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Attenuation of PI3K/AKT signaling pathway by *Ocimum gratissimum* leaf flavonoid-rich extracts in streptozotocin-induced diabetic male rats

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A R T I C L E I N F O

Keywords:
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A B S T R A C T

Diabetes is a group of medical conditions characterized by the body's inability to effectively control blood glucose levels, due to either insufficient insulin synthesis in type 1 diabetes or inadequate insulin sensitivity in type 2 diabetes. According to this research, the PI3K/AKT pathway of Ocimum gratissimum leaf flavonoid-rich extracts in streptozotocin-induced diabetic rats was studied. We purchased and used a total of forty (40) male Wistar rats for the study. We divided the animals into five (5) different groups: normal control (Group A), diabetic control (Group B), low dose (150 mg/kg body weight) of Ocimum gratissimum flavonoid-rich leaf extract (LDOGFL) (Group C), high dose (300 mg/kg body weight) of Ocimum gratissimum flavonoid-rich leaf extract (HDOGFL) (Group D), and 200 mg/kg of metformin (MET) (Group E). Streptozotocin induced all groups except Group A, which serves as the normal control group. The experiment lasted for 21 days, following which we sacrificed the animals and harvested their brains for biochemical analysis on the 22nd day. We carried out an analysis that included reduced glutathione (GSH), glutathione transferases (GST), catalase (CAT), glutathione peroxidase (GPx), and superoxide dismutase (SOD), along with GLUT4, MDA, pro-inflammatory cytokines, NO, neurotransmitters, cholinergic enzyme activities, cardiolipin, and the gene expression of PI3K/AKT. The obtained result indicates that the flavonoid-rich extracts of O. gratissimum significantly enhanced the levels of GSH, GST, CAT, GPx, and SOD, as well as GLUT4 and cardiolipin. The levels of GSH, GST, CAT, GPx, and SOD, as well as GLUT4 and cardiolipin, were significantly increased by gratissimum. Moreover, the extracts decrease the levels of MDA, pro-inflammatory cytokines, NO, neurotransmitters, and cholinergic enzyme activities. Additionally, the flavonoid-rich extracts of O. gratissimum significantly improved the AKT and PI3K gene expressions in diabetic rats. gratissimum had their AKT and PI3K gene expressions significantly ($p < 0.05$) improved. The

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findings indicate that O. gratissimum leaf flavonoids have the potential to treat diabetes mellitus. gratissimum leaf flavonoids possess therapeutic potential in themselves and can be applied in the management of diabetes mellitus. Although further analysis can be carried out in terms of isolating, profiling, or purifying the active compounds present in the plant's extract.

1. Introduction

Diabetes mellitus stands as a global health challenge, with a steadily increasing prevalence and profound implications for affected individuals and healthcare systems worldwide [1]. Characterized by persistent hyperglycemia, diabetes is not merely a disorder of glucose homeostasis but a complex metabolic condition entailing multiple pathophysiological processes. Among the key contributors to diabetic complications are oxidative stress, chronic inflammation, and impaired insulin signaling pathways [2]. Diabetic neuropathy, a prevalent consequence associated with diabetes, is distinguished by the occurrence of nerve impairment that may impact a range of physiological processes [3]. The occurrence of this phenomenon may be attributed to the prolonged elevation of blood glucose levels and other metabolic variables that are linked to diabetes. Depending on the specific kind of neuropathy, this ailment may result in various symptoms, including numbness, discomfort, muscular weakness, and autonomic disorders such as digestive troubles and cardiovascular abnormalities [4]. The therapy of diabetic neuropathy encompasses the regulation of blood glucose levels, the mitigation of other risk factors such as elevated cholesterol and blood pressure, and the adoption of a health-conscious lifestyle including consistent physical activity and weight control [3].

The PI3K (phosphatidylinositol 3-kinase) and AKT (protein kinase B) pathways are very important for keeping glucose levels stable in the body [5]. They are important parts of insulin-mediated glucose uptake and metabolic regulation. With an emphasis on comprehending insulin receptor signaling, the study of the phosphoinositide-3-kinase–pro-ein kinase B/AKT (PI3K-PKB/AKT) pathway and the function of activating receptor tyrosine kinases (RTKs) began in earnest in the early 1980s [6]. This research ultimately led to the discovery of the essential elements and mechanism of insulin receptor signaling. Proteins called insulin receptor substrate (IRS) interact with PI3K and turn on PKB/AKT through PDK1. After discovering a link between PI3K and PKB/AKT activation and cancer development, researchers conducted numerous studies to investigate the regulation of this pathway. These studies identified negative regulators such as protein phosphatase-2 (PP2A), phosphatase and tensin homolog (PTEN), and the pH-domain leucine-rich-r peat-containing protein phosphatases (PHLPP1/2) [7,8]. The PI3K-PKB/AKT pathway strictly controls and exhibits extreme conservation through a series of steps. Class-1A PI3Ks, which are linked to their regulatory subunits or adaptor molecules like IRS proteins, are directly stimulated by activated receptors [7,8]. Through its catalytic domain, PI3K is activated, converting phosphatidylinositol-(3,4)-bisphosphate (PIP2) lipids into phosphosphatidylinositol-3,4,5-trisphosphateP3). In the "activation loop," PKB/AKT connects to PIP3 at the cell membrane. This lets PDK-1 get to Thr-308 and phosphorylate it, which turns on PKB/AKT to a certain extent [9,10]. As scientists look for new ways to treat illnesses, natural compounds, especially flavonoids, which are bioactive secondary metabolites found in many plant species [11], have gotten a lot of attention.

Flavonoids exhibit a wide range of biological actions, including anti-inflammatory, anti-diabetic, and antioxidant capabilities. Scientists are devoting a great deal of attention to these substances because they have shown promise in reducing the problems linked to diabetes [12,13]. In this context, the present study delves into the intricate interplay between flavonoid-rich extracts from the leaves of Ocimum gratissimum, also referred to sto asnt leaf or clove basil [14], and the PI3K/AKT pathways in the context of diabetes. Diabetes research has widely employed Streptozotocin (STZ)-induced diabetic rat models due to their relevance in mimicking the pathophysiological aspects of human diabetes [15]. Using this model, the goal of this study is to find out how Ocimum gratissimum leaf extracts can help with important parts of diabetes, such as oxidative stress, inflammation, insulin signaling, and neurological symptoms, with a focus on how they affect the important PI3K/AKT pathways [16]. The potential therapeutic value in diabetes management motivates the choice of *Ocimum gratissimum*, a plant renowned for its rich flavonoid content, as the source of extracts [17–19].

Additionally, exploring its impact on a multitude of parameters, encompassing redox stress, cholinergic enzyme activities, neurotransmitter levels, glucose transporter levels, Gene expressions of AKT and PI3K, nitric oxide levels, purinergic enzyme activities, cardiolipin levels, and brain histoarchitecture, allows for a comprehensive evaluation of its therapeutic potential [20]. The many aspects of diabetes and the complexity of the PI3K/AKT pathways make this study very useful for looking into flavonoid-rich extracts from Ocimum gratissimum leaves as a possible addition to existing diabetes treatments [21]. The results of this study may not only shed light on how the effects we've seen happen, but they may also help us come up with new ways to deal with diabetes and its complications, especially neuropathy, which will ultimately help people who are dealing with this difficult metabolic disorder.

2. Materials and methods

2.1. Plant Materials Source and authentication

Plant Materials Source and Authentication Leaves of *Ocimum gratissimum* were gathered at an open area of the Nigerian Forestry Research Institute in Ibadan, Oyo state. senior taxonomist at the Forestry Research Institute of Nigeria (FRIN), Ibadan, Nigeria, identified and authenticated the leaf with a voucher number of 113741.

2.2. Chemicals, reagents and enzyme kits

Chemicals, reagents and enzyme kits methanol, sulphuric acid, absolute ethanol, fructose, concentrated ammonium hydroxide, dilute ammonium hydroxide, streptozotocin (STZ), formalin, sodium citrate buffer, and phosphate buffer were obtained from Sigma-Aldrich Germany, while all the reagents used were of analytical grade. Also, the enzyme kits used were from Randox Laboratory (Crumlin, United Kingdom).

2.3. Processing of Ocimum gratissimum

For two weeks, *Ocimum gratissimum* leaves were allowed to air dry at room temperature. Then the air-dried leaves were ground into a powder using an electronic blender.

2.3.1. Extraction of flavonoid extract of Ocimum gratissimum

This was carried out using a procedure described by Obafemi et al. [22], briefly a known gram of the powered sample in 80 % methanol for 72 h to de-fat it. The next step was to filter with muslin fabric. We used a rotary evaporator to concentrate the produced clear filtrate. After that, 20 g of the residue was dissolved in 200 mL of 10 % H_2SO_4 and heated to 100 °C 30 min in a water bath to initiate hydrolysis. The mixture was left on ice for 15 min in order to cause the flavonoid aglycones to precipitate. We dissolved the flavonoid aglycones in 50 mL of heated 95 % ethanol and then filtered the mixture into a 100 mL volumetric flask that was

completely filled with 95 % ethanol. Then, a rotary evaporator was used to concentrate it. We then precipitated the filtrate using concentrated ammonium hydroxide. To extract the flavonoid extracts, we let the entire solution settle, collected the precipitate, and washed it with diluted ammonium hydroxide. The extract was kept at 4 °C in a refrigerator.

2.4. Experimental animals

Experimental Animals A total of 40 male Wistar rats with averan averageght of 120 ± 20 g were acquired from Show-Gold Animal HousHouse infin, Oye-Ekiti, Ekiti stStateNigeria. Groups of five (5) were kept in a standard laboratory environment with a temperature of 22 ± 20 °C and a 12-h light/dark cycle. We acclimated the animals for two weeks. The rules and standards set forth by the Faculty of Science Ethics Committee were followed in this work.

2.5. Induction of Diabetes

Induction of Diabetes: rats were fed with normal rat's pellets chow, the rats to be induced were given 20 % w/v fructose water for two weeks while the normal control rats (un-induced) were given only water, previously described by Salau et al. [22]. 12 h before the induction of streptozotocin (STZ), their feeds were removed from each cage and left with only fructose solution and water respectively. STZ at a dose of 40 mg/kg body weight already dissolved in sodium citrate at a pH of 7.4 was administered intraperitoneally to the experimental animals, after which an equal volume of citrate buffer was administered to the normal control group (un-induced rats). The animals with fasting blood glucose ≥250 mg/dL confirmed at 48 h of induction with the aid of a Glucometer considered diabetic and used for this study [23].

2.6. Experimental Design and Animal Treatment

Experimental Design and Animal Treatment: Following STZ induction, we divided the animals into five groups, each containing eight rats. After the rats were precisely weighed, they were divided into the following groups according to body weight.

Group I: Ratsratst were not induced (Normal control)
Group II: Diabetics without treatment (diabetic control)
Group III: Diabetics administered low a dose (150 mg/kg body weight) of Ocimum gratissimum flavonoid-rich leaf extract (LDOGFL);
Group IV: Diabetics administered high dose (300 mg/kg body weight) of Ocimum gratissimum flavonoid-rich leaf extract (HDOGFL); and
Group V: Diabetics administered 200 mg/kg metformin (MET).

Notably, we administered Ocimum gratissimum flavonoid-rich leaf extract and metformin to the respective animals by oral gavage between 9 a.m. and 10: 30 a.m. daily for 21 days.

2.7. Collection and tissue processing

On the 22nd day of the experiment, the rats were sacrificed under halothane as anesthesia. We immediately extracted blood samples from each rat through cardiac puncture. Their blood was collected into a plain bottle and was centrifuged (Table Top Medical Electric Centrifuge Machine) for a period of 5 min minutes1,500 rpm. We immediately extracted the serum and refrigerated it until we needed it for further analysis. Each animal's brain was collected, washed in normal saline, cleaned using filter paper, weighed using a weighing balance, and homogenized in 0.1 M potassium phosphate buffer at pH 6.5. The homogenized samples were centrifuged at 4000 rpm for 15 min before being used for further analysis [24].

2.8. Biochemical parameters studied

2.8.1. Redox stress biomarker assays

2.8.1.1. Determination of malondialdehyde level. The study focused on the biochemical parameters. The mixture was fully heated for 20 min min at 100 °C using water bath. After cooling, it was centrifuged for 10 min 78 g (4000 rpm). After centrifuging for an additional 10 min the absorbance of the supernatant was measured at a wavelength of 540 nm against a reference blank of distilled water [25].

2.8.1.2. Determination of superoxide dismutase activity. Misra and Fridovich [26] outlined the procedure for measuring superoxide dismutase activity. A 0.5 mL tissue homogenate was diluted using a 1:10 dilution ratio in 4.5 mL of distilled water. In order to equilibrate in a spectrophotometric cuvette, an aliquot of 0.2 mL of diluted blood sample was added to 2.5 mL of 0.05 M carbonate buffer (pH 10.2). We then added 0.3 mL of substrate (0.3 mM epinephrine) and 0.2 mL of distilled water to initiate the reaction. We observed the rise in absorbance at 480 nm every 30 s for 150 s.

2.8.1.3. Determination of glutathione-s-transferase activity. We combined an aliquot of the material and buffer containing 1 mM GSH with 1 mM CNDB. We measured the absorbance at 340 nm as a function of time after administering CNDB [27].

2.8.1.4. Determination of catalase activity. The procedure outlined by Beers and Sizer [28] was employed to measure the catalase activity. After adding hydrogen peroxide (0.036 % w/w, 2.9 mL), we diluted the homogenate (0.1 mL) accordingly. We prepared the blank using potassium phosphate buffer (50 mM, pH 7.0; 3.0 mL). We read the absorbance at 240 nm.

2.8.1.5. Determination of glutathione peroxidase activity. 500 μL of the brain homogenate sample was combined with 500 μL of 0.1 M phosphate buffer (pH 7.4), 100 μL of 10 mM sodium azide, 200 mL of 4 mM GSH, and 100 μL of 2.5 mM H2O2. Following this, 600 μL of distilled water was added and thoroughly mixed. After 3 min of incubation at 37 °C, 0.5 mL of 10 % TCA was added, and the reaction mixture was centrifuged for 5 min minutes3000 rpm. One mL of the supernatants was mixed with 2 mL of K_2HPO_4 and 1 mL of 0.04 % DTNB. The absorbance was then measured at 412 nm against a blank [29].

2.8.1.6. Determination of reduced glutathione level. The method outlined by Ellman [30] was used to calculate the amount of GSH present in the sample homogenate. Briefly, 0.1 mL of 25 % trichloroacetic acid (TCA) was mixed with 1.0 mL of liver homogenate, and the precipitate was extracted by centrifuging the mixture at 5000 g for 10 min. 2 mL of 0.6 mM DTNB produced in 0.2 M, phosphate buffer (pH 8.0) was mixed with 0.1 mL of supernatant. We measured the absorbance at 412 nm.

2.8.2. Pro-inflammatory biomarker assays

2.8.2.1. Determination of interleukin-1β (IL-1β), interleukin-6 (IL-6) and tumor necrosis factor-α (TNF- α) levels. Interleukin-6 (IL-6) and tumor necrosis factor-α (TNF-α) levels Briefly, each well received 100 μL of standard or sample. It was incubated at 37 °C for 90 min. Removing the liquid, 100 μL of biotinylated detection antibody was added. It was incubated at 37 °C for 1 h. Aspirated and washed 3 three times. Hence,00 μL of HRP conjugate and was incubated at 37 °C for 30 min. Aspirated and washed 5 five times. Then, 90 μL of the substrate reagent was added and incubated at 37 °C for 15 min. The Stop solution (50 μL) was added and the absorbance was read at 450 nm [31].

2.8.3. Cholinergic enzyme biomarker assays

2.8.3.1. Determination of acetylcholinesterase (AChE) activity. The total volume of reaction mixture of 1 mL contained phosphate buffer (0.1 M, pH 8.0), DTNB (10 mM), 50 μL cytosol and acetylthiocholine iodide (150 mM). Change in absorbance was monitored at 412 nm for 3 min [32].

2.8.3.2. Determination of butyrylcholinesterase (BChE) activity. Phosphate buffer (0.1 M, pH 8.0), DTNB (10 mM), 50 μL cytosol, and butyrylthiocholine iodide (150 mM) were all present in the reaction mixture of 1 mL total volume. At 412 nm, the change in absorbance was observed for 3 min [33].

2.8.4. Selected neurotransmitter biomarker assays

The levels of dopamine, norepinephrine and serotonin were determined by following their respective Randox commercial kits.

2.8.5. Cerebral GLUT-4, nitric oxide and cardiolipin assays

The concentration of GLUT4 was determined according to the method of Li et al. [34]. The determination of cerebral nitric oxide (NO) concentration was done using the procedure of Miranda et al. [35]. The cerebral cardiolipin concentration was carried out using the procedure reported by Fajardo et al. [36].

2.8.6. Gene expression study of AKT and PI3K

2.8.6.1. Isolation of total RNA. Tissue samples were treated with the Quick-RNA MiniPrepTM Kit to extract total RNA. After being treated with DNAse I, the DNA contamination was eliminated. Using an A&E Spectrophotometer (A&E Lab. UK), the RNA was quantified at 260 nm and its purity was verified at 260 nm and 280 nm.

2.8.6.2. cDNA conversion. Reverse transcriptase reaction was used to convert 1 μg of DNA-free RNA to cDNA using a cDNA synthesis kit based on ProtoScript II first-strand technology (New England BioLabs). The reaction was carried out in three steps: 65 °C for 5 min, 42 °C for 1 h, and 80 °C for 5 min [37].

2.8.6.3. PCR amplification and agarose gel electrophoresis. The appropriate primers (Inqaba Biotec, Hatfield, South Africa) were used in OneTaqR2X Master Mix (NEB) polymerase chain reaction (PCR) for the amplification of the gene of interest. A 25 μL reaction mixture comprising cDNA, primers (forward and reverse, Table 1), and Ready Mix Taq PCR master mix was used for the PCR amplification process. As long as the following is true: 30 cycles of amplification (denaturation at 95 °C for 30 s, annealing for 30 s, and extension at 72 °C for 60 s) preceded the first denaturation at 95 °C for 5 min. The final extension was conducted at 72 °C for 10 min. One percent agarose gel was used to resolve the amplicons. The GAPDH gene was used to normalize the relative level of expression of each gene, and quantification of band intensity was done using "image J″ software [37].

Table 1
Primer sequences.

	Forward primer	Reverse primer
AKT	5′-TCACCTCTGAGACCGACACC-3′	5′-ACTGGCTGAGTAGGAGAACTGG-3′
PI3K	5′-AACACAGAAGACCAATACTC-3′	5′-CAATCCACAACTCGCTCCAA-3′
GAPDH	5′-GCAAGGATACTGAGAGCAAGAG-3′	5′-CATCTCCCTCACAATTCCATCC-3′

2.8.7. Purinergic enzyme biomarker assays

2.8.7.1. Determination of Na^+/K^+-ATPase, ca^{2+}/mg^{2+}-atpase and Mg^{2+}-ATPase activities. A test tube containing 400 μL of 200 mM NaCl/40 mM KCl/60 mM Tris (pH 7.4) was pipetted. Following that, 240 μl of distilled water, 20 μL of EGTA (20 mM), 20 μL of $MgCl_2.6H_2O$ (80 mM), and 20 μL of suitably diluted tissue supernatant were added. After mixing, this was incubated for 5 min at 37 °C. Next, 100 μL of 8 mM ATP was added. After mixing, this was incubated for 30 min at 37 °C. Subsequently, 2000 μL of reagent C and 200 μL of SDS (5 %) were added. For the purpose of developing color, the mixture was left to stand at room temperature for 30 min. The blank was made in the same way, but instead of using 20 μL of tissue supernatant, 20 μL of distilled water was used. At 820 nm, the test's absorbance was measured in relation to the blank. The concentration of inorganic phosphate was then calculated by extrapolating the measured absorbance from the phosphate calibration curve [38].

2.8.7.2. Determination of ecto-nucleoside triphosphate diphosphohydrolase (ENTPDase) activity. The method described by Akomolafe et al. [39] was followed in order to determine this. Briefly, 20 μL of the reaction samples were incubated for 10 min at 37 °C with a mixture that contained 200 μL of the reaction buffer (1.5 mM $CaCl_2$, 5 mM KCl, 0.1 mM EDTA, 10 mM glucose, 225 mM sucrose, and 45 mM Tris-HCl). After that, the reaction mixture was given 20 μL of 50 mM ATP, and it was left to incubate for 20 min at 37 °C in a shaker. After adding 200 μL of 10 % TCA, 200 μL of 1.25 % ammonium molybdate, and some freshly made 9 % ascorbic acid, the reaction was stopped. The mixture was left to stand for 10 min on ice. The absorbance at 600 nm was measured.

2.8.8. Histopathological examination

This was carried out using hematoxylin and eosin (H&E) staining as described by Blume et al. [40]. We submerged the brain tissues in a 10 % neutral-buffered formalin solution for 48 h to preserve them. The tissue was then subjected to dehydration using escalating concentrations of ethanol for a duration of 15 min per concentration, followed by embedding in paraffin wax. Following that, the specimens were divided into sections with a thickness of 4 μm before being subjected to staining with hematoxylin and eosin (H&E). We examined the H&E sections using a light microscope (Olympus CX31, Tokyo, Japan).

2.9. Statistical analysis

All the experimental results were presented as mean ± S.D (n = 5). Statistical significances were examined by ANOVA followed by Tukey's multiple comparison (post-hoc test) using a software GraphPad Prism (Version 5.0). $p < 0.05$ was fixed as statistically significant.

3. Results

3.1. Effect of Ocimum gratissimum flavonoid-rich extract on activities/levels of redox stress biomarkers in the brain of STZ-induced diabetic rats

Fig. 1 shows how O. gratissimum flavonoid-rich extract changed the levels and activities of biomarkers for redox stress in the brains of rats that were given STZ to make them diabetic. Compared to the normal group, the diabetic group that wasn't treated had a significant ($p < 0.05$) rise in lipid peroxidation (measured by MDA levels) and a significant ($p < 0.05$) drop in the activities of antioxidant enzymes (glutathione-S-transferase, GST; catalase, CAT; glutathione peroxidase, GPx; and superoxide dismutase, SOD). There was also a significant ($p < 0.05$) drop in the level of reduced glutathione (GSH) in the DC group. However, these misdemeanors were significantly ($p < 0.05$) attenuated in a dose-dependent fashion by the administration of low and high doses of flavonoid-rich extracts of *O. gratissimum,* which looked very much like

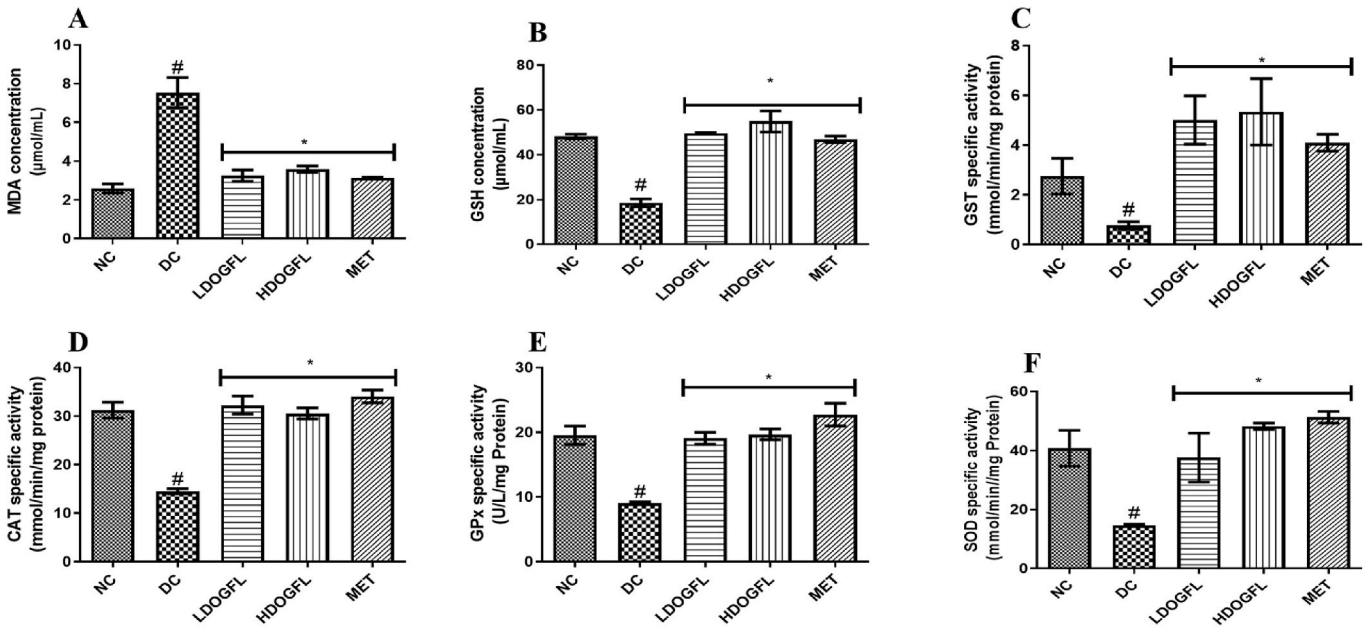

Fig. 1. Effect of *Ocimum gratissimum* flavonoid-rich extract on activities/levels of redox stress biomarkers in the brain of STZ-induced diabetic rats Each value is a mean of eight determinations ±SD. #p < 0.05 vs. NC, *p < 0.05 vs. DC **Legend**: NC: Normal Control, DC: Diabetic Control, LDOGFL: Low dose (150 mg/kg body weight) of flavonoid-rich extract of *Ocimum gratissimum,* HDOGFL: High dose (300 mg/kg body weight) of flavonoid-rich extract of *Ocimum gratissimum*, MET: metformin group, MDA: Malondialdehyde, GSH: Reduced glutathione GST: Glutathione-S-Transferase, CAT: Catalase, GPx: Glutathione Peroxidase, and SOD: Superoxide dismutase.

the standard (metformin)-administered group.

3.2. *Effect of Ocimum gratissimum flavonoid-rich extract on levels of pro-inflammatory biomarkers in the brain of STZ-induced diabetic rats*

By looking at Fig. 2, it is clear that giving STZ to diabetic rats caused

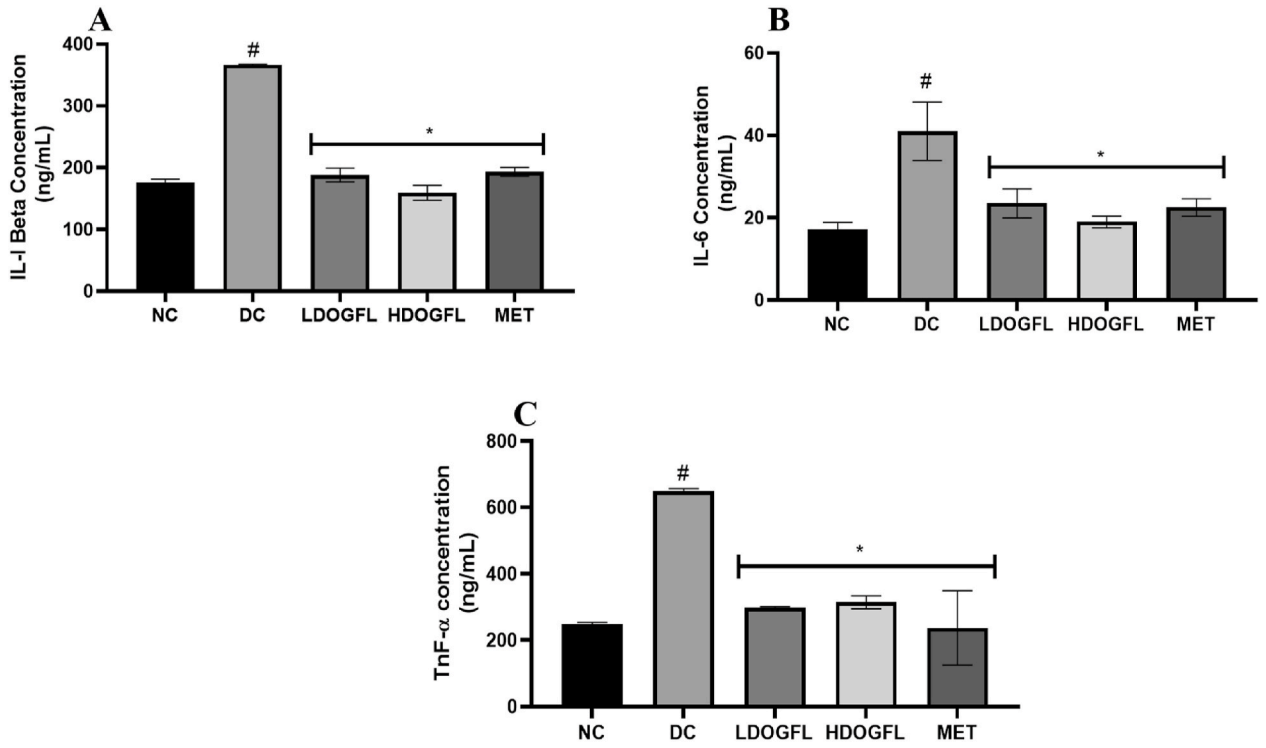

Fig. 2. Effect of *Ocimum gratissimum* flavonoid-rich extract on levels of pro-inflammatory biomarkers in the brain of STZ-induced diabetic rats
Each value is a mean of eight determinations ± SD. #p < 0.05 vs. NC, *p < 0.05 vs. DC **Legend**: NC: Normal Control, DC: Diabetic Control, LDOGFL: Low dose (150 mg/kg body weight) of flavonoid-rich extract of *Ocimum gratissimum,* HDOGFL: High dose (300 mg/kg body weight) of flavonoid-rich extract of *Ocimum gratissimum*, MET: metformin, IL-1beta: Interleukin-1 beta, IL-6: Interleukin-6 and TnF-α: Tumor Necrosis Factor-alpha.

a significant ($p < 0.05$) increase in the levels and concentrations of brain chemicals that cause inflammation, such as IL-1β, IL-6, and TNF-α. We compared this to the normal control rats (NC). Pro-inflammatory biomarkers rose more slowly ($p < 0.05$) when 150 and 300 mg/kg of flavonoid-rich O. gratissimum extract and 200 mg/kg of metformin were given.

3.3. Effect of Ocimum gratissimum flavonoid-rich extract on cholinergic enzyme activities in the brain of STZ-induced diabetic rats

Fig. 3 shows the effect of O. gratissimum flavonoid-rich extract on cholinergic enzyme activities in STZ-induced diabetic rats' brains. The activities of cholinergic enzymes (acetylcholinesterase, AChE, and butyrylcholinesterase, BChE) were notably ($p < 0.05$) increased in the diabetic untreated group (DC) when compared with the normal control rats (NC) as a result of STZ intoxication. Conversely, the harmful increase of cholinergic enzyme biomarkers was reversed significantly ($p < 0.05$) as a result of the administration of *O. gratissimum* flavonoid-rich extract and metformin in a near-normal situation.

3.4. Effect of Ocimum gratissimum flavonoid-rich extract on selected neurotransmitter levels in the brain of STZ-induced diabetic rats

Fig. 4 reveals a significant ($p < 0.05$) increase in selected neurotransmitter (dopamine, norepinephrine, and serotonin) concentration (depicting elevated excitotoxicity levels) due to STZ intoxication in the brain of diabetic untreated rats (DC) when compared with those in the normal control group (NC). Nonetheless, this anomalous increase in neurotransmitter concentration was significantly ($p < 0.05$) tempered in a dose-dependent manner by the administration of low and high doses of flavonoid-rich extracts of *O. gratissimum* as well as standard metformin.

3.5. Effect of Ocimum gratissimum flavonoid-rich extract on level/concentration of nitric oxide and cardiolipin in the brain of STZ-induced diabetic rats

As shown in Figs. 5–7, when compared to normal, the administration of STZ to the diabetic untreated rats resulted in a significant ($p < 0.05$) increase in the level of nitric oxide (NO) and a concurrent significant ($p < 0.05$) decrease in the concentrations of cerebral glucose transporter-4 (GLUT-4) and brain cardiolipin. Even so, giving metformin along with low and high doses of flavonoid-rich extracts of Ocimum gratissimum was able to significantly ($p < 0.05$) reverse and eventually normalize the negative trend of NO, GLUT-4, and cardiolipin in the brain.

3.6. Effect of Ocimum gratissimum flavonoid-rich extract on levels of relative gene expressions of AKT and PI3K in the brain of STZ-induced diabetic rats

Fig. 8 reveals the relative gene expression patterns of the protein kinase-B (AKT) and phosphoinositide-3-kinase (PI3K) genes in the brains of STZ-induced diabetic rats and the resultant restorative effect of *O. gratissimum* flavonoid-rich extract. Using glycealdehyde-3 phosphate dehydrogenase (GAPDH) as the housekeeping gene, there was a significant ($p < 0.05$) down-regulation of the Akt and PI3K genes in the diabetic control (DC) group compared to the normal control (NC) group. The administration of *O. gratissimum* flavonoid-rich extract and standard significantly ($p < 0.05$) up-regulated and restored the relative gene expression of the genes (Akt and PI3K) in a dose-dependent manner.

3.7. Effect of Ocimum gratissimum flavonoid-rich extract on purinergic enzyme activities in the brain of STZ-induced diabetic rats

As shown in Fig. 9, the administration of STZ led to a significant ($p < 0.05$) diminution in the activities of purinergic enzymes (Ecto-nucleoside triphosphate diphosphohydrolase, E-NTPDase, Na^+/K^+ATPase, Ca^{2+}/Mg^{2+}ATPase, and Mg^{2+}ATPase) in the diabetic untreated rats when compared with those in the normal group. These lethal decreases in purinergic enzyme activities were ameliorated significantly ($p < 0.05$) by the post-treatment of flavonoid-rich extracts of *O. gratissimum* (150 mg/kg bwt and 300 mg/kg bwt) as well as metformin (200 mg/kg bwt) as standard drugs.

3.8. Effect of Ocimum gratissimum flavonoid-rich extract on histoarchitectural modifications in the brain (hippocampus) of STZ-induced diabetic rats

Fig. 10 displays the impact of an O. gratissimum flavonoid-rich extract on the histoarchitectural changes in the hippocampus of rats that were made diabetic with STZ. Deleterious histological modifications were observed in the photomicrograph of the diabetic untreated group (DC) when compared with the normal group (NC). Nevertheless, these observed delinquencies were notably reversed to a near-normal condition by the administration of low and high doses of flavonoid-rich extracts of *O. gratissimum* and standard upon close perusal of the photomicrographs of the diabetic-treated rats.

4. Discussion

The PI3K/AKT pathway plays a crucial role in glucose regulation, and bioactive compounds from medicinal plants, such as phenolics and flavonoids, show promise in managing diabetes and related complications [46]. Overall, diabetes is a major global health issue, and

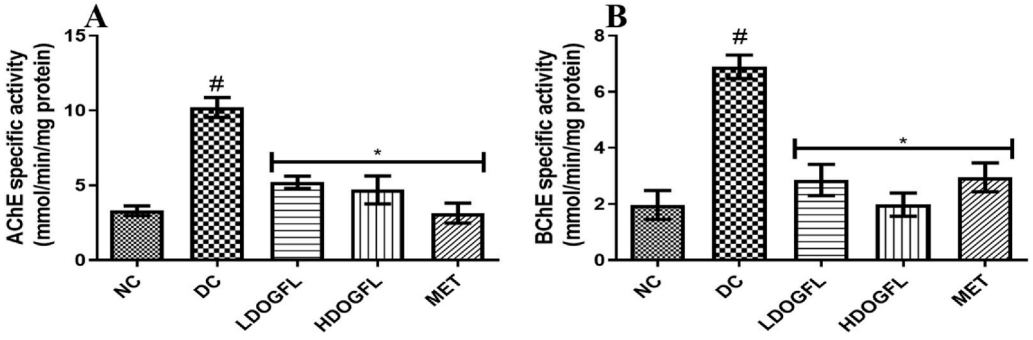

Fig. 3. Effect of Ocimum gratissimum flavonoid-rich extract on cholinergic enzyme activities in the brain of STZ-induced diabetic rats
Each value is a mean of eight determinations ± SD. #$p < 0.05$ vs. NC, *$p < 0.05$ vs. DC **Legend**: NC: Normal Control, DC: Diabetic Control, LDOGFL: Low dose (150 mg/kg body weight) of flavonoid-rich extract of *Ocimum gratissimum,* HDOGFL: High dose (300 mg/kg body weight) of flavonoid-rich extract of *Ocimum gratissimum*, and MET: metformin.

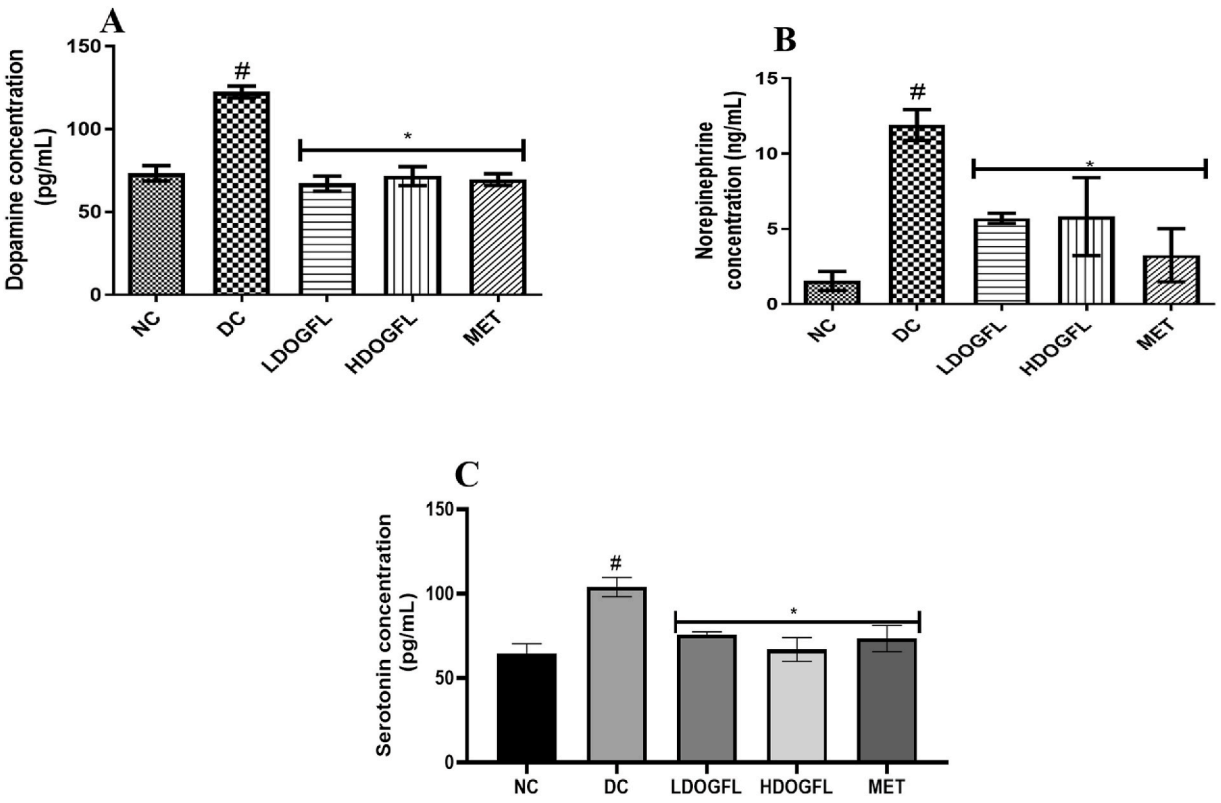

Fig. 4. Effect of Ocimum gratissimum flavonoid-rich extract on selected neurotransmitter levels in the brain of STZ-induced diabetic rats
Each value is a mean of eight determinations ± SD. #p < 0.05 vs. NC, *p < 0.05 vs. DC
Legend: NC: Normal Control, DC: Diabetic Control, LDOGFL: Low dose (150 mg/kg body weight) of flavonoid-rich extract of *Ocimum gratissimum,* HDOGFL: High dose (300 mg/kg body weight) of flavonoid-rich extract of *Ocimum gratissimum*, and MET: metformin.

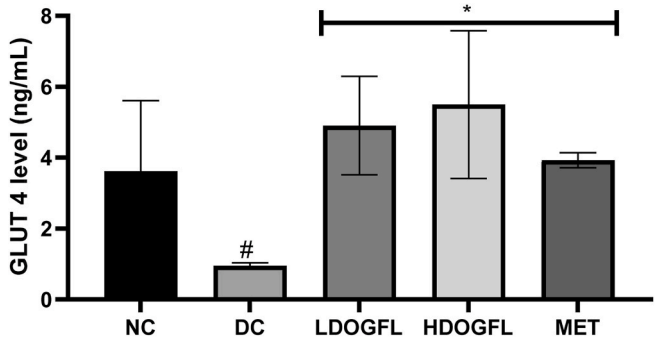

Fig. 5. Effect of *Ocimum gratissimum* flavonoid-rich extract on level/concentration of GLUT-4, in the brain of STZ-induced diabetic rats
Each value is a mean of eight determinations ± SD. #p < 0.05 vs. NC, *p < 0.05 vs. DC
Legend: NC: Normal Control, DC: Diabetic Control, LDOGFL: Low dose (150 mg/kg body weight) of flavonoid-rich extract of *Ocimum gratissimum,* HDOGFL: High dose (300 mg/kg body weight) of flavonoid-rich extract of *Ocimum gratissimum*, and MET: metformin.

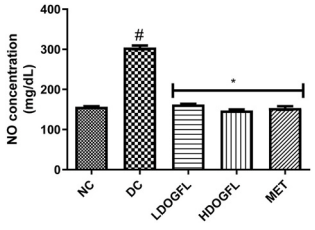

Fig. 6. Effect of *Ocimum gratissimum* flavonoid-rich extract on level/concentration of Nitric oxide in the brain of STZ-induced diabetic rats
Each value is a mean of eight determinations ± SD. #p < 0.05 vs. NC, *p < 0.05 vs. DC **Legend**: NC: Normal Control, DC: Diabetic Control, LDOGFL: Low dose (150 mg/kg body weight) of flavonoid-rich extract of *Ocimum gratissimum,* HDOGFL: High dose (300 mg/kg body weight) of flavonoid-rich extract of *Ocimum gratissimum*, MET: metformin.

understanding its molecular mechanisms, like PI3K/AKT, and exploring natural remedies offer potential avenues for diabetes management and treatment [47]. This study provides valuable insights into the potential therapeutic effects of *Ocimum gratissimum* flavonoid-rich extracts on various aspects of diabetes-related complications in STZ-induced diabetic rats, encompassing a range of parameters, including redox stress biomarkers, pro-inflammatory markers, cholinergic enzyme activities, neurotransmitter levels, glucose transporter expression, gene expressions of AKT and PI3K, purinergic enzyme activities, and brain histopathology.

Antioxidants are versatile molecules that play a crucial role in inhibiting or impeding free radical reactions, thereby effectively delaying or preventing cellular damage [49]. The study found that STZ-induced diabetes led to a significant ($p < 0.05$) increase in lipid peroxidation, accompanied by a significant ($p < 0.05$) decrease in the activities of vital antioxidant enzymes (GST, CAT, GPx, SOD) and a significant ($p < 0.05$) reduction in reduced glutathione (GSH) levels, which was in tandem with literature [48]. These defense mechanisms are not only ubiquitous across living organisms but also exist in various forms, both enzymatic and non-enzymatic, within cells and in extracellular environments. Underlying normal biochemical processes, heightened environmental exposures, and increased consumption of dietary substances like xenobiotics can give rise to the generation of

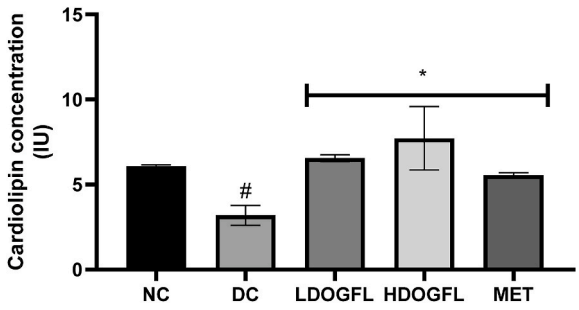

Fig. 7. Effect of *Ocimum gratissimum* flavonoid-rich extract on level/concentration of cardiolipin in the brain of STZ-induced diabetic rats
Each value is a mean of eight determinations ± SD. #p < 0.05 vs. NC, *p < 0.05 vs. DC
Legend: NC: Normal Control, DC: Diabetic Control, LDOGFL: Low dose (150 mg/kg body weight) of flavonoid-rich extract of *Ocimum gratissimum,* HDOGFL: High dose (300 mg/kg body weight) of flavonoid-rich extract of *Ocimum gratissimum*, and MET: metformin.

reactive oxygen species (ROS) and reactive nitrogen species (RNS) [15,50]. These highly reactive ROS and RNS are accountable for oxidative stress, a significant contributor to a range of pathological conditions. Oxidative stress prompts alterations in cellular components, thereby fostering the development of diseases [51]. However, the detrimental impacts of oxidative stress can be effectively mitigated by reinforcing cellular defenses through the action of antioxidants [52]. Some specific compounds function as *in-vivo* antioxidants by augmenting the body's innate antioxidant defenses, which include the activation of genes responsible for encoding enzymes like superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx), ultimately elevating the levels of endogenous antioxidants [53,54]. These antioxidant enzymes facilitate the conversion of harmful oxidative byproducts into hydrogen peroxide (H_2O_2), which is subsequently converted into harmless water through a multi-step process involving essential co-factors such as copper, zinc, manganese, and iron. Conversely, non-enzymatic antioxidants function by disrupting free radical chain reactions [55,56]. These changes reflect an environment of heightened oxidative stress in diabetic untreated rats [57]. However, the administration of *O. gratissimum* flavonoid-rich extract effectively attenuated these abnormalities, exhibiting dose-dependent improvement comparable to metformin treatment and in accordance with the literature [58]. This observation underscores the potent antioxidant properties of the extract, which may probably counteract the oxidative stress associated with diabetes [59].

In the brains of diabetic untreated rats, STZ-induced diabetes triggered a significant ($p < 0.05$) increase in pro-inflammatory biomarkers, including interleukin-1β (IL-1β), interleukin-6 (IL-6), and tumor necrosis factor-α (TNF-α), corroborated by several previous literature [60]. Several researchers have put forward the groundbreaking concept by demonstrating that the pro-inflammatory cytokine, TNF-α, could trigger insulin resistance, challenging conventional thinking [61,62]. This revelation led to a paradigm shift, highlighting fat tissue as a source of cytokines and bioactive substances, not limited to TNF-α but also encompassing leptin, IL-6, resistin, MCP-1, PAI-1, and others [63]. While adiponectin, produced by fat, decreases with increased adiposity, TNF-α, IL-6, MCP-1, visfatin, and PAI-1 are expressed at high levels not only in adipocytes but also in activated macrophages and other cells [64]. These cytokines, along with chemokines like MCP-1, play crucial roles in creating the sub-acute inflammatory state linked to obesity, ultimately contributing to insulin resistance and type-2 diabetes. Moreover, research focusing on intracellular pathways activated by inflammation, rather than individual cytokines, has reshaped our understanding of insulin resistance, emphasizing the involvement of JNK and IkKβ, which can be targeted to improve insulin resistance [65]. This inflammation-induced pathway realignment has momentous implications for addressing the complex interplay of obesity, inflammation, and insulin resistance [66]. This inflammatory upsurge, as a result of STZ intoxication, can contribute to neuroinflammation, a common complication in diabetes [67]. However, the administration of *O. gratissimum* flavonoid-rich extract mitigated these pro-inflammatory markers significantly ($p < 0.05$), mirroring the effects of metformin treatment. These findings highlight the potential anti-inflammatory properties of the extract, suggesting its role in reducing neuroinflammation associated with diabetes [68].

The activities of cholinergic enzymes: acetylcholinesterase (AChE) and butyrylcholinesterase (BchE), were notably ($p < 0.05$) increased in the brains of diabetic untreated rats due to STZ intoxication. AChE and BChE are essential enzymes in the nervous system, primarily responsible for terminating acetylcholine (ACh) neurotransmission at cholinergic synapses. In normal brain physiology, AChE is located at synapses, breaking down ACh to prevent prolonged receptor stimulation [69]. BChE, found in peripheral tissues, may act as a backup enzyme or regulator of cholinergic signaling [70]. In impaired brain physiology, reduced or elevated AChE activity is associated with neurodegenerative disorders, causing ACh accumulation or deficiency and eventual cognitive deficits [71]. Toxic exposures can lead to cholinergic over or under-stimulation. BChE outside the brain detoxifies chemicals. These

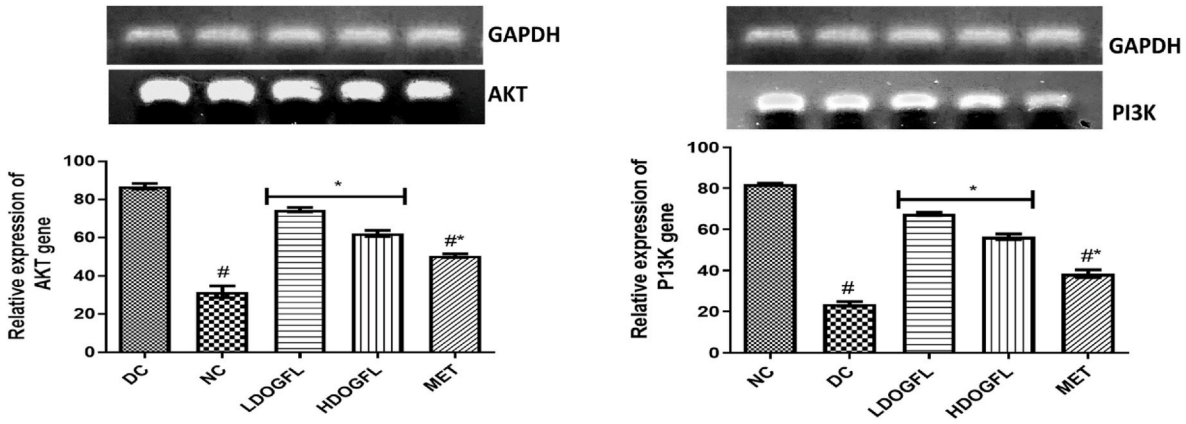

Fig. 8. Relative gene expressions of Akt and PI3K levels of flavonoid-rich extracts from *Ocimum gratissimum* leaf in streptozotocin-induced diabetic rats
Each value is a mean of eight determinations ± SD. #p < 0.05 vs. NC, *p < 0.05 vs. DC
Legend: NC: Normal Control, DC: Diabetic Control, LDOGFL: Low dose (150 mg/kg body weight) of flavonoid-rich extract of *Ocimum gratissimum,* HDOGFL: High dose (300 mg/kg body weight) of flavonoid-rich extract of *Ocimum gratissimum*, MET: Metformin, PI3K: Phosphatidylinositol 3-kinase, Each gel was cropped from different parts of the same gel.

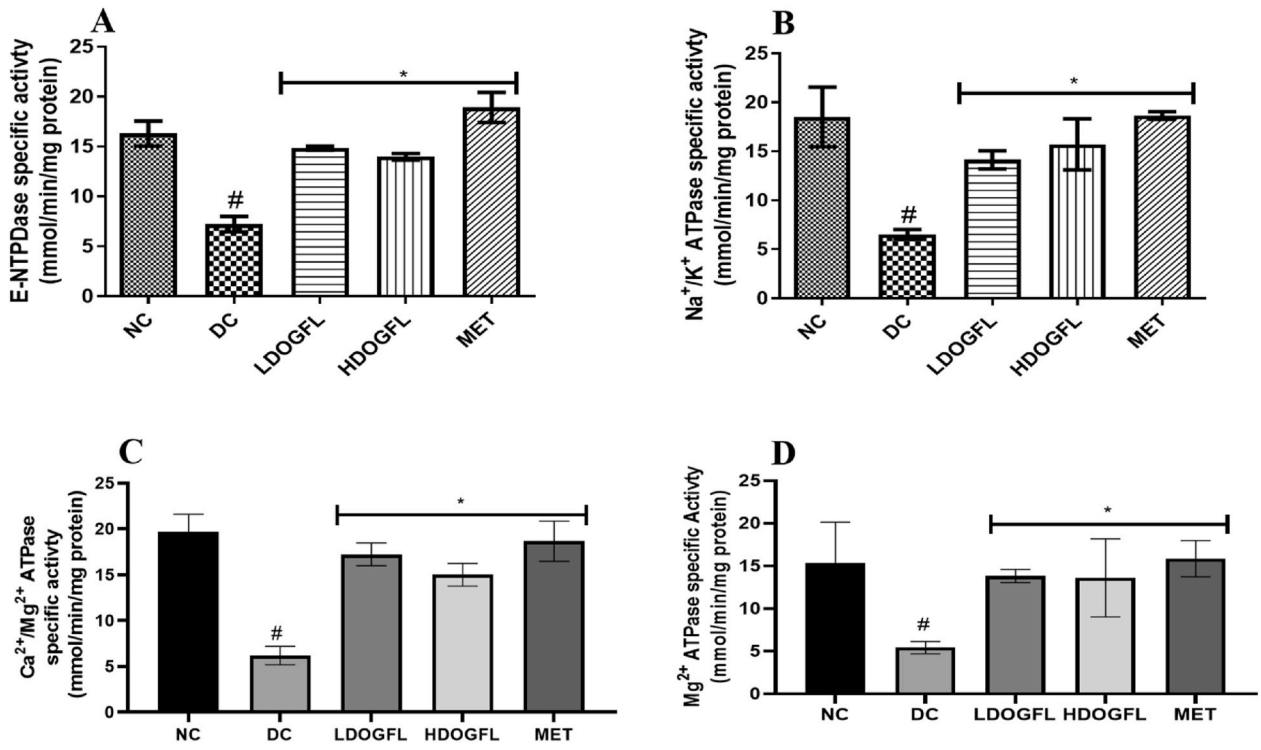

Fig. 9. Effect of *Ocimum gratissimum* flavonoid-rich extract on purinergic enzyme activities in the brain of STZ-induced diabetic rats
Each value is a mean of eight determinations ±SD. #$p < 0.05$ vs. NC, *$p < 0.05$ vs. DC **Legend**: NC: Normal Control, DC: Diabetic Control, LDOGFL: Low dose (150 mg/kg body weight) of flavonoid-rich extract of *O. gratissimum,* HDOGFL: High dose (300 mg/kg body weight) of flavonoid-rich extract of *O. gratissimum*, MET: metformin, E-NTPDase: Ecto-nucleoside triphosphate diphosphohydrolase.

enzymes play crucial roles in neurological conditions and toxic exposures, impacting treatment and management strategies. Elevated cholinergic enzyme activities can lead to impaired cholinergic neurotransmission, which is implicated in cognitive dysfunction observed in diabetes [72]. However, the administration of *O. gratissimum* flavonoid-rich extract effectively ($p < 0.05$) reversed these harmful increases, approaching near-normal levels. This indicates the potential of the extract to restore cholinergic function and ameliorate cognitive deficits associated with diabetes [73].

STZ-induced diabetes caused a significant ($p < 0.05$) increase in selected neurotransmitter levels, such as dopamine, norepinephrine, and serotonin in the brain. Dopamine and serotonin are neurotransmitters with distinct roles in brain function [75]. Dopamine, primarily known for its involvement in the reward system and motor control, may have neuroprotective properties, especially in Parkinson's disease. It's not typically associated with excitotoxicity but can indirectly influence it in certain conditions like schizophrenia [76]. Serotonin, on the other hand, plays a role in mood regulation and has potential neuroprotective effects, particularly against depression, and possesses anti-inflammatory properties. While not directly linked to excitotoxicity, serotonin receptors throughout the brain can modulate neurotransmitter systems, potentially influencing excitotoxicity in specific contexts [77,78]. These roles are complex and context-dependent, reflecting the intricate nature of neurotransmitter function in the brain [79]. These neurochemical changes, as a result of STZ intoxication, may contribute to neurological complications seen in diabetes [80]. However, the administration of the flavonoid-rich extract effectively tempered these abnormal increases in neurotransmitter concentrations in a dose-dependent manner, akin to metformin treatment. These results suggest the extract's neuroprotective potential by modulating neurotransmitter imbalances associated with diabetes [80].

Diabetic untreated rats exhibited a significant ($p < 0.05$) rise in nitric oxide (NO) levels in the brain, along with notable ($p < 0.05$) decreased concentrations of cerebral glucose transporter-4 (GLUT-4) and brain cardiolipin which is in agreement with the literature [81–83]. Nitric oxide (NO) serves as a vital signaling molecule, regulating processes like blood vessel dilation, neurotransmission, and immune function. In diabetes, particularly type-2 diabetes, NO's significance lies in its role in vasodilation, promoting the relaxation of blood vessels to enhance blood flow [83]. However, diabetes often leads to an increase in NO production and activity, resulting in impaired endothelial function and increased oxidative stress [84]. This endothelial dysfunction contributes to diabetic vascular complications, including cardiovascular disease and neuropathy [85]. Strategies to normalize NO levels can help mitigate these complications. Another crucial player in diabetes is GLUT-4, a glucose transporter protein primarily found in muscle, fat cells and brain. Its primary function is to facilitate the uptake of glucose into these cells upon insulin signaling. In type-2 diabetes, insulin resistance is common, causing impaired GLUT-4 translocation to the cell membrane and reduced glucose uptake [86,87]. This leads to elevated blood glucose levels. Cardiolipin, a unique phospholipid located in the inner mitochondrial membrane, plays a critical role in maintaining mitochondrial structure and energy production [88]. In diabetes, particularly type-2 diabetes, mitochondrial dysfunction and oxidative stress are prevalent and so, changes in cardiolipin composition may contribute to this dysfunction, resulting in reduced energy production and increased production of reactive oxygen species (ROS) [89,90]. Both factors are linked to the development of diabetes and its connected complications. In essence, understanding the roles of NO, GLUT-4, and cardiolipin sheds light on the intricate mechanisms underlying diabetes and its related health issues [91]. These changes occasioned by STZ intoxication reflect elevated free radical production, vascular and mitochondrial dysfunction as well as impaired glucose metabolism in diabetes [92]. However, treatment with *O. gratissimum* flavonoid-rich extract and metformin effectively ($p < 0.05$) normalized these parameters, indicating potential improvements in vascular/endothelial function,

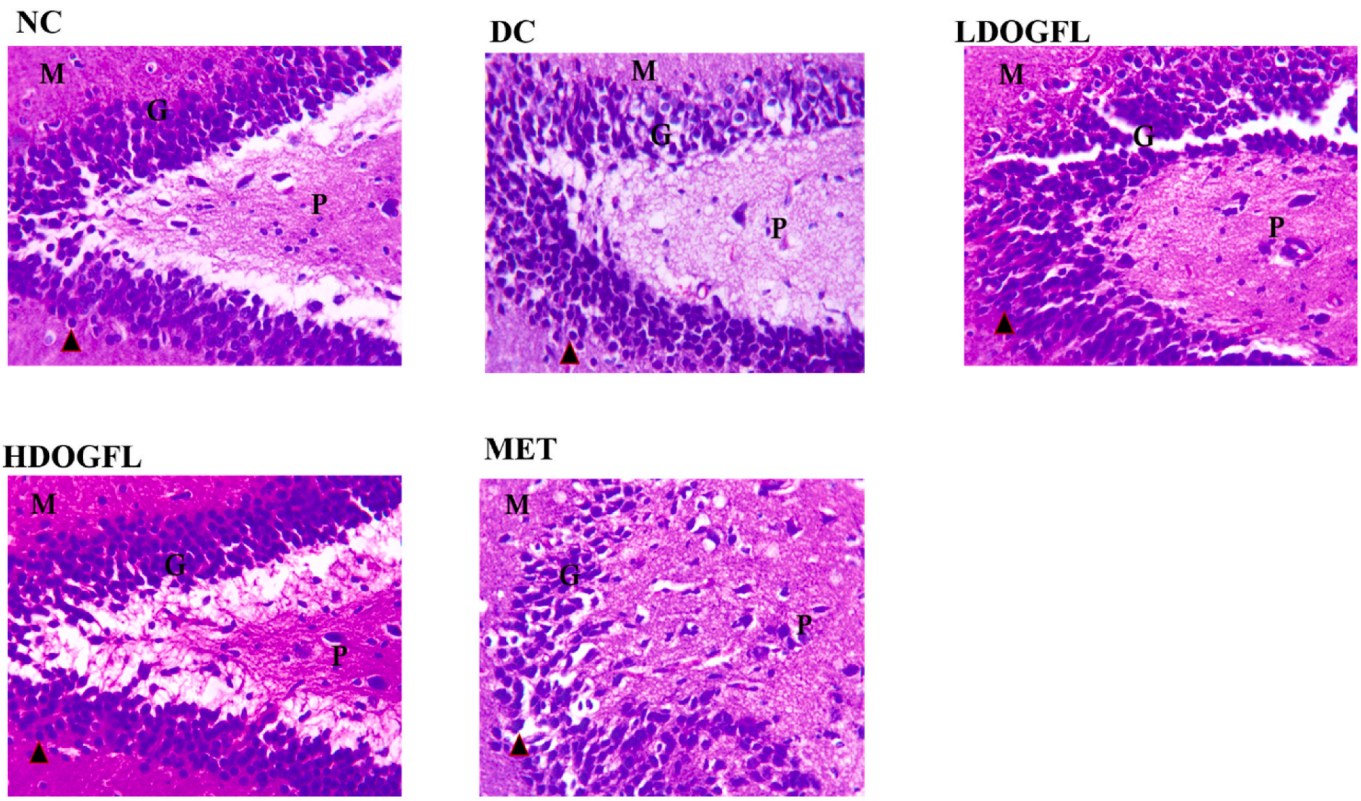

Fig. 10. Brain (hippocampus) histoarchitectural examination of flavonoid-rich extracts from *Ocimum gratissimum* leaf in streptozotocin-induced diabetic rats Stained with H&E (Mag. x800), Scale 5μM

Legend: NC: Normal Control, DC: Diabetic Control, LDOGFL: Low dose (150 mg/kg body weight) of flavonoid-rich extract of *O. gratissimum,* HDOGFL: High dose (300 mg/kg body weight) of flavonoid-rich extract of *O. gratissimum*, MET: metformin. G: Granular layer cells, M: Molecular layer, G: Granular layer, P: Polymorphic layer.

antioxidant-free radical balance and glucose transport in diabetic rats [93].

The relative gene expressions of protein kinase-B (AKT) and phosphoinositide-3-kinase (PI3K) were significantly ($p < 0.05$) downregulated in the brains of diabetic untreated rats compared to the normal control group. This above trend was in perfect agreement and tandem with previous literature [86,87]. Gene regulation plays a crucial role in diabetes management by influencing how genes are turned on or off to control insulin production, glucose metabolism, and other key processes [94]. Understanding and modulating gene expression through lifestyle changes, medications, and emerging therapies can help individuals better manage their blood sugar levels, improve insulin sensitivity, and reduce the risk of diabetes-related complications [95]. This personalized approach to diabetes care holds promise for more effective and tailored treatments in the future [86,96]. PI3K and AKT are essential components of the insulin signaling pathway, regulating glucose metabolism. PI3K, activated by insulin binding, produces PIP3, a key messenger. PIP3 recruits AKT to the cell membrane, where it becomes active [97,98]. AKT, a protein kinase, plays pivotal roles, including promoting glucose uptake, glycogen synthesis, protein production, inhibiting gluconeogenesis, and supporting cell survival [99,100]. Dysregulation of this pathway underlies insulin resistance in type-2 diabetes. This downregulation reflects impaired insulin signaling in diabetes [101]. Conversely, in agreement with literature, treatment with the flavonoid-rich extract and metformin significantly ($p < 0.05$) upregulated and restored the relative gene expressions of AKT and PI3K in a dose-dependent manner probably by their binding to the promoter region of the genes. This suggests that the extract may improve insulin signaling in diabetic rats by modulating these key genes [102–104].

The activities of purinergic enzymes, including Ecto-nucleoside triphosphate diphosphohydrolase (E-NTPDase), Na^+/K^+ATPase, Ca^{2+}/Mg^{2+}ATPase, and Mg^{2+}ATPase, were significantly ($p < 0.05$) diminished in diabetic untreated rats, indicating impaired purinergic signaling in the brain. In normal physiology, E-NTPDase help regulate purinergic signaling by breaking down ATP and ADP into AMP, impacting processes like neurotransmission and immune responses [105]. In diabetes, changes in purinergic signaling may affect insulin secretion and immune system regulation [106]. Na^+/K^+ATPase maintain cellular ion balance, crucial for nerve impulses and muscle contraction. In diabetic state, disruptions in Na^+/K^+ATPase activity can lead to electrolyte imbalances, nerve dysfunction, and muscle dysfunction [105–10108]. Ca^{2+}/Mg^{2+}ATPase regulate calcium and magnesium ion levels in cells, important for muscle contraction and intracellular signaling. In diabetes, calcium dysregulation is linked to complications like nerve damage and cardiovascular problems [109,110]. In regular physiology, Mg^{2+}ATPase help generate cellular energy through ATP hydrolysis. Diabetes-related magnesium imbalances can impact this enzyme activity, contributing to insulin resistance and cardiovascular issues [111,112]. These enzymes play vital roles in maintaining cellular function, and changes in their activities can contribute to diabetes-related complications [108,112]. However, administration of the *O. gratissimum* flavonoid-rich extract effectively ($p < 0.05$) ameliorated these reductions, similar to the effects of metformin. These results suggest that the extract may enhance purinergic signaling in diabetic rats, potentially impacting neurological function [113].

Histological examination of the hippocampus in diabetic untreated rats revealed deleterious modifications, including vacuolations and severe loss of granular layer cells, in tandem with previous studies [114,115]. However, treatment with the flavonoid-rich extract and metformin restored the histoarchitecture to near-normal conditions. This suggests that the extract may have a protective effect on the brain's structural integrity, potentially preserving cognitive function [116].

5. Conclusion

In summary, the findings from this comprehensive study shed light on the remarkable therapeutic prospects of *Ocimum gratissimum* flavonoid-rich extract in alleviating a wide array of complications associated with diabetes in streptozotocin (STZ)-induced diabetic rats. The extract demonstrated its potential through multifaceted actions, including potent antioxidant effects, anti-inflammatory properties, restoration of cholinergic function, modulation of neurotransmitter imbalances, improved vascular function and glucose transport, and the reinstatement of critical molecular signaling pathways like AKT and PI3K. Furthermore, the extract exhibited the capacity to enhance purinergic signaling and safeguard brain structural integrity, hinting at its neuroprotective qualities. The results of the study suggest that there is a need for more investigation into the molecular processes that are involved, the discovery of chemicals that are powerful, and the possibility that this natural extract might be used for the management of diabetes and the complicated problems that are associated with it. In addition, this study is not without limitation as we were unable to determine the flavonoid present using HPLC because we don't have this facility as at the time was research was conducted.

Institutional review board statement

Not applicable.

Ethical approval

All experimental protocols were approved FUOYE Faculty of Science Ethics Committee (FUOYEFSC 201122-REC2022/008)

Informed consent statement

Not applicable.

Consent for publication

Not applicable.

CRediT authorship contribution statement

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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.

Data availability

Data will be made available on request.

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