic effects of *T. occidentalis* and its use for the treatment of diabetes mellitus have been well-reported (Jimoh, 2017; Eseyin et al., 2018; Ojimmelukwe, P. C., 2022).

Additionally, the phytochemical constituents of *Telfairia occidentalis*, previous research reveals, inhibited activities of α -glucosidase and α -amylase (enzymes associated with T2DM) in adult Wistar rats in a concentration-dependent manner (Jimoh, 2017). However, *T. occidentalis*' ability to treat streptozotocin-induced diabetes and neurodegeneration resulting from hippocampal assault in rats has not adequately been investigated in previous research works hence; this study delved into the effects of ethanol extract of *T. occidentalis* leaf on neurodegeneration in diabetes mellitus.

MATERIALS AND METHODS

EQUIPMENTS

Microtome (Laboid: LBM- RM2), Hot plate (A39-CVL004, Japan), Floating Bath (Water Bath: MH-8504), Rotary evaporator (Buchi), dissecting kit, Centrifuge (Denly, Model BS 400), Top Loader Balance (Metlet Telodo, Mg 126). Soxhlet Extractor, oral cannula, Y-maze and Open Field Apparatuses, Petri dish, Motic Scanner, 2 ml sterile disposable syringes and needles, cotton wool, 2 ml plain bottles, and Fine Test Glucometer,

CHEMICALS AND REAGENTS

Insulin (ACTRAPID INJECTION 100 IU/ml, SANOFI PHARMA (INDIA), PTE LTD) was purchased at Mohad's Pharmaceutical, OAUTHC, Ile-Ife. STREPTOZOTOCIN with batch number (S131213/CAS 18883-66-4; Cayman Pharmaceutical Company, 1180 East Ellsworth Road, Ann Arbor, Michigan 48108, USA) was purchase from Bridge Biotech, Ilorin, Kwara State, Nigeria, varying doses of ethanol, citric acid, sodium citrate, 5% Detrose, normal saline and distilled water.

PLANT MATERIAL

Fresh leaves of *T. occidentalis* (fluted pumpkin) were obtained from Odo-Ogbe, Ile-Ife, and was authenticated by a taxonomist in the Department of Botany, Obafemi Awolowo University, Ile Ife; a voucher specimen number (IFE 18341) was assigned and plant deposited in the Department herbarium for future reference.

PLANT EXTRACTION & PREPARATION

The fresh leaves of *T. occidentalis* were removed from the stem, washed in distilled water, air-dried at room temperature, weighed at intervals to obtain a stable weight to ensure proper dryness, and blended to powdery form with the use of a mechanical blender. The extraction of the plant constituents was done using Soxhlet Extraction method with ethanol as solvent. The extract was filtered using Whatman No. 1 filter paper (0.2 mm) to remove residues. The filtrate was concentrated using a rotary evaporator and freeze-dried. The dried extract was stored in a desiccator until it is ready for use.

ANIMAL CARE AND MANAGEMENT

Forty-two adult male Wistar rats with an average weight of 160g-180 g were used for this study. The rats were procured from the animal holding of College of Health Sciences, OAU, Ile-Ife. The rats were housed in plastic cages, kept under standard laboratory condition of temperature, humidity and light in the Animal House at the Department of Anatomy and Cell Biology of OAU, Ile-Ife; fed on standard laboratory rat chow and constituted High Fat Diet, (Sunshine Mill, Oshogbo, Osun State, Nigeria) and given access to clean water.

EXPERIMENTAL DESIGN

The forty-two rats were randomly assigned into six groups (A-F) of seven rats each, and allowed to acclimatize for one week. Group A (normal control) rats were given single dose of citrate buffer solution and fed on standard laboratory rat chow throughout the experiment.

Groups B, C, D and F were fed on constituted High Fat Diet (HFD) for four weeks and given Streptozotocin (35 mg/kg body weight), intraperitoneally, dissolved in 0.1M freshly prepared solution of citrate buffer at the pH of 4.5, for 5 days under fasting condition.

Group E (Prophylactic group) rats were fed on constituted High Fat Diet for four weeks and given *ELETO* (300 mg/kg body weight) orally for two weeks before the administration of Streptozotocin (35 mg/kg body weight), intraperitoneally, dissolved in 0.1M freshly prepared solution of citrate buffer at the pH of 4.5, for 5 days under fasting condition.

Three days post STZ-induction and confirmation of diabetic state across the groups, *ELETO* and Insulin administration started. Group B was the STZ Negative Control, Group C (low dose) received 300 mg/kg body weight of *ELETO* for two weeks, Group D (High Dose) received 600 mg/kg body weight of *ELETO* for two weeks, and Group F rats were given 5 IU/kg body weight of insulin intraperitoneally for two weeks.

EXPERIMENTAL DESIGN

SN	Group (n =7)	Feed/Diet	Treatment/Dosage	Administration	Duration
1	A	Normal Rat Chow	Citrate buffer (1ml/kg)	I.P	5 days
2	B	HFD	STZ(35mg/kg)	I.P	5 days
3	C	HFD	STZ(35mg/kg/day)+ <i>ELETO</i> (300 mg/kg)	I.P + Oral	5 days+2wks
4	D	HFD	STZ(35mg/kg/day)+ <i>ELETO</i> (600mg/kg)	I.P + Oral	5 days+2wks
5	E	HFD	<i>ELETO</i> (300mg/kg/day)+STZ (35 mg/kg)	Oral + I.P	5 days+2wk
6	F	HFD	STZ(35mg/kg/day)+Insulin (5IU/Kg)	I.P + Oral	5 days +2wks

INDUCTION OF DIABETES MELLITUS

Diabetes was induced in overnight fasted Wistar rats by five days low-dose intraperitoneal injection of 35 mg/kg body weight of Streptozotocin dissolved in 0.1M freshly prepared solution of citrate buffer at the pH of 4.5 as review in the works of Zhang *et al.*, (2020), Eleonore, (2023) and Ghasemi

& Jeddy, (2023). To avoid hypoglycemic shock, the experimental animals of Groups (B-F) were given 5% dextrose (glucose) as drinking water overnight on the first day. On the 8th day following STZ induction, blood sample was acquired from the caudal portion of the tail of rats and measured with blood glucose levels of rats above 215 mg/dL (11.1 mmol/L) automatically considered diabetic as review also in the works of Aje *et al.*, (2020) and Agunloye *et al.*, (2022).

FASTING BLOOD GLUCOSE RECORD

Blood was taken from the caudal portion of the tail of the rats after induction to determine fasting blood glucose level, and this was done throughout the period of the experiment. A fine test glucometer was used to evaluate blood samples for glucose levels. Each rat was monitored and the tail was rubbed to ensure that there was enough blood at the tip of the tail. The tail's end was cleansed with an alcohol swab and pierced with a lancet. The first drop of the blood was discarded, and the tail was cleansed, and the next drop was collected using a compatible glucose test strip. Each reading was then recorded appropriately.

NEUROBEHAVIORAL STUDY

Neurobehavioral study was carried out 24 hours after the last administration of ELETO using the Y-maze and Open-field Test. The Y-maze test evaluated spatial memory, exploration, and anxiety-like behavior in animals by observing their navigation through a Y-shaped maze; while open field test assessed locomotor activity, anxiety-like behavior, and exploratory behavior in a large, enclosed area. The rats were accustomed to both Open Field and Y-maze apparatus prior to the start of the exercise. To eliminate any aroma indications left by the previous subject rat, both the Open Field and Y-maze apparatuses main chamber were scrubbed with 95% ethanol prior to usage and before each future test. The ethanol was allowed to evaporate entirely prior to testing.

PERCENTAGE WEIGHT CHANGE

Weight of the rats was taken weekly using digital weighing balance, and percentage weight change was calculated as followed:

$$\% W = \frac{\text{Final weight} - \text{Initial weight}}{\text{Initial weight}} \times 100\%$$

The relative brain weight was determined as followed:

$$\text{Relative Organ Weight} = \frac{\text{Organ Wt.}}{\text{Body Wt.}} \times 100\%$$

TISSUE PROCESSING

Tissue was processed via paraffin wax embedding method of Drury and Wallington (1980). Sections of 5µm thickness was produced from the tissue blocks and stained.

STAINING

Histological staining

- Hematoxylin and Eosin - demonstration of general histoarchitecture of hippocampus

Histochemical staining

- Bielschowsky's Silver Stain – demonstration of nerve fibers (axons and myelin sheath), senile plaques and tangles.
- Cresyl Violet Fast (CVF) – demonstration of nissl bodies

SACRIFICE AND SAMPLE COLLECTION

At the end of the experiment, the rats were sacrificed under diethyl ether anesthesia and the brain of the animals were completely excised, and weighed on a top loader electronic analytical balance. The brain was fixed in Neutral Buffer Formalin (NBF) by total immersion for 24 hours and later gross in a cross-coronal section, fixed in NBF for further histological analysis. At sacrifice, blood samples were collected with 2 ml syringe by cardiac puncture, stored in 5 ml plain bottles and left for 20 minutes at room temperature to clot.

PHOTOMICROGRAPHY

Tissue slides were scanned with Motic scanner with SVS format and the scanned slides were viewed, analyzed and measured.

IMAGE ANALYSIS

Integrated morphology analysis was undertaken using the image J software.

STATISTICAL ANALYSIS

Data were analyzed using one-way analysis of variance (ANOVA), followed by Tukey’s test for multiple comparison. Results were expressed as Means \pm S.E.M. $p < 0.05$ was considered statistically significant

RESULTS

Fig. 4.1: Graph Showing Body Weight Progression during the experiment.

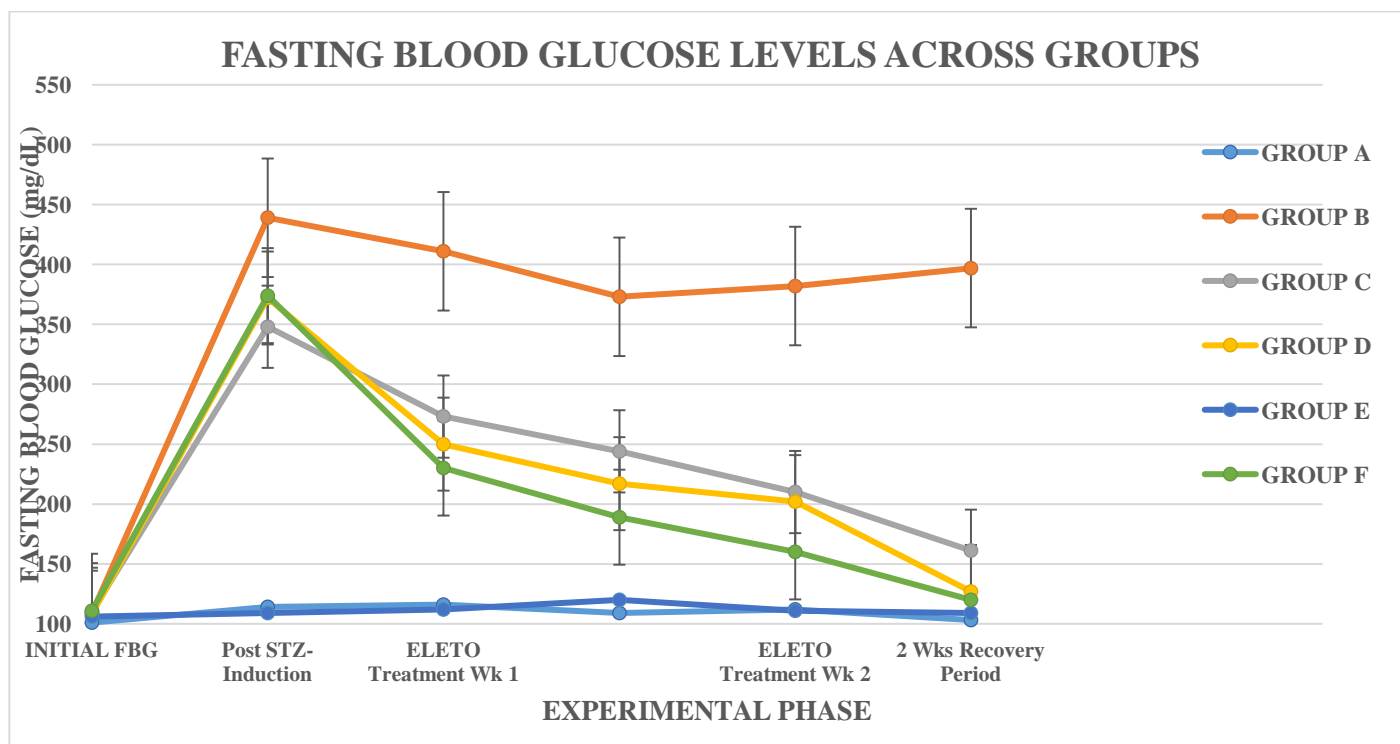


Fig.4.2: Effect of ELETO on the Fasting Blood Glucose level before induction, after induction, 1st, & 2nd week of treatment and 2 weeks Post Treatment of STZ-induced diabetic Wistar rats.

NEUROBEHAVIORAL TEST

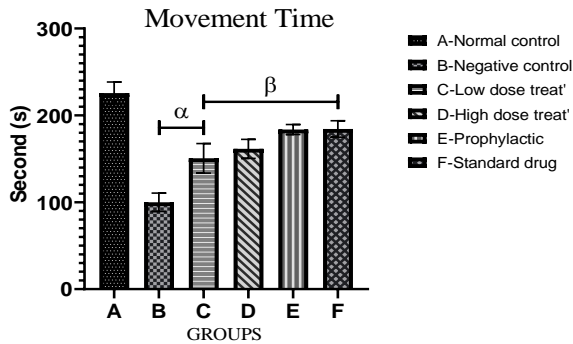


Fig. 4.6: Effects of Ethanol Leaf Extract of *Telfairia occidentalis* (ELETO) on Movement Time in Open Field Neurobehavioral Test of STZ-induced Diabetic Wistar Rat after last Treatment. Each bar represents Mean \pm SEM. The bar with (α) is significantly different from normal control; (β) is significantly different from negative control, using one way ANOVA, Tukey test at $P < 0.05$.

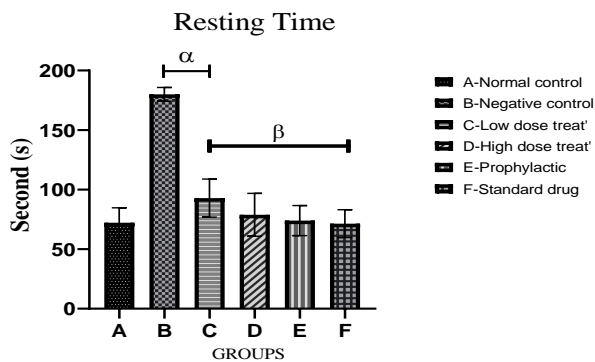


Fig. 4.7: Effects of Ethanol Leaf Extract of *Telfairia occidentalis* (ELETO) on Resting Time in Open Field Neurobehavioral Test of STZ-induced Diabetic Wistar Rat after last Treatment. Each bar represents Mean \pm SEM. The bar with (α) is significantly different from normal control; (β) is significantly different from negative control, using one way ANOVA Tukey test at $P < 0.05$.

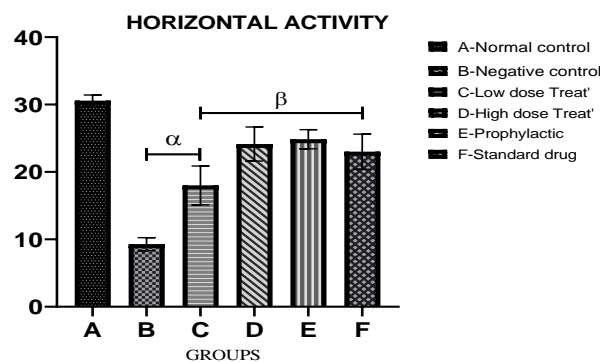


Fig. 4.8: Effects of Ethanol Leaf Extract of *Telfairia occidentalis* (ELETO) on Horizontal activity in Open Field Neurobehavioral Test of STZ-induced Diabetic Wistar Rat after last Treatment. Each bar represents Mean \pm SEM. The bar with the superscrip (α) is significantly different from the normal control; (β) is significantly different from the negative control, using one way ANOVA, Tukey test at $P < 0.05$.

NEUROBEHAVIORAL TEST

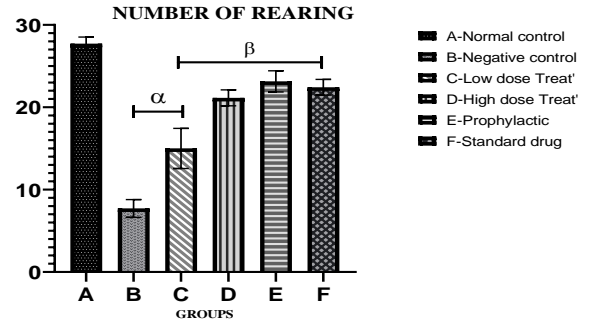


Fig. 4.10: Effects of Ethanol Leaf Extract of *Telfairia occidentalis* (ELETO) on Number of Rearing in Open Field Neurobehavioral Test of STZ-induced Diabetic Wistar Rat after last Treatment. Each bar represents Mean \pm SEM. The bar with (α) is significantly different from normal control; (β) is significantly different from negative control, using one way ANOVA Tukey test at $P < 0.05$.

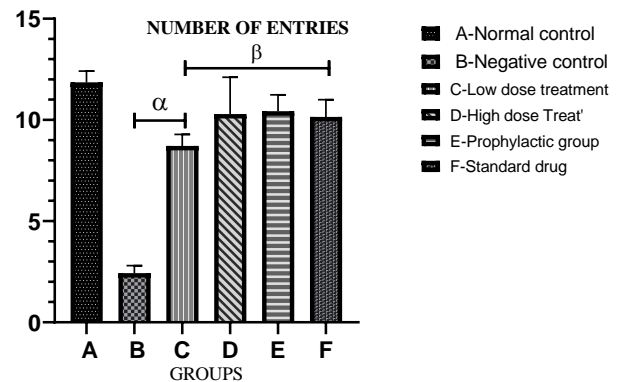


Fig. 4.13: Effects of Ethanol Leaf Extract of *Telfairia occidentalis* (ELETO) on Number of Entries in Y-Maze Neurobehavioral Test of STZ-induced Diabetic Wistar Rat after last Treatment. Each bar represents Mean \pm SEM. The bar with (α) is significantly different from normal control; (β) is significantly different from negative control, using ANOVA Tukey test at $P < 0.05$.

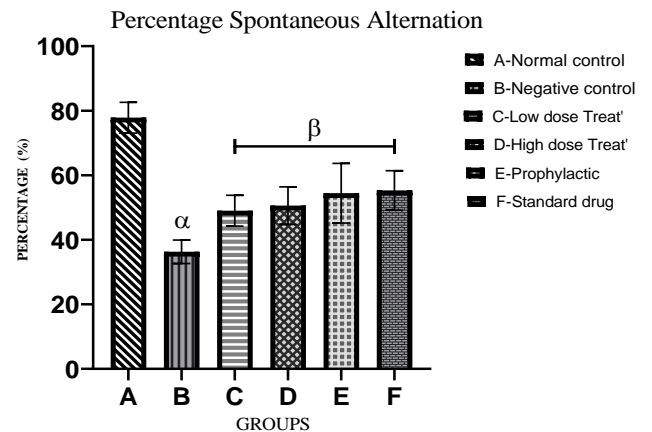


Fig. 4.14: Effects of Ethanol Leaf Extract of *Telfairia occidentalis* (ELETO) on Percentage Spontaneous Alternation in Y-Maze Neurobehavioral Test of STZ-induced Diabetic Wistar Rat after last Treatment. Each bar represents Mean \pm SEM. The bar with (α) is significantly different from normal control; (β) is significantly different from negative control using one way ANOVA Tukey test at $P < 0.05$.

HISTOMORPHOMETRY ANALYSIS

HISTOMORPHOMETRY ANALYSIS

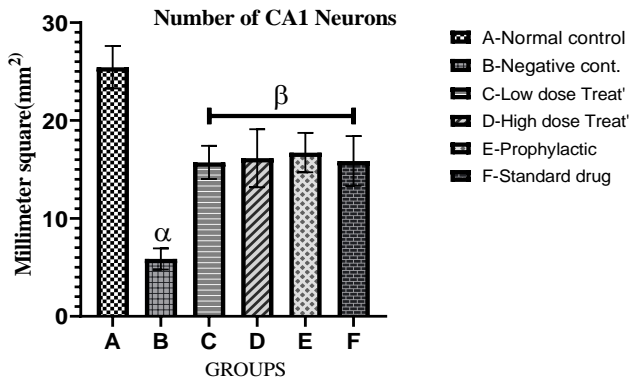


Fig. 4.19: Effects of Ethanol Leaf Extract of *Telfairia occidentalis* (ELETO) on Total Neuronal Density of CA1 STZ-induced Diabetic Wistar Rats. Each bar represents Mean ± SEM. The bar with (α) is significantly different from normal control; (β) is significantly different from negative control, using one way ANOVA Tukey test at P <0.05.

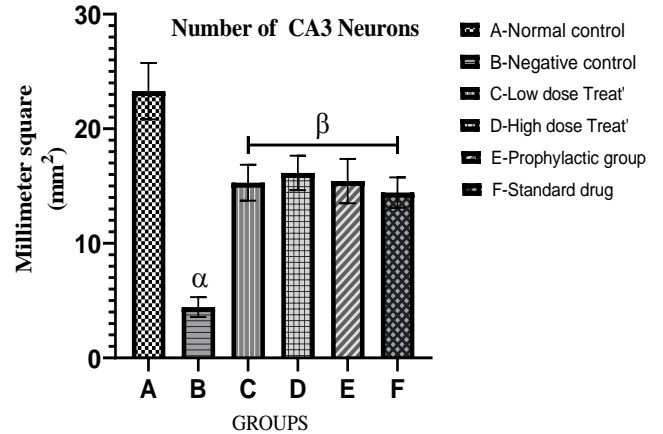


Fig. 4.21: Effects of Ethanol Leaf Extract of *Telfairia occidentalis* (ELETO) on Total Neuronal Density of CA3 of STZ-induced Diabetic Wistar Rats. Each bar represents Mean ± SEM. The bar with (α) is significantly different from normal control; (β) is significantly different from negative control, using one way ANOVA Tukey test at P <0.05.

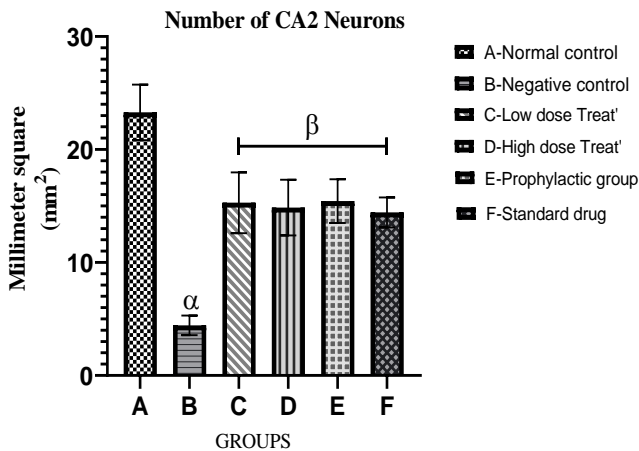


Fig. 4.20: Effects of Ethanol Leaf Extract of *Telfairia occidentalis* (ELETO) on Total Neuronal Density of CA2 STZ-induced Diabetic Wistar Rats. Each bar represents Mean ± SEM. The bar with (α) is significantly different from normal control; (β) is significantly different from negative control, using one way ANOVA Tukey test at P <0.05.

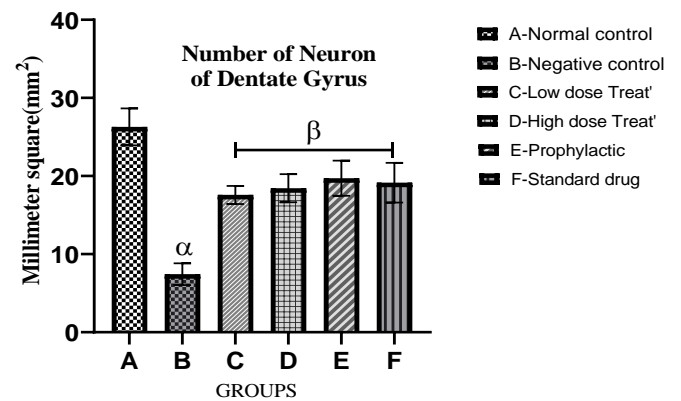


Fig. 4.22: Effects of Ethanol Leaf Extract of *Telfairia occidentalis* (ELETO) on Total Neuronal Density of Dentate Gyrus of STZ-induced Diabetic Wistar Rats. Each bar represents Mean ± SEM. The bar with (α) is significantly different from normal control; (β) is significantly different from the negative control, using one way ANOVA Tukey test at P <0.05.

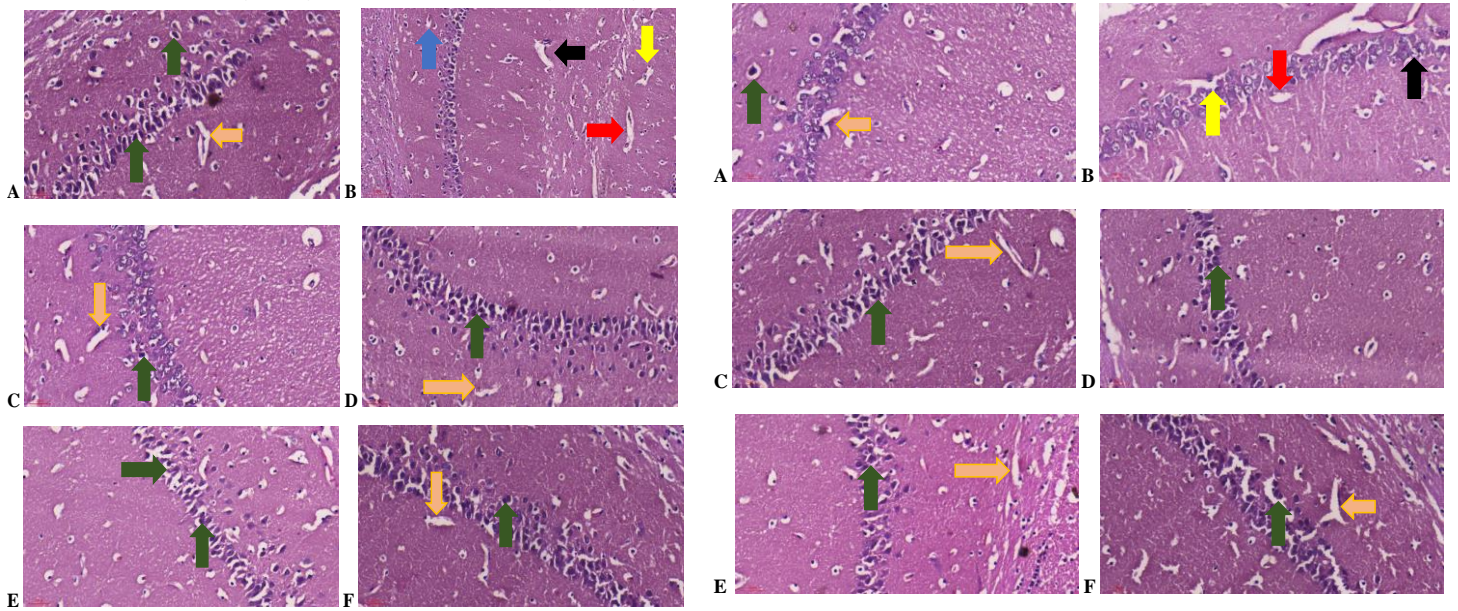


Plate 1: CA1 Region

Plate 2: CA2 Region

Selected photomicrographs of CA1 & CA2 regions of hippocampus of group A, B, C, D, E, & F. The red arrow identifies neuronal apoptosis, black arrow identifies eosinophilic necrosis, the yellow arrow identifies pyknotic nucleus amid associated vacuolations, and the blue arrow shows karyolytic neuronal cells. By contrast, the green arrow identifies relatively normal neurons, and the orange arrow identifies myelin sheath. (H & E X 400, Scale bar 60 μm).

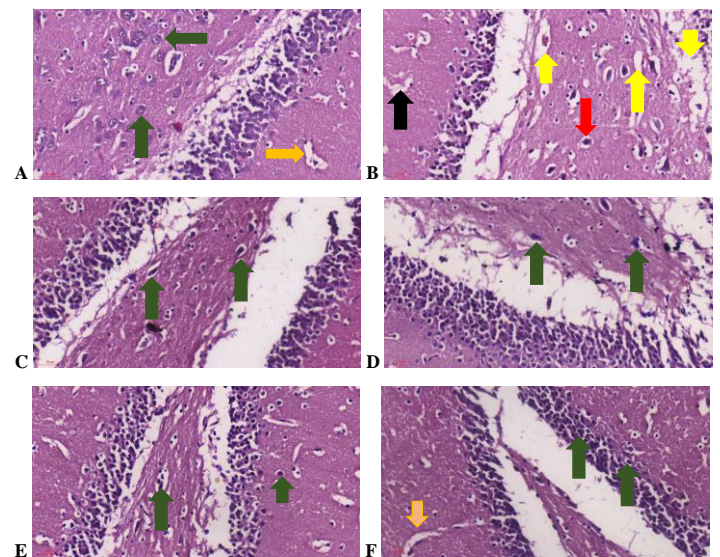
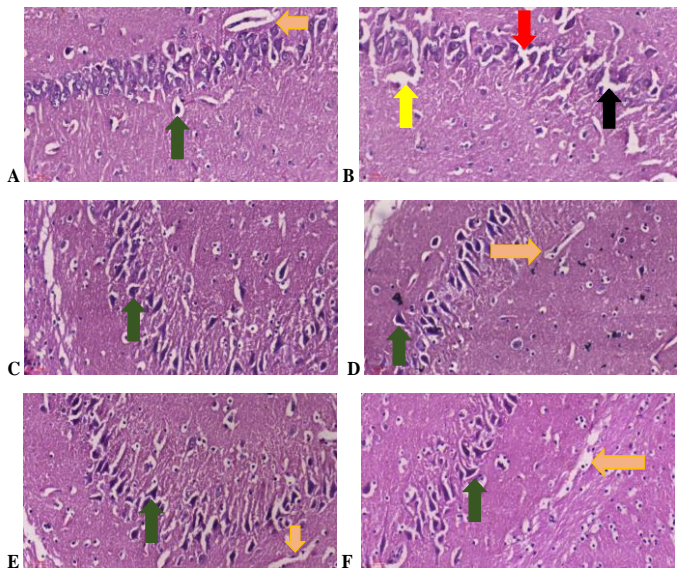


Plate 3: CA3 Region

Plate 4: DG Region

Selected photomicrographs of CA3 & DG regions of hippocampus of group A, B, C, D, E, & F. The red arrow identifies neuronal apoptosis, black arrow identifies eosinophilic necrosis, the yellow arrow identifies pyknotic nucleus amid associated vacuolations, and the blue arrow shows karyolytic neuronal cells. By contrast, the green arrow identifies relatively normal neurons, and the orange arrow identifies myelin sheath. (H & E X 400, Scale bar 60 μm).

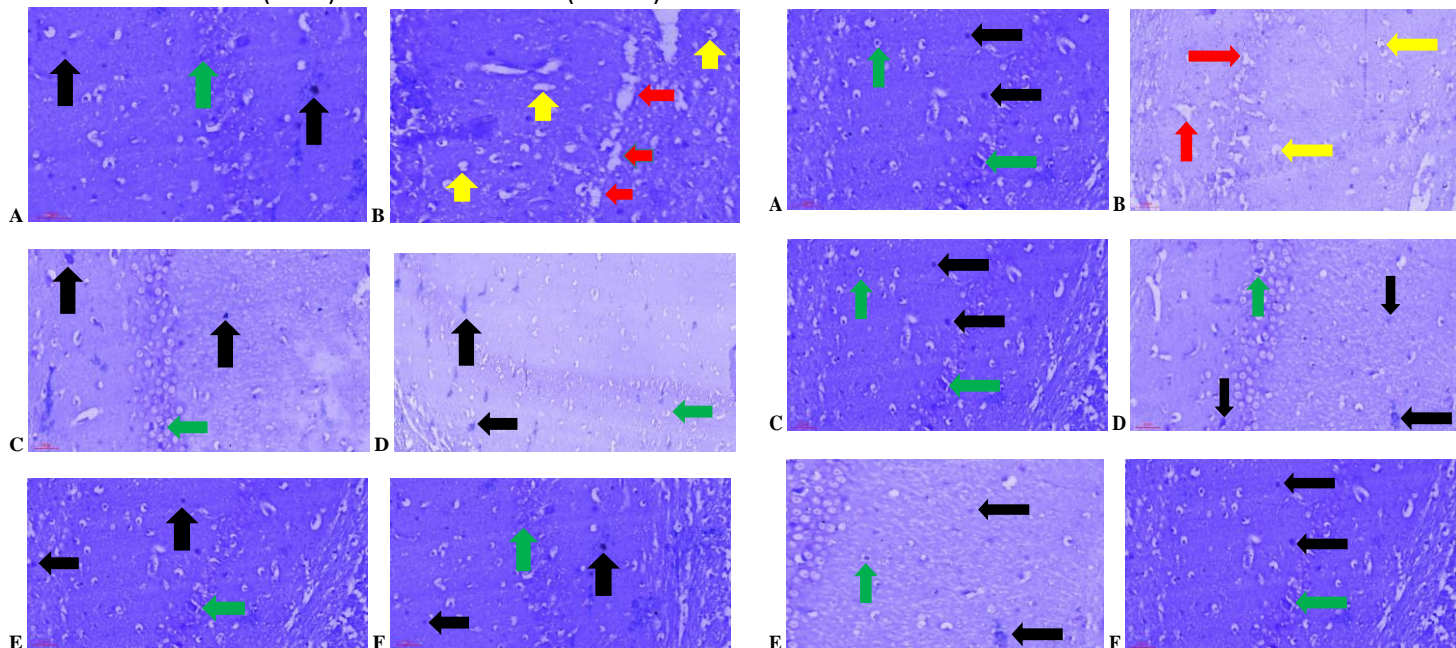


Plate 5: CA1 Region

Plate 6: CA2 Region

Selected photomicrographs of CA1 & CA2 regions of hippocampus of group A, B, C, D, E, & F. The red arrow identifies degenerated neurons amid associated vacuolations. By contrast, the green arrow identifies relatively normal neurons, and the black arrow identifies nissl substance. Cresyl Fast Blue X 400 (Scale bar 60 μm).

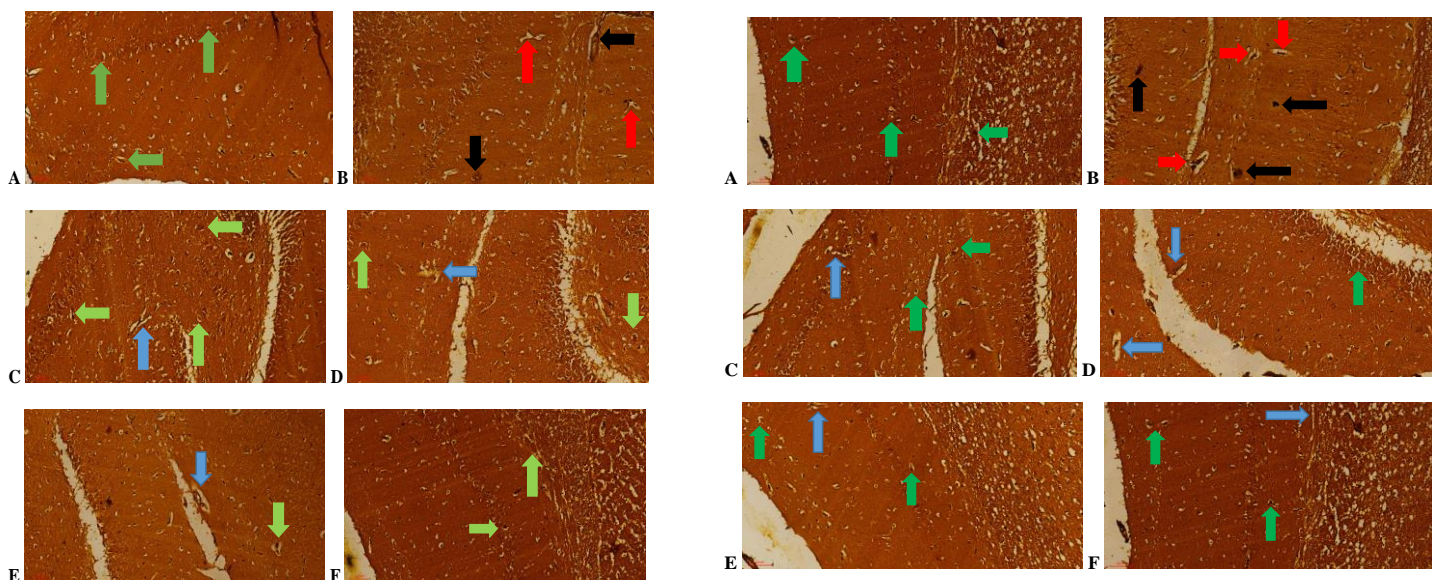


Plate 7: CA1 Region

Plate 8: CA2 Region

Selected photomicrographs of CA1 & CA2 regions of hippocampus of group A, B, C, D, E & F. The black arrow identifies senile plaques; the red arrow identifies degenerated neurons amidst associated vacuolations. By contrast, the green arrow identifies relatively normal neurons, and the blue arrow identifies nerve fiber and area of synapsis. Bielschowsky x 400 (Scale bar 60 μm).

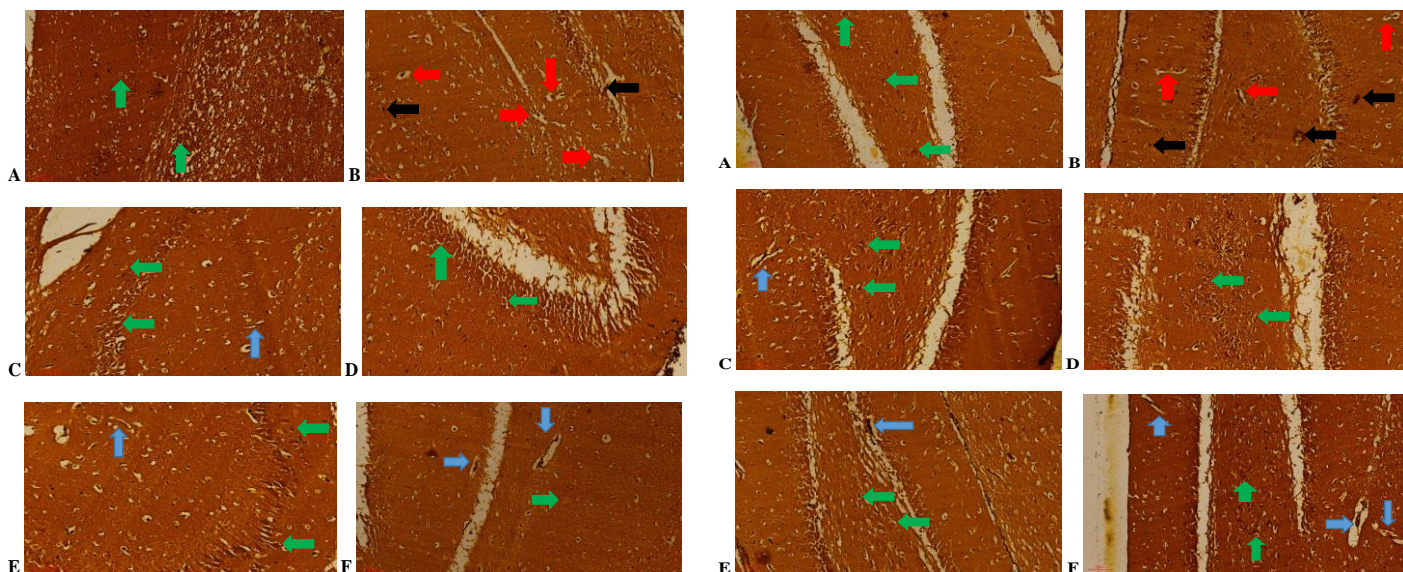


Plate 9: CA3 Region

Plate 10: DG Region

Selected photomicrographs of CA3 & DG regions of hippocampus of group A, B, C, D, E & F. The black arrow identifies senile plaques; the red arrow identifies degenerated neurons amidst associated vacuolations. By contrast, the green arrow identifies relatively normal neurons, and the blue arrow identifies nerve fiber and area of synapsis. Bielschowsky x 400 (Scale bar 60 μ m).

DISCUSSION

Diabetes mellitus, a multifaceted condition, is known to disrupt normal glucose metabolism resulting in chronic hyperglycemia and glycosuria, and this disruption not only affects glucose metabolism but also leads to increase oxidative stress, which damages multiples organs, contributing to approximately 80% of global morbidity and mortality (Khandra *et al.*, 2024; World Health Organization, 2023; Sohail *et al.*, 2024).

In this present study, both High Fat Diet (HFD) and streptozotocin (STZ) were employed to induce diabetes in animals. The HFD was utilized to cause obesity and peripheral insulin resistance, while STZ was employed to selectively damage the pancreatic beta cell and induce hyperglycemia. This method of inducing diabetes is well-established, and has been used in numerous works (Zhang *et al.*, 2020; Eleonore, 2023). The dose of STZ used in this study (35 mg/kg b.w) is consistent with recent studies that used STZ to induce diabetes in animals (Ghasemi & Jeddy, 2023; Bauer *et al.*, 2023). The five days allowed for the selective destruction of pancreatic beta cells and the induction of hyperglycemia. Hyperglycemia state was confirmed on the 8th day post induction with fasting blood glucose ranging from 450 mg/dl (25 mmol/L) and above, and at fourth week post treatment, hyperglycemia state was sustained at 300 mg/dl (16 mmol/L) and above across the diabetic group. This finding is accordance with recent work of (Zhang *et al.*, 2020; Ghasemi & Jeddy, 2023) that demonstrated that rats fed with HFD and given low doses intraperitoneal injection of STZ caused hyperglycemia in Wistar rats and was sustained at 260 mg/dL in diabetic group throughout the experimental period.

However, rats that were administered with Streptozotocin (STZ-induced diabetes) experienced a significant drop in body weight across the experimental groups (B, C, D, & F) compared with the normal control group (A) and prophylactic group (E). This notable weight loss reflects the well-documented ability of diabetes mellitus to cause metabolic disruption by breaking down tissues proteins and slowing down nutrient absorption (Novelli *et al.*, 2020; Dilworth *et al.*, 2023).

Numerous studies have revealed and emphasised *T. occidentalis's* potential phytochemical profile, especially its anti-inflammatory and antioxidant qualities, which have been demonstrated to be useful in reducing oxidative stress (Elejere *et al.*, 2019; Femi-Akinlosotu *et al.*, 2022). These activities are probably responsible for the observed considerable drop in fasting blood glucose levels and stable weight recovery when comparing the low and high dose groups (C and D) to the untreated diabetic group B. This clearly indicates that *ELETO's* high flavonoid and phenolic contents may have a significant effect in preventing hyperglycaemia, particularly at larger doses (Kayode and Kayode, 2011; Udoudoh *et al.*, 2020). This result is in line with earlier findings that *T. occidentalis* ethanol leaf extract had strong antioxidant activity, decreased blood glucose levels, and increased body weight in rats with STZ-induced diabetes (Onuoha *et al.*, 2017; Udoudoh *et al.*, 2020). One intriguing finding was that Wistar rats in Group E, administered with 300 mg/kg body weight of *ELETO* for two weeks prior to STZ induction, exhibited no significant increase in blood glucose levels. Moreover, these rats showed a substantial increase in body weight. This observation suggests that *ELETO*, rich in antioxidants, may possess prophylactic properties that prevent hyperglycemia when administered for an extended period. This study finding aligns with the works of (Lesile *et al.*, 2015; Jimoh, 2017) that reported a total inhibition of α -amylase activity, one of the key enzymes linked to T2DM, by *T. occidentalis* leaves extract; and that the extract significantly hindered a rise in blood glucose at 400 mg/kg body weigh when administered. However, the findings of this study differs with the work of Adesi *et al.*, (2012) who reported that *T. occidentalis* leaves significantly increased blood glucose levels in Wistar rats.

The study's findings on insulin, a well-established therapeutic agent for regulating blood levels, provide a valuable comparison. Notably, the insulin-treated Group F exhibited significant reduction in blood glucose levels and improved weight recovery, similar to those treated with *ELETO*. However, this study's findings suggest that *ELETO*, particularly at higher doses, may offer similar protective and restorative effects as insulin in diabetes-related condition; implying that *ELETO* could potentially serve as a complementary or alternative therapy for managing diabetes mellitus. The findings of this study corroborate with recent report of (Onyeka *et al.*, 2017; Aworunse *et al.*, 2018) that animals treated with insulin and *ELETO* exhibited significant improvements in blood glucose levels, insulin sensitivity and pancreatic beta-cell function.

Previous research showed that Diabetes mellitus disrupts not only glucose regulation but also has profound effects on the brain, particularly the hippocampus (Biessel and Staekenborg, 2018). This study conducted neurobehavioral tests, Open Field and Y-maze, to assess the animals' exploratory activity, anxiety level and spatial memory, providing insights into the cognitive and emotional consequences of diabetes, and how *ELETO* treatment could improve the damage caused by the disease. For the open field test, rats of group B (STZ negative control) exhibited significant decrease in the number of horizontal and vertical crossings, rearing, movement time and increase rest time compared to rats of group A (normal control). However, the *ELETO* dose-dependent

treated groups (C,D, and E) as well as rats of insulin treated group (F) exhibited significant increase in horizontal and vertical activities, movement time and decrease rest time compared to the STZ negative control rats of group (B). The findings of this study suggest that diabetes mellitus significantly contributed to cognitive impairment while *ELETO* dose-dependent treatment as well as insulin treatment improve animals' ability to explore due to decrease anxiety as a result of *ELETO* antioxidant levels and insulin neuroprotective effects. These findings corroborate with previous studies of (Owoeye & Gabriel, 2016; Eru *et al.*, 2021) that reported similar findings , including decreased horizontal and vertical activities, movement time and rearing in diabetic animals, while highlighting the neuroprotective effects of *ELETO* in mitigating oxidative stress due to its high antioxidant levels and improving neuronal condition (Owoeye & Gabriel, 2016).

The Y-maze test was carried out to assess whether *ELETO* and insulin administration could restore or improve spatial learning and short term memory, impaired by diabetes mellitus in rats. For the Y-maze test, rats of group B (STZ negative control) exhibited significant decrease in the number of entries and spontaneous alternation percentage compared to rats of group A (normal control). However, the *ELETO* dose-dependent treated groups (C, D, and E) as well as rats of insulin treated group (F) exhibited significant increase in the number of entries and spontaneous right percentage when compared to group B (negative control). This shows that *ELETO* and insulin administration was able to reverse the damage on the part of the brain relating to its inability to navigate, causing the rats to develop an enhanced will to explore new environment. The findings of this study corroborates with recent studies by (Owoeye & Gabriel, 2016; Eru *et al.*, 2021) that documented how diabetic rats administered with *ELETO* and insulin showed improved cognitive ability by navigating through the maze test, exploring new environment.

Histomorphometry analysis of hippocampal stained sections subjected to image J software and subsequent input of data obtained in Graph-pad prism showed a significant reduction of pyramidal neurons of CA1-CA3 regions of STZ-induced diabetes treated group B when compared to the normal control and *ELETO* treated groups; and further reduction in the number of granular neurons of the Dentate Gyrus reflects the damage of STZ-induced diabetes cause by oxidative stress on neuronal integrity.

The gradual increase of pyramidal neurons of CA regions and that of granular neurons of DG suggest that *T. occidentalis* restore normal neurons due to its antioxidant properties, and this is in line with recent research of Leon et al., 2021 who reported neuronal restoration after STZ-induction and *T. occidentalis* treatment.

The histopathological examination of hippocampal sections from the STZ-induced diabetic treated rats in this study further confirmed the extent of brain damage caused by oxidative stress.

In the normal control group, the hippocampal sections exhibited normal histoarchitectural features, including the hippocampal formation, Dentate Gyrus and Subiculum. The hippocampus proper is formed of Cornu Ammonis, which are the CA1-CA4. The CA1 and CA2 are formed of zones of small pyramidal neurons while the zones of CA3-CA4 are formed of zones of large pyramidal neurons. CA4 projects in the concavity of Dentate Gyrus that is formed of small granular neurons. The subiculum is outward continuation of CA1 region. This normal hippocampal architecture is

essential for its optimal function, including learning and memory, glucose metabolism, and other cognitive function.

In contrast, the hippocampal sections of rats administered with streptozotocin alone exhibited significant histoarchitectural distortions. These included marked changes in all regions in the form of disorganization and cell loss of small pyramidal cells, shrinkage in the size of the large pyramidal cells, chromatolysis, and marked vacuolation of the granular and molecular layers which exhibited enlarged neurons and excess glial cells. Neuronal necrosis, which is the death of neurons, was marked by pyknosis (shrinkage of the nucleus), and karyolysis (dissolution of the nucleus). Pyknosis is often considered a hallmark of irreversible cellular injury and indicates severe damage to the hippocampal neurons (Ajiboye *et al.*, 2024). The necrotic changes observed in the present study aligned with the work of (Tanbek *et al.*, 2022), who talked similar neuronal nuclear alterations, including pyknosis, in the hippocampal cells of rats administered with streptozotocin. Vacuolation reflects impaired hippocampal cells functions and brain attempts to isolate damaged or dysfunctional organelles within the cells.

In contrast to the untreated group B, neuropathological alterations revealed by histological analysis of hippocampus sections revealed that the majority of neurones in the pyramidal layer of the normal control group A had intact cytoarchitecture and densely stained Nissl bodies. Since a decline in Nissl bodies signifies neuronal degeneration, they are crucial to the health of neurons. The disintegration and dispersion of Nissl bodies, or chromatolysis, suggests that chemicals like STZ can induce brain damage. This is demonstrated in the negative control group B, which displayed dispersed and destroyed Nissl bodies in the pyramidal layer neurones.

Neuropathological changes from histological examination of hippocampal tissue sections (H & E; Bielschowsky's silver stain) from the STZ-induced diabetic rats also revealed in this present study a pronounced loss of myelin sheath, as well as numerous senile plaques, tangles. However, the administration of *ELETO* in doses of 300 and 600 mg/kg body weight to streptozotocin-induced diabetes treated rats produced a significant antioxidant, ameliorative and reparative effects in a dose-dependent manner. Hippocampal sections of rats treated with *ELETO* showed reduced histoarchitectural distortions compared to the STZ-induced diabetes only group. Specifically, there was a preservation of small and large pyramidal cells, marked reduction in neuronal necrosis and vacuolations, preserved myelin sheath, etc. These findings suggest that *ELETO* not only enhanced antioxidant enzyme activity but also preserved the structural integrity of the hippocampus cells. The dose-dependent ameliorative effect of *ELETO* observed in this study indicates that higher doses of the extract provide a greater protection against oxidative damage, as confirmed by improved hippocampal architecture at the highest dose of 600 mg/kg. The observed effects of *ELETO* in this study are in line with the works of several studies that have shown the efficacy of plant-based extracts in protecting against chemical-induced brain damages. For instance, *T. occidentalis* has been reported to contain bioactive compounds such as flavonoids, polyphenols, and other antioxidants that scavenge free radical and reduce oxidative damage in the brain (Eseyin *et al.*, 2018; Christopher *et al.*, 2022). The ability of *ELETO* to mitigate oxidative stress, promote myelin sheath repair, and restore normal hippocampal histoarchitecture align with previous studies that emphasize the protective effects of natural plant extracts against brain damage (Salman *et al.*, 2021).

CONCLUSION

It can be concluded from this study that *T. occidentalis* extract showed both prophylactic and ameliorative potentials through its ability to restore near normal hippocampal histoarchitecture.

Finally, *T. occidentalis* is better as a prophylactic agent than curative agent against STZ-induced diabetic hippocampal damage.

RECOMMENDATION

Further studies and clinical trial should be conducted to explore the exact isolate of *T. occidentalis* which confers onto it the prophylactic and ameliorative potentials in preventing hippocampal damage and cognitive decline in STZ-induced diabetic Wistar rats.

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