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Effects of *Ficus exasperata* on neurotransmission and expression of BDNF, tau, ACHE and BACE in diabetic rats

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ABSTRACT

Diabetes mellitus, a chronic metabolic disorder, has significant global health implications, particularly due to its neurological complications, such as diabetic neuropathy. This condition increases the risk of neurodegenerative diseases by affecting peripheral nerves and cognition. *Ficus exasperata*, known for its neuroprotective properties, shows promise as a therapeutic option for addressing these complications. This study evaluates the effects of methanol extract of *Ficus exasperata* (MEFE) on neurotransmission and the expression of Tau, brain-derived neurotrophic factor (BDNF), acetylcholinesterase (ACHE), and Beta-Site Amyloid Precursor Protein Cleaving Enzyme (BACE) in alloxan-induced diabetic Wistar rats. The controlled experimental design involved 20 Wistar rats divided into four groups (n = 5): control, diabetic untreated, diabetes + MEFE (200 mg/kg), and diabetes + insulin (0.3 IU). The methanol extract was prepared using cold maceration, and an aliquot was subjected to gas chromatography-mass spectrometry. Constituents of MEFE were docked with neurologic receptors. Blood glucose levels were measured using the glucose oxidase method, and neurotransmitter levels, antioxidants, oxidative stress markers, and the expression of Tau, BDNF, ACHE, and BACE were assessed using standard procedures and qRT-PCR. Data were analyzed using one-way ANOVA at $P < 0.05$. Results indicated that MEFE significantly reduced fasting blood glucose levels compared to untreated diabetic rats. *In silico* docking identified kaur-16-ene, a constituent of MEFE, as having the highest binding affinity for NMDA, TrkB, mAchR and nAchR receptors, indicating its neuroprotective potential. MEFE also enhanced antioxidant enzyme levels (SOD, GPx, catalase) while reducing oxidative stress markers (MDA, 8-OHdG). Gene expression analysis revealed that MEFE modulates the expression of Tau, BDNF, ACHE, and BACE, suggesting its potential to influence neurodegenerative pathways associated with diabetic neuropathy. *Ficus exasperata* demonstrates significant therapeutic potential in managing diabetic neuropathy and related cognitive impairments by modulating neurotransmission, protein expression, and antioxidant defenses.

1. Introduction

Diabetes mellitus is a chronic, multifaceted metabolic disorder marked by persistent hyperglycemia and has become an increasing global health concern. As of 2021, it is estimated that more than 500 million adults are living with diabetes, and this number is projected to reach 700 million by 2050 [1]. The pathophysiology of diabetes encompasses a myriad of complications, including cardiovascular diseases, neuropathy, nephropathy, and retinopathy, which contribute significantly to morbidity and mortality [2]. Among these complications, diabetic neuropathy, a disorder affecting the peripheral nerves, presents a substantial burden due to its prevalence and the profound impact on patients' quality of life. Diabetic neuropathy can manifest as sensory loss, pain, and autonomic dysfunction, and it is also associated with cognitive impairments and an increased risk of neurodegenerative diseases [3]. The central nervous system (CNS) is particularly vulnerable to the detrimental effects of diabetes [4]. Chronic hyperglycemia and insulin resistance can lead to alterations in brain metabolism, oxidative stress, inflammation, and impaired neurotransmission, all of which

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Abbreviations

MEFE	Methanol extract of *Ficus exasperata*
SOD	Superoxide dismutase
GPx	Glutathione peroxidase
CAT	Catalase
GR	Glutathione Reductase
MDA	Malondialdehyde
8-OHdG	8-hydroxy-2′-deoxyguanosine
BDNF	Brain-Derived Neurotrophic Factor
Tau	Tau Protein (also known as Microtubule-Associated Protein Tau)
ACHE	Acetylcholinesterase
BACE	Beta-Site Amyloid Precursor Protein Cleaving Enzyme
NMDA receptor	N-Methyl-D-Aspartate receptor
TrkB receptor	Tropomyosin receptor kinase B
mAchR receptor	Muscarinic Acetylcholine Receptor
nAchR receptor	Nicotinic Acetylcholine Receptor

contribute to neurodegeneration and cognitive deficits [5]. The search for effective therapeutic strategies to mitigate these neurological complications is of paramount importance. In this study, medicinal plants have gained attention for their potential neuroprotective properties, attributed to their rich content of bioactive compounds with antioxidant, anti-inflammatory, and neurotrophic activities. *Ficus exasperata*, commonly known as the sandpaper tree, is a medicinal plant native to Africa and widely used in traditional medicine for treating various ailments [6]. The plant belongs to the *Moraceae* family and is recognized for its distinctive rough leaves, which have earned it the common name. Traditional uses of *Ficus exasperata* include the treatment of inflammatory conditions, hypertension, wounds, and microbial infections [7]. The plant's therapeutic potential is largely attributed to its diverse phytochemical constituents, including flavonoids, alkaloids, tannins, and saponins. These bioactive compounds have demonstrated a range of pharmacological activities, such as antioxidant, anti-inflammatory, analgesic, and antimicrobial effects [8]. Recent scientific investigations have started to explore the neuroprotective properties of *Ficus exasperata*, particularly its potential to modulate neurotransmission and influence the expression of key proteins involved in neurodegeneration and neuronal plasticity [9]. This study aims to build upon this emerging body of research by investigating the effect of *Ficus exasperata* on neurotransmission and the expression of Tau, brain-derived neurotrophic factor (BDNF), acetylcholinesterase (ACHE), and Beta-Site Amyloid Precursor Protein Cleaving Enzyme (BACE) in diabetic rats. Tau protein is a microtubule-associated protein that plays a critical role in maintaining the stability of the neuronal cytoskeleton [10]. In pathological conditions, Tau can undergo hyperphosphorylation, leading to the formation of neurofibrillary tangles, a hallmark of neurodegenerative diseases such as Alzheimer's disease [11]. Hyperglycemia and insulin resistance associated with diabetes can exacerbate Tau pathology, contributing to cognitive impairments. Therefore, understanding how *Ficus exasperata* influences Tau expression and phosphorylation status in diabetic conditions is of significant interest. BDNF is a neurotrophin essential for neuronal survival, growth, differentiation, and synaptic plasticity. It plays a crucial role in learning and memory processes, and its expression is often reduced in neurodegenerative diseases and diabetic neuropathy [12]. Enhancing BDNF signaling has been proposed as a therapeutic strategy to combat neurodegeneration and promote cognitive function. Investigating the effect of *Ficus exasperata* on BDNF expression could provide valuable insights into its potential as a neuroprotective agent in diabetes. Recent studies have explored the impact of *Ficus exasperata* on Beta-Site Amyloid Precursor Protein Cleaving Enzyme (BACE), a key enzyme involved in the pathogenesis of Alzheimer's disease, particularly in diabetic conditions [13]. Diabetes, a metabolic disorder characterized by chronic hyperglycemia, has been linked to an increased risk of neurodegenerative diseases, including Alzheimer's [14]. This present study aim to uncover the potential therapeutic benefits of *Ficus exasperata* in mitigating neurodegenerative processes associated with diabetes. The alloxan-induced diabetic rat model is widely used in diabetes research due to its ability to mimic the pathophysiological changes observed in human diabetes, including hyperglycemia, insulin deficiency, and diabetic complications [15]. By administering *Ficus exasperata* extracts to diabetic rats and analyzing changes in neurotransmitter levels and the expression of the aforementioned proteins, this research aims to elucidate the potential neuroprotective mechanisms of the plant. The outcomes of this study could have significant implications for the development of novel therapeutic strategies targeting diabetic neuropathy and cognitive impairments. *Ficus exasperata* could serve as a basis for the development of new treatments derived from natural products.

2. Materials and methods

2.1. Pant collection, identification and extraction process

Ficus exasperata leaves were collected in April 2022, during a period of increased light intensity of 12–14 h per day, which favors the synthesis of essential natural components in the plant's sap, flowers, seeds, bark, leaves, and pods. The leaves were sourced from Ondo State and authenticated at the University of Ibadan's Department of Botany using voucher specimen number UIH: 240407. After air-drying, the leaves were milled into powder and soaked in 99.9 % methanol (Sigma-Aldrich Co., LLC, Missouri, US). Methanol was selected for its ability to mix with water, its polarity, and its low boiling point, which facilitates easy removal after extraction and allows it to dissolve both polar and non-polar substances. The extraction process followed the cold maceration procedure outlined by Kapalavavi et al. (2021) [16]. Approximately 21.3 kg of Ficus exasperata Vahl leaves were cleaned under running water and air-dried for four weeks at room temperature (25 °C). Afterward, 5.1 kg of dried leaves were ground, yielding 3.4 kg of powder. This powder was then soaked in 5 L of methanol for 72 h and subsequently filtered. The filtrate was concentrated using a rotary evaporator (Union Laboratory, California, US) at 60 °C. Finally, the extracted material was further dried in a water bath, resulting in a pasty, dark-green extract (96 g) with a yield of 2.82 %.

2.2. Study design

There were twenty male Wistar rats assigned to four groups of five rats each. The Group I (control group), represents non-diabetic group which receives 0.3 mL of distilled water, Group II (diabetes untreated group) which receives 0.3 mL distilled water, Group III was diabetic group treated with Methanol Extract of *Ficus exasperata* (MEFE) at a dosage of 200 mg/kg, Group IV was diabetic group which received treatment with insulin at a dosage of 0.3 IU, a typical pharmacological intervention for managing diabetes. Diabetes mellitus was induced in the animals via intraperitoneal injection (*i.p*) of alloxan monohydrate at a dose of 200 mg/kg which is a standard way to cause diabetes in experimental animals by destroying the pancreatic cells that produce insulin. Treatments with MEFE and insulin were administered for a period of 28 days. A dose of 200 mg/kg of *Ficus exasperata* was selected based on previous studies [6]. In order to establish an efficient concentration for the targeted outcomes, this dosage was established by exploratory investigations and dose-response tests. The experiment lasted for 28-day duration to enable a thorough evaluation of the long-term impacts of *Ficus exasperata* on neurotransmission and other pertinent physiological parameters in the test animals.

2.3. Animal care and handling

The University of Medical Sciences Ethical Review Committee in Ondo State approved all animal treatments, ensuring that the study complied with ethical norms and guidelines. These study was carried out in compliance with the Declaration of Helsinki, which establishes moral guidelines for animal-based medical research [17]. The Wistar rats (150 ± 10 g) utilized in this investigation were fed a regular chow diet up until the point of sacrifice. The rats were fasted overnight to determine their physiological status with greater accuracy. The rats were humanely put to sleep after receiving 0.5 mg/kg of ketamine, to reduce their pain and suffering, and then subjected to cervical dislocations. The rat's skull was then carefully opened, and the brain was gently removed using precise dissection tools. A 50 mg sample of brain tissue was removed, kept at −20 °C in an RNAlater, and then utilized to analyze the mRNA expression of neurological biomarkers. Thereafter, the remaining brain tissue were weighed and then homogenized into a mixture for further experimental assays using phosphate-buffered saline. The relative brain weight was calculated by dividing the brain weight by the final body weight and multiplying the result by 100. The homogenized samples were separated into various cellular components by cold centrifugation (4 °C) at 5000 rpm for 5 min. The supernatant was then collected with a micropipette for further biochemical examination. This procedure ensures that the brain samples were consistently and precisely prepared in order to generate reproducible experimental findings.

2.4. Gas chromatography-mass spectrometry analysis of methanol extract of Ficus exasperata

For GC-MS analysis, the methanol extract of *Ficus exasperata* was redissolved in methanol. The sample was evaporated and then run down a chromatographic column in a gas chromatograph to separate the different chemicals for examination. The mass-to-charge ratios of these separated chemicals were then used by mass spectrometry to identify and quantify them, resulting in a comprehensive chemical profile of methanol extract of *Ficus exasperata* [18].

2.5. Assay of antioxidant enzymes

Superoxide dismutase activity was measured using a spectrophotometric assay at 37 °C, where the enzyme's ability to inhibit the reduction of nitroblue tetrazolium was monitored at 560 nm [19]. Glutathione peroxidase activity was assessed by measuring the rate of NADPH oxidation at 340 nm, typically after incubating the reaction mixture at 37 °C for 5 min [20]. Catalase activity was determined by monitoring the decomposition of hydrogen peroxide at 240 nm, after an initial incubation period of 10 min at room temperature [21]. Glutathione reductase activity was measured by the decrease in absorbance at 340 nm due to NADPH oxidation, with the reaction mixture incubated at 25 °C for 30 min [22]. MDA levels were quantified using the thiobarbituric acid reactive substances (TBARS) assay, where samples were mixed with TBA and incubated at 95 °C for 60 min, and the absorbance of the resulting pink complex was read at 532 nm [23]. Lastly, 8-OHdG was measured using an ELISA, with samples and standards incubated at 37 °C for 1 h, followed by the addition of a detection antibody and subsequent absorbance reading at 450 nm [24].

2.6. Determination of neurotransmitter level

Acetylcholine levels were quantified using a colorimetric assay, where the sample was incubated at 37 °C for 30 min with reagents that produce a measurable color change, read at 540 nm [25]. Glutamate levels were determined using an enzymatic assay, with samples incubated at 25 °C for 20 min, and the resulting color change measured at 450 nm [26]. Serotonin and dopamine levels were assessed using high-performance liquid chromatography (HPLC) with electrochemical detection, requiring samples to be prepared and maintained at 4 °C before analysis [27]. GABA levels were determined by an enzymatic assay, where the reaction mixture was incubated at 37 °C for 60 min, with the absorbance read at 340 nm [26]. Total protein was measured using the Bradford protein assay, with samples incubated with Coomassie Brilliant Blue dye at room temperature for 10 min, and the absorbance measured at 595 nm [28].

2.7. Gene expression analysis

Total RNA was extracted from the rat brain tissues using an RNA extraction kit, followed by the synthesis of cDNA through reverse transcription using a reverse transcriptase enzyme. The qPCR reactions were then set up with specific primers (Table 1) for Tau, BDNF, ACHE, and BACE genes, along with a suitable DNA polymerase and a SYBR Green for fluorescence detection. The qPCR reactions were performed in a thermal cycler with an initial denaturation at 95 °C for 10 min, followed by 40 cycles of denaturation at 95 °C for 15 s, annealing at 60 °C for 30 s, and extension at 72 °C for 30 s. The fluorescence emitted during the amplification was measured in real-time, and the expression levels of the target genes were quantified relative to a housekeeping gene (GAPDH), allowing for precise determination of gene expression changes [29].

2.8. In silico docking study

In-silico docking was performed using a systematic approach starting with sourcing the ligands of interest from the PubChem database in SDF format. The crystal structures of the target receptors, namely the NMDA receptor, TrkB receptor, mAchR receptor, and nAchR receptor, were obtained from the RCSB-PDB database and prepared using Biovia Studio Visualizer tools. This preparation involved the removal of water molecules, inhibitors, and co-crystallized compounds, as well as the addition of missing hydrogen atoms. Further receptor preparation was conducted using UCSF Chimera tools to ensure optimal structure for docking. Molecular docking analysis was carried out with Pyrex software, where the PDB and SDF formats of the proteins and ligands were imported for docking simulations. Binding affinities were calculated and ranked, with the interactions of the highest affinity ligands being visualized using Discovery Studio Visualizer [30].

2.9. Statistical analysis

Statistical analysis was performed using one-way analysis of variance (ANOVA), followed by Tukey's multiple comparison post-hoc test, utilizing GraphPad Prism version 10.3.0. The data were expressed as mean ± SEM, with significance defined at $p < 0.05$.

3. Results

Table 3 Shows the effect of methanol extract of *Ficus exasperata* on body and brain weight. Data were expressed as mean ± SEM (n = 5, all groups) and analyzed using one-way ANOVA followed by Tukey multiple comparism Post-hoc test. Values are considered significant at $p < 0.05$*, $p < 0.01$**, $p < 0.001$*** and $p < 0.0001$****

Table 1
The primer sequences of target genes.

Gene		Primer Sequence (5′ → 3′)
Tau protein (Tau)	F	GAGGAGAGCCCAATGTTTCATA
	R	GCCTGCAGACCATACATCTATAC
Brain-Derived Neurotrophic Factor (BDNF)	F	TTGAGCACGTGATCGAAGAGC
	R	GTTCGGCATTGCGAGTTCCAG
Acetylcholinesterase (ACHE)	F	TCCTCCTTGGACGTGTATGA
	R	GGTAGAGCCAAGAAGCCAAA
Beta-Site Amyloid Precursor Protein Cleaving Enzyme (BACE)	F	ATGAGGCCCAGTGTGGTGGA
	R	CAGCACGCGCAGGGCCGCGG

3.1. Chemical composition and impact of methanol extract of Ficus exasperata on weight and glucose levels

Table 2 reveals the chemical composition of *Ficus exasperata* Vahl leaf extract (MEFE), which has demonstrated significant interactions with neurologic receptors in the brain through *in silico* docking studies (see Fig. 1). In the central nervous system, receptors like NMDA, TrKb, mAchR, and nAchR play crucial roles in neurotransmission. Previous research identified kaur-16-ene, a constituent of MEFE, as a key compound responsible for its biological properties [31]. In this study, kaur-16-ene exhibited the highest binding affinity with NMDA receptor (−9.0 kcal/mol), TrKb receptor (−11.3 kcal/mol), and nAchR receptor (−8.7 kcal/mol). For mAchR receptor, caryophyllene oxide showed the highest binding affinity with a vina score of −8.5 kcal/mol. These interactions are mediated primarily by alkyl and van der Waals forces, alongside interactions with the amino acid residues of these receptors (Fig. 6). The binding affinity of kaur-16-ene and caryophyllene oxide surpassed that of standard neurotransmitters acetylcholine and glutamate, indicating stronger interactions with these target proteins/receptors, as larger negative docking scores reflect higher binding affinity. There was significant increase ($p < 0.01$) in body weight in diabetic group treated with insulin (0.3 IU) when compared to diabetic untreated. However there was no significant difference ($p > 0.05$) in body weight in diabetic treated with MEFE when compared to diabetic untreated group. In addition, no significant difference ($p > 0.05$) was observed in brain weight in diabetic group treated with MEFE when compared to diabetic untreated group. Fasting Blood glucose (FBG) decreased significantly ($p < 0.0001$) in diabetic group treated with MEFE when compared to diabetic untreated (Fig. 2). Furthermore, there was significant decrease ($p < 0.0001$) in Fasting blood glucose in diabetic group treated with insulin (0.3IU) when compared to diabetic untreated. The observed decrease in FBG in insulin treated group was comparable with the level of glucose in the control group.

Table 3
Shows the effects of Methanol extract of *Ficus exasperata* on body and brain weights.

Groups	Initial Body Weight	Final Body Weight (g)	Body Weight Difference (g)	Brain Weight (g)	Relative brain Weight (%)
Control	126.20 ± 4.35	158.0 ± 6.7	31.80	1.37 ± 0.09	0.87
Diabetes Untreated	138.00 ± 3.16	127.40 ± 2.9	10.60	1.43 ± 0.12	1.12
Diabetes + *Ficus exasperata* (200 mg/kg)	129.00 ± 8.53	129.90 ± 9.0	0.90	1.49 ± 0.21	1.15
Diabetes + Insulin (0.3 IU)	141.20 ± 4.15	194.30 ± 7.2**	53.10	1.46 ± 0.14	0.75

3.2. Effect of methanol extract of Ficus exasperata on brain antioxidant enzymes

The activity of superoxide dismutase enzyme increased significantly ($p < 0.0001$) following treatment with MEFE (200 mg/kg) compared to diabetic untreated. This increase was comparable with the control group. Diabetic group treated with insulin (0.3 IU) also showed significant increase at $p < 0.001$ (Fig. 3a). Glutathione peroxidase increased significantly ($p < 0.0001$) in diabetic group treated with MEFE (200 mg/kg) when compared to diabetic untreated group. However a significant decrease ($p < 0.0001$) in glutathione peroxidase was observed in diabetic group treated with insulin compared to diabetic untreated (Fig. 3b). Treatment with MEFE (200 mg/kg) caused significant increase ($p < 0.0001$) in catalase activity when compared to diabetic untreated. Catalase activity also increased significantly ($p < 0.0001$) in diabetes + insulin (0.3 IU) when compared to diabetic untreated group (Fig. 3c). The activity of glutathione reductase increased significantly ($p < 0.0001$) in diabetic group treated with MEFE (200 mg/kg) when compared to diabetic untreated. This observed increased was comparable with the control group. Diabetic group treated with insulin (0.3 IU) also showed significant increase at $p < 0.05$ when compared to diabetic untreated group (Fig. 3d). Malondialdehyde decreased significantly ($p < 0.0001$) in diabetic groups treated with MEFE (200 mg/kg) and Insulin (0.3 IU) when compared to diabetic untreated group (Fig. 3e). Furthermore, 8-OHdG decreased significantly ($p < 0.01$) in diabetic group treated with MEFE (200 mg/kg). There was also significant decrease ($p < 0.001$) in 8-OHdG in diabetes group treated with insulin

Table 2
Chemical profile of methanol extract of *Ficus exasperata* and its binding affinity with neurologic receptors.

S/N	RT (min)	Compound	NMDA receptor Kcal/mol	TrKb receptor Kcal/mol	mAchR receptor Kcal/mol	nAchR receptor Kcal/mol
1	11.550	**Cycloisolongifolene, 8,9-dehydro-**	−7.4	−9.0	−8.0	−8.3
2	12.209	**Eudesma-4(15),7-dien-1.beta –ol**	−8.0	−9.0	−8.1	−7.9
3	13.714	**Neophytadiene**	−6.7	−7.4	−5.5	−6.0
4	14.143	**9-Eicosene, (E)**	−6.0	−6.8	−5.0	−6.0
5	14.604	**Pentadecanoic acid, 14-methyl-**	−5.0	−6.9	−4.9	−5.7
6	15.009	**n-Hexadecanoic acid**	−6.2	−6.3	−4.9	−6.3
7	15.266	**Hexadecanoic acid, ethyl ester**	−6.3	−6.6	−4.6	−5.6
8	15.598	**7-Tetradecyne**	−4.8	−6.3	−5.6	−5.2
9	15.857	**Kaur-16-ene**	−9.0	−11.3	−8.4	−8.7
10	16.217	**10,13-Octadecadienoic acid, methyl**	−6.9	−7.0	−5.0	−6.0
11	16.272	**9-Octadecenoic acid (Z)-**	−6.6	−7.1	−5.1	−5.8
12	16.327	**11-Octadecenoic acid, methyl ester**	−6.0	−6.8	−5.1	−5.2
13	16.382	**Oxirane, decyl-**	−5.2	−5.6	−4.8	−5.4
14	16.504	**Methyl stearate**	−6.4	−6.6	−4.9	−5.5
15	16.597	**Linoelaidic acid**	−5.6	−7.4	−5.7	−6.6
16	16.650	**cis-Vaccenic acid**	−4.7	−4.2	−6.1	−6.3
17	16.831	**Linoleic acid ethyl ester**	−6.5	−7.1	−5.3	−6.1
18	16.880	**Ethyl Oleate**	−5.1	−7.0	−5.3	−5.5
19	17.100	**Octadecanoic acid, ethyl ester**	−4.9	−6.7	−5.5	−5.0
20	17.633	**Alpha-Farnesene**	−7.3	−7.6	−5.7	−6.5
21	17.872	**Hexane, 1-chloro-5-methyl-**	−4.7	−4.7	−4.1	−4.4
22	18.689	**Caryophyllene oxide**	−7.2	−8.9	−8.5	−7.4
23	19.217	**Bicyclo [5.1.0] octane, 8-methylene-**	−6.0	−6.3	−5.7	−6.0
	Standard	**Acetylcholine**	−4.0	−4.1	−4.2	−4.5
	Standard	**Glutamate**	−5.1	−4.6	−4.9	−5.0

Fig. 1. Shows *Ficus exasperata* Vahl leaves (*White fig*).

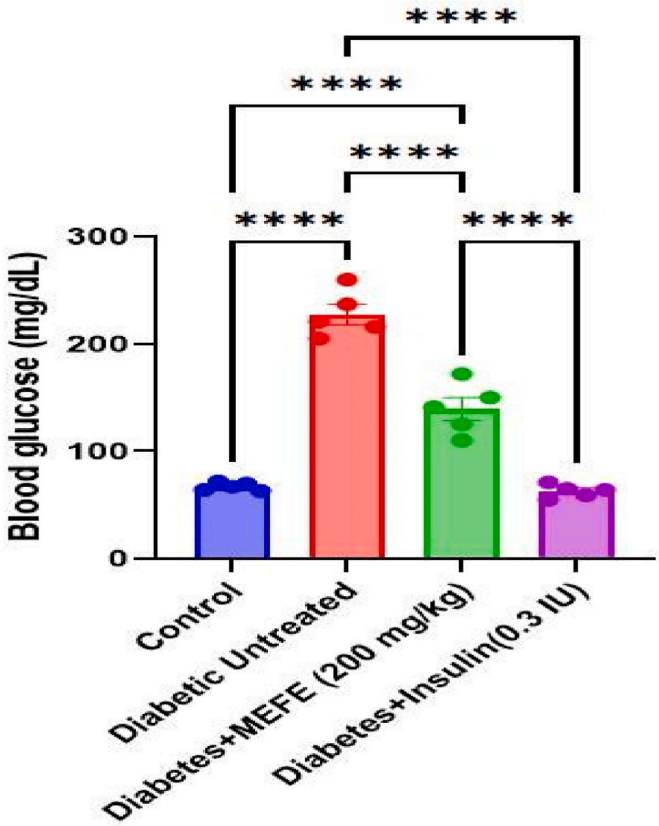

Fig. 2. Shows the effect of methanol extract of *Ficus exasperata* on blood glucose concentration. Data were expressed as mean ± SEM (n = 5, all groups) and analyzed using one-way ANOVA followed by Tukey multiple comparism Post-hoc test. Values are considered significant at $p < 0.05$*, $p < 0.01$**, $p < 0.001$*** and $p < 0.0001$****.

(0.3 IU) when compared with diabetic untreated group (Fig. 3f).

3.3. Effect of methanol extract of Ficus exasperata on brain neurotransmitters

Acetylcholine increased significantly ($p < 0.0001$) in diabetes + MEFE (200 mg/kg) when compared to diabetic untreated. Furthermore acetylcholine increased significantly ($p < 0.05$) in diabetes + insulin (0.3 IU) group when compared to control group (Fig. 4a). Glutamate decreased significantly ($p < 0.0001$) in diabetic untreated when compared to control. However, there was significant increase ($p < 0.0001$) in glutamate level in diabetes + MEFE (200 mg/kg) when compared to diabetic untreated (Fig. 4b). Total protein increased significantly in diabetes + MEFE (200 mg/kg) when compared to diabetic untreated (Fig. 4c). The observed increase in protein in diabetes + MEFE (200 mg/kg) was significantly higher ($p < 0.01$) compared to control. There was significant decrease ($p < 0.001$) in dopamine in diabetic untreated compared to control. Dopamine level increased significantly in diabetic group treated with MEFE (200 mg/kg) and insulin (0.3 IU) compared to diabetic untreated (Fig. 4d). There was significant decrease ($p < 0.01$) in the level of GABA in diabetic untreated when compared to control. However, GABA level increased significantly ($p < 0.01$) in diabetes + MEFE (200 mg/kg) when compared to diabetic untreated (Fig. 4e). There was significant decrease ($p < 0.0001$) in serotonin level in diabetic untreated group when compared to control group. However, serotonin level increased significantly ($p < 0.01$) in diabetes + MEFE (200 mg/kg) when compared to diabetic untreated.

3.4. Effect of methanol extract of Ficus exasperata on mRNA expression of target genes in the brain

We observed significant increase ($p < 0.05$) in mRNA expression of brain derived neurotrophic factor (BDNF) in diabetes + MEFE (200 mg/kg) when compared to diabetic untreated (Fig. 5) (Supplementary material 1). The expression of Tau protein increased significantly ($p < 0.0001$) in diabetic untreated when compared to control group. However, Tau protein mRNA expression decreased significantly in ($p < 0.0001$) in diabetes + MEFE (200 mg/kg) when compared to diabetic unteated. The mRNA of acetylcholinesterase was significantly expressed ($p < 0.0001$) in diabetes + MEFE (200 mg/kg) when compared to diabetic untreated. Furthermore, the mRNA expression of Beta-Site Amyloid Precursor Protein Cleaving Enzyme (BACE) increased significantly ($p < 0.01$) in diabetic untreated when compared to control. However, the mRNA expression of BACE decreased significantly ($p < 0.0001$) in diabetes + MEFE (200 mg/kg) when compared to diabetic untreated.

3.5. Histological assessment of the brain tissue in diabetic rats treated with methanol extract of Ficus exasperata

Fig. 7 shows the structural changes in photomicrograph in the brain tissue. The photomicrograph of the brain tissue appeared to have normal architecture in the control group. However, the nucleus of cell in diabetic untreated appeared condensed and deeply stained due to cell damage. The cytoplasm showed vacuolation, indicating cellular stress or degeneration. Small vacuolation of the cytoplasm was also observed in diabetes + MEFE (200 mg/kg) though treatment with *Ficus exasperata* appeared to reduce the size of these vacuoles. No vacuolation was observed following treatment with insulin (0.3 IU).

4. Discussion

In this study, we investigated the effects of *Ficus exasperata* Vahl leaf extract on neurotransmitters and its potential impact on the mRNA expression of BDNF, Tau, ACHE, and BACE in diabetic rats. There was no significant change in brain or body weight following treatment with *Ficus exasperata*, suggesting that its mechanisms of action may primarily

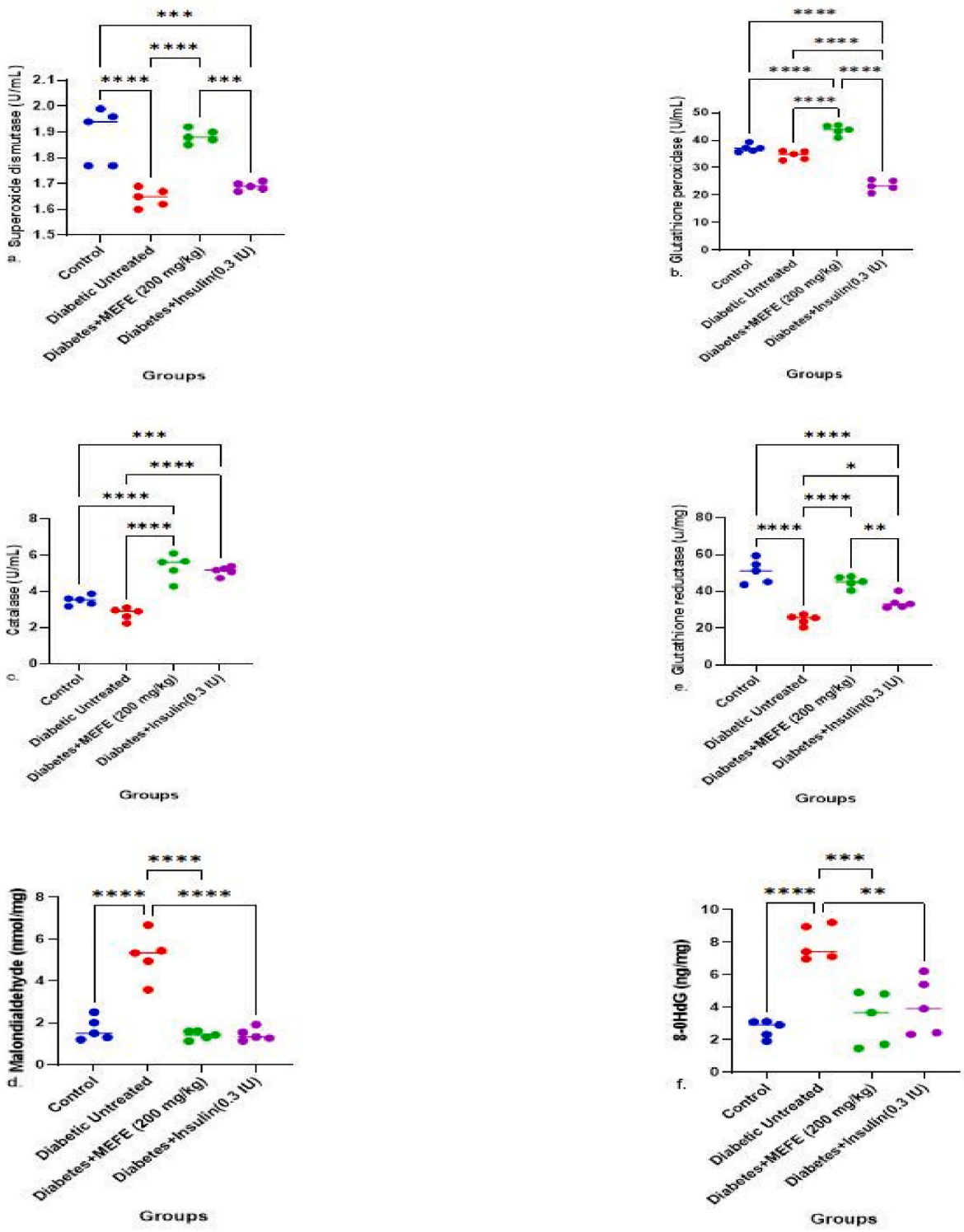

Fig. 3. Show the effect of methanol extract of *Ficus exasperata* on antioxidant activities (a) Superoxide dismutase (b) Glutathione peroxidase (c) Catalase (d) Glutathione Reductase (e) Malondialdehyde (f) 8-hydroxy-2′-deoxyguanosine Data were expressed as mean ± SEM (n = 5, all groups) and analyzed using one-way ANOVA followed by Tukey multiple comparism Post-hoc test. Values are considered significant at $p < 0.05$*, $p < 0.01$**, $p < 0.001$*** and $p < 0.0001$****.

target metabolic pathways that do not directly affect overall body or brain weight [6,32]. This findings is in tandem with the report of Bafor, 2009 [33] who stated that no significant changes in body weight was produced following treatment with *Ficus exasperata.* Blood glucose concentration decreased significantly in diabetic rats treated with MEFE (200 mg/kg) though this effect was not comparable with insulin, the standard antidiabetic agent used in this study. *Ficus exasperata* could have caused a significant decrease in blood glucose by enhancing insulin sensitivity, which allows cells to more effectively take up glucose from the blood [34,35]. Additionally, its chemical constituents might stimulate insulin secretion from the pancreatic beta cells, increasing the hormone's availability to regulate blood sugar levels [34]. *Ficus exasperata* may also have inhibited carbohydrate-digesting enzymes like alpha-amylase and alpha-glucosidase, slowing down glucose absorption

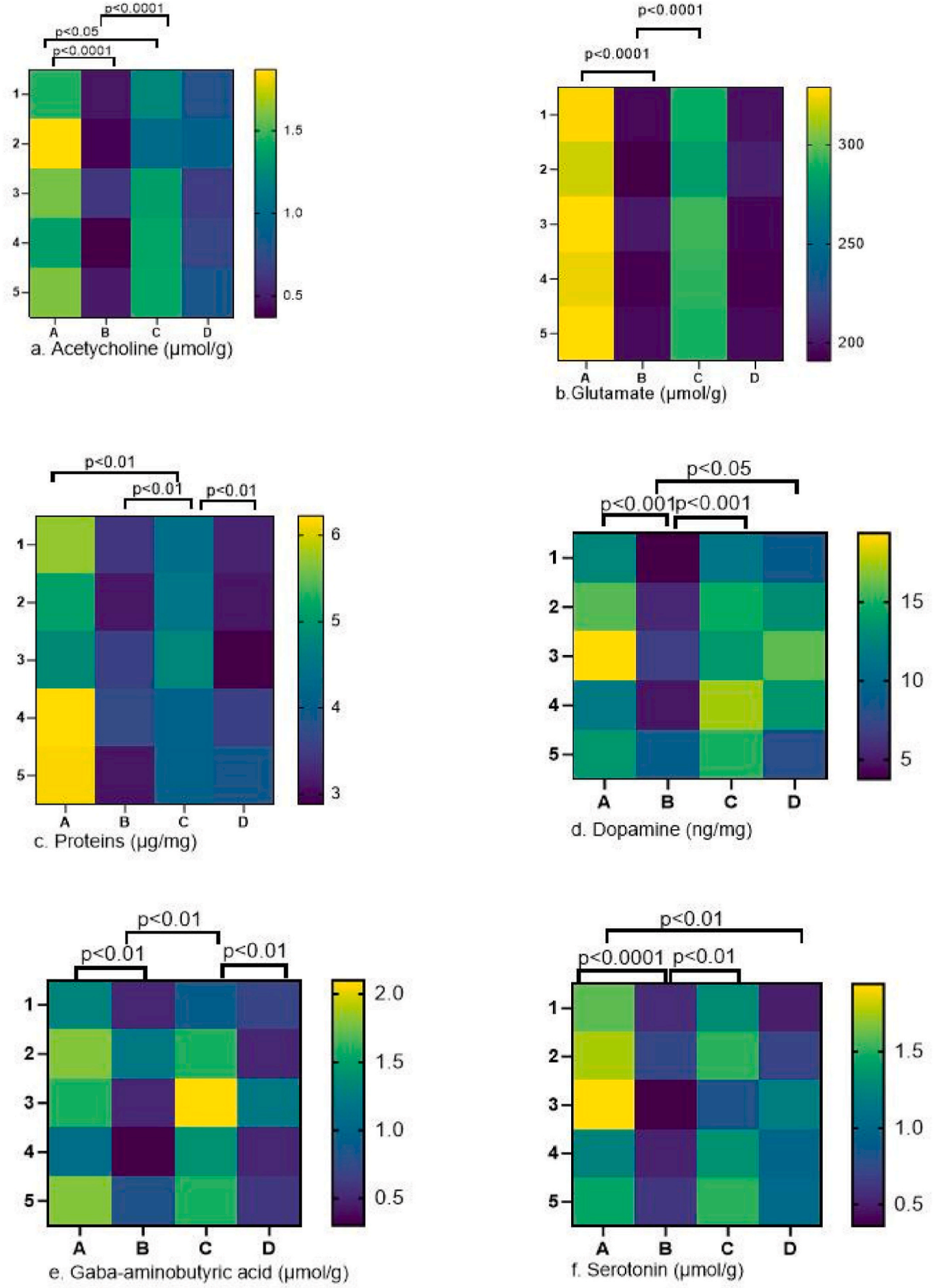

Fig. 4. Show the effect of methanol extract of *Ficus exasperata* on mRNA expression of (a) Acetylcholine (b) Glutamate (c) Total protein (d) Dopamine (e) Gaba-aminobutyric acid (f) Serotonin. A (Control), B (Diabetes untreated), C (Diabetes + MEFE (200 mg/kg), D (Diabetes + Insulin (0.3 IU) Data were expressed as mean ± SEM (n = 5, all groups) and analyzed using one-way ANOVA followed by Tukey multiple comparism Post-hoc test. Values are considered significant at $p < 0.05$*, $p < 0.01$**, $p < 0.001$*** and $p < 0.0001$****.

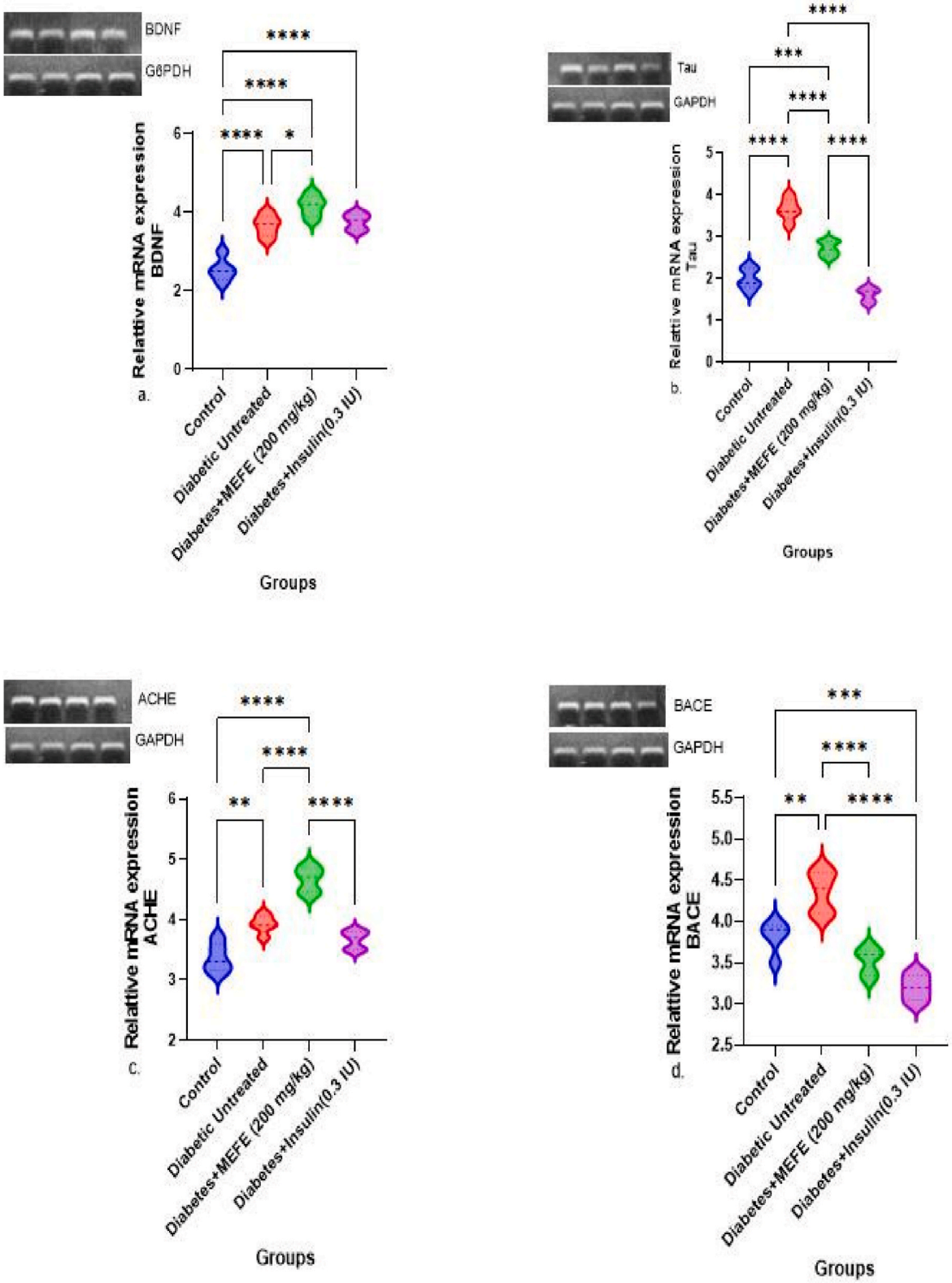

Fig. 5. Show the effect of methanol extract of *Ficus exasperata* on mRNA expression of (a) Brain Derived Neurotrophic Factor (b) Tau protein (c) Acetylcholinesterase (d) Beta-Site Amyloid Precursor Protein Cleaving Enzyme Data were expressed as mean ± SEM (n = 5, all groups) and analyzed using one-way ANOVA followed by Tukey multiple comparism Post-hoc test. Values are considered significant at $p < 0.05$*, $p < 0.01$**, $p < 0.001$*** and $p < 0.0001$****.

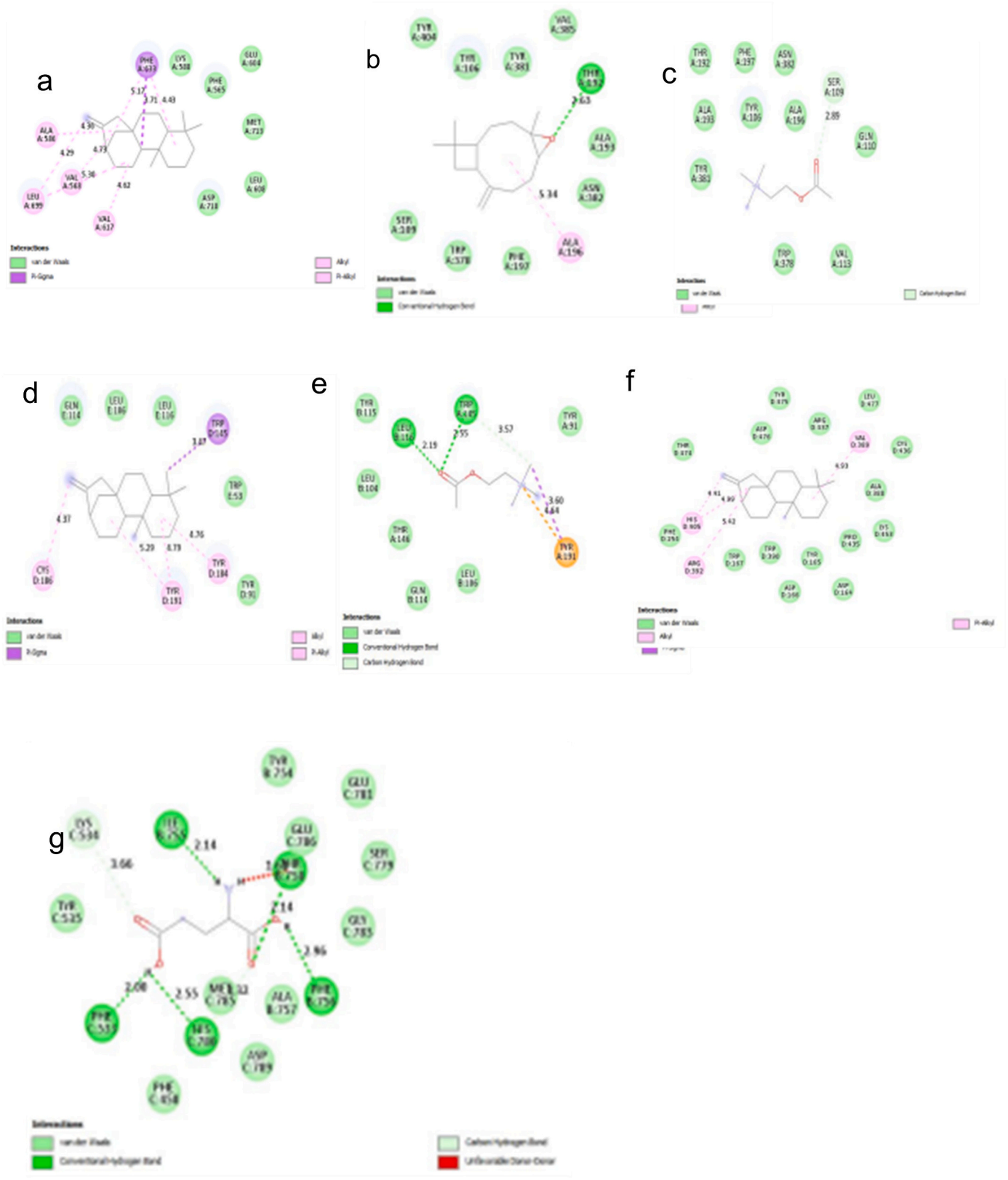

Fig. 6. Show the molecular interaction between a. Kaur-16-ene and TrkB Receptor b. Caryophyllene oxide and Muscarinic Acetylcholine Receptor (mAChR) c. Acetylcholine and Muscarinic Acetylcholine Receptor (mAChR) d. Kaur-16-ene and Nicotinic Acetylcholine Receptor (nAChR) e. Kaur-16-ene and N-methyl-D-aspartate receptor (NMDA) and f. Glutamate and N-methyl-D-aspartate receptor (NMDA).

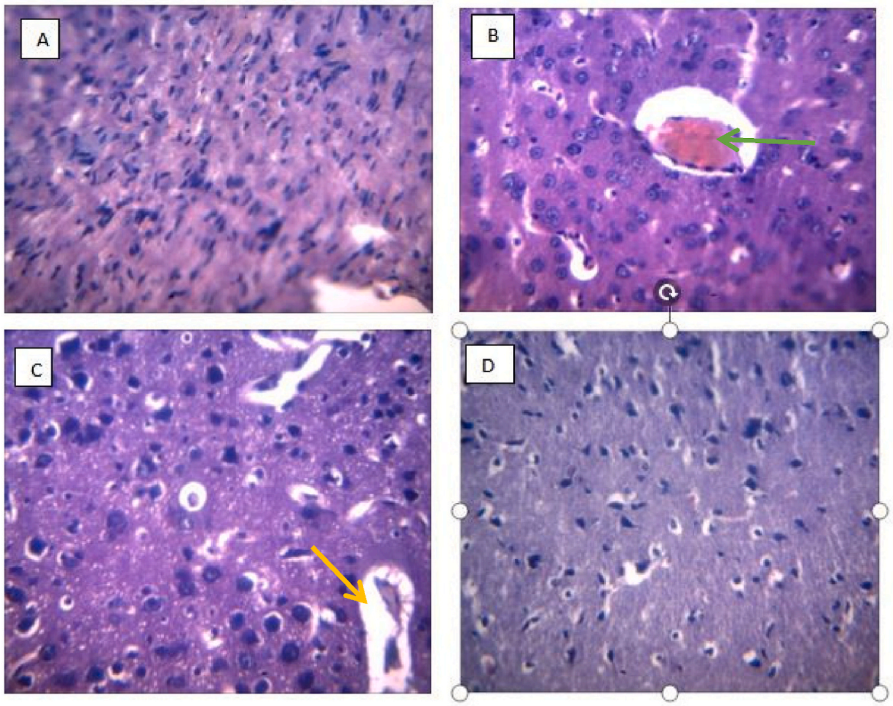

Fig. 7. The photomicrograph of the brain tissue. A (Control) showed normal architecture of the brain tissue with dark stained nucleus B (Diabetic untreated) showed brain tissue with deeply stained cytoplasm and vacuolation (Green colour), C (diabetes + 200 mg/kg of MEFE) showed brain tissue with small vacuolation of the cytoplasm (Yellow Arrow), and D (diabetes + 0.3 IU of insulin) showed normal architecture of brain tissue. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

in the intestines and preventing sharp spikes in blood sugar. Though we have not seen studies where the impact of *Ficus exasperata* on carbohydrate digesting enzymes were previously reported, this might be a potential area of further research. *Ficus exasperata* caused significant increase in antioxidant enzymes including superoxide dismutase (SOD), catalase, glutathione peroxidase, and glutathione reductase. This effect might be due to the rich content of its chemical constituents, which possess strong antioxidant properties [34]. These constituents may have enhanced the body's endogenous antioxidant defense system by upregulating the expression of antioxidant enzymes or by directly scavenging reactive oxygen species (ROS). This boost in antioxidant activity may helps to neutralize free radicals, reduce oxidative stress, and protect cells from damage [35]. *Ficus exasperata* could have caused a significant decrease in malondialdehyde (MDA) and 8-hydroxy-2′-deoxyguanosine (8-OHdG) by mitigating oxidative stress through its antioxidant-rich constituents [36]. These antioxidants neutralize reactive oxygen species (ROS), preventing them from causing lipid peroxidation and DNA damage, which are reflected by lower levels of MDA and 8-OHdG [37].

In diabetic conditions, pathophysiological changes can occur in neurotransmitter systems, leading to dysregulation of acetylcholine, glutamate, dopamine, GABA, and serotonin pathways [38]. Chronic hyperglycemia can impair acetylcholine synthesis and release, reducing cholinergic signaling, which is crucial for cognitive function [39]. Glutamate levels may increase abnormally, leading to excitotoxicity and neuronal damage due to overstimulation of glutamate receptors [40]. Dopamine production and release may be disrupted, affecting reward pathways and potentially contributing to mood disorders [41]. GABA levels could decrease, weakening inhibitory control and leading to increased neuronal excitability [42]. Serotonin synthesis might be impaired, contributing to mood disturbances and reduced neurogenesis [43]. These neurotransmitter imbalances disrupt normal neurotransmission, leading to cognitive deficits, mood disorders, and neurodegenerative changes associated with diabetes.

The methanol extract *of Ficus exasperata* (200 mg/kg) might have caused a significant increase in acetylcholine and glutamate by enhancing their synthesis or reducing their breakdown, possibly through the inhibition of enzymes like acetylcholinesterase, which degrades acetylcholine, or by modulating glutamate metabolism. In contrast to our findings, Bafor et al. (2011) [44] investigated the effects of an aqueous extract of *Ficus exasperata* on an isolated uterus and observed notable decrease with oxytocin production, while the levels of acetylcholine and other neurohormones remained unaffected by the extract. This increase in neurotransmitter levels would enhance neurotransmission by promoting stronger and more sustained signaling at synapses. Acetylcholine is critical for learning, memory, and muscle activation, while glutamate is the primary excitatory neurotransmitter involved in synaptic plasticity and cognitive functions [45]. Therefore, the elevation of these neurotransmitters could improve cognitive function and neural communication.

Ficus exasperata might have caused a significant increase in dopamine and brain total protein by promoting dopamine synthesis or release, possibly through the upregulation of enzymes like tyrosine hydroxylase, which is involved in dopamine production. The increase in brain total protein could reflect enhanced protein synthesis or reduced protein degradation, supporting overall neuronal health and function. This report is in tandem with Adekeye et al., 2020 [46] who reported an increase in motor activity and improved neurofunction in mice following administration of *Ficus exasperata.* Higher dopamine levels would enhance neurotransmission, particularly in pathways related to reward, motivation, and motor control [47]. The increase in brain total protein could also support the synthesis of neurotransmitter receptors, enzymes, and structural proteins, thereby strengthening synaptic connections and improving neural communication [48].

Ficus exasperata could have caused a significant increase in GABA and serotonin by enhancing their synthesis or release, potentially through the modulation of key enzymes such as glutamic acid decarboxylase for GABA and tryptophan hydroxylase for serotonin. The extract chemical constituents might also inhibit the reuptake or

degradation of these neurotransmitters, leading to higher extracellular levels. This findings support the report of Mandal et al., 2000 [49] who had earlier reported that another family of moraceae called *Ficus racemosa* increased serotonin and GABA levels, thus exhibiting anti-inflammatory potentials. GABA, as the primary inhibitory neurotransmitter, would reduce neuronal excitability and promote calming effects, while serotonin, involved in mood regulation, would enhance feelings of well-being [50]. The increased levels of both neurotransmitters would contribute to balanced neurotransmission, supporting mood stability and reducing anxiety.

Ficus exasperata could have caused significant increase in BDNF and decreased in Tau protein mRNA expression in the brain by activating transcription factors or signaling pathways such as CREB (cAMP response element-binding protein) that regulate these genes [51]. *Ficus exasperata* might also enhance neuronal activity, leading to increased demand for BDNF, which in turn stimulates their gene expression. With reference to pubmed search, this is the first time *Ficus exasperata* will be reported to impact the expression of these genes. Elevated BDNF mRNA supports neuroplasticity, synapse formation, and neurotransmitter release, while decreased Tau mRNA expression aids in maintaining the stability and function of microtubules essential for intracellular transport [52]. These changes could enhance neurotransmission, thereby supporting cognitive function and neural resilience.

Ficus exasperata could have caused significant increase in ACHE and decrease in BACE mRNA expression in the brain by activating specific signaling pathways or transcription factors that regulate these genes [53]. *Ficus exasperata* might also influence neuronal activity or stress responses, leading to upregulation of their enzymes [54]. However, Adeyemi et al., 2024 [47] had earlier reported the inhibitory action of aqueous extract *Ficus exasperata* on acetylcholinesterase which is in contrast with our finding. The increase in ACHE mRNA expression from this study would result in higher levels of acetylcholinesterase, which breaks down acetylcholine, potentially reducing cholinergic neurotransmission. An decreased BACE mRNA expression could lead to increased production of beta-secretase, an enzyme involved in the generation of beta-amyloid, which is associated with neurodegenerative processes [55]. While these changes could be part of a complex regulatory mechanism, they might also impact neurotransmission by altering the balance between excitatory and inhibitory signals, potentially influencing cognitive function and neural health [56].

Kaur-16-ene, one of the constituents of *Ficus exasperata* demonstrated particularly high binding affinities with NMDA, TrkB, and nAchR receptors, while caryophyllene oxide showed significant affinity for the mAchR receptor. These interactions are primarily mediated by alkyl and van der Waals forces, alongside specific amino acid residues within the receptor sites [57]. Notably, the binding affinities of kaur-16-ene and caryophyllene oxide suggesting that these compounds could potentially modulate neurotransmission more effectively by stabilizing receptor-ligand interactions [58]. This enhanced binding suggests a strong potential for these compounds to influence neurological pathways, potentially offering therapeutic benefits or modulating receptor activity in a way that surpasses endogenous neurotransmitters.

The study utilized a dosage of 200 mg/kg in animals, with no adverse effects observed at this level, suggesting a favorable safety profile within this range as reported also in previous studies [6]. To approximate a human equivalent dose (HED), this dosage was adjusted based on body surface area considerations, yielding an approximate HED of 32 mg/kg. This preliminary HED provides a reference point for evaluating potential safety and applicability in humans, although further studies are necessary to confirm these findings.

5. Conclusion

Our study demonstrates that *Ficus exasperata* Vahl leaf extract exerts significant effects on neurotransmitter levels, antioxidant enzyme activity, and mRNA expression of key biomolecules in diabetic rats. The extract's ability to reduce blood glucose, and modulate neurotransmitter systems highlights its potential as a therapeutic agent for managing diabetic neuropathies. The observed increases in antioxidant enzymes and decreases in oxidative stress markers suggest a protective role against oxidative damage, which is particularly beneficial in diabetes-induced neurodegeneration. Furthermore, the elevation of acetylcholine, glutamate, dopamine, GABA, and serotonin supports the extracts potential to improve cognitive and mood-related functions, while the modulation of BDNF and Tau protein expression may contribute to neuroprotection and synaptic resilience. Although no adverse effects were noted at the 200 mg/kg dosage used in the animals, translating this to an approximate human equivalent dose of 32 mg/kg requires further safety validation. Furthermore, this study's small sample size may limit the generalizability of the findings. Hence, future studies are warranted to explore the specific mechanisms by which *Ficus exasperata* impacts carbohydrate metabolism, neurotransmitter regulation, and gene expression, and to assess its efficacy and safety in human clinical trials.

CRediT authorship contribution statement

Olorunsola Israel Adeyomoye: Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Juliana Bunmi Adetunji:** Writing – review & editing, Visualization, Validation, Software, Resources, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Olugbemi Temitope Olaniyan:** Writing – review & editing, Writing – original draft, Visualization, Supervision, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Charles Oluwaseun Adetunji:** Writing – original draft, Visualization, Validation, Supervision, Project administration, Methodology, Formal analysis, Data curation. **Ogunmiluyi Oluwafunmbi Ebenezer:** Writing – review & editing, Writing – original draft, Visualization, Supervision, Methodology.

Informed consent statement

Not Applicable.

Institutional Review Board statement

The study protocol was approved by the Institutional Review Board of University of Medical Sciences, Ondo City, Nigeria.

Data availability statement

The data presented in this study are available on request from the corresponding author.

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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.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.metop.2024.100333.

References

[1] GBD 2021 Diabetes Collaborators. Global, regional, and national burden of diabetes from 1990 to 2021. with projections of prevalence to 2050: a systematic analysis for the Global Burden of Disease Study 2021 [published correction appears in Lancet 2023 Sep 30;402:1132. https://doi.org/10.1016/S0140-6736(23)02044-5. 10408. Lancet. 2023;402(10397):203-234. doi:10.1016/S0140-6736(23)01301-6.

[2] Shojima N, Yamauchi T. Progress in genetics of type 2 diabetes and diabetic complications. J Diabetes Investig 2023;14(4):503–15. https://doi.org/10.1111/jdi.13970.

[3] Cardoso SM, Correia SC, Carvalho C, Moreira PI. Mitochondria in Alzheimer's disease and diabetes-associated neurodegeneration: license to heal. Handb Exp Pharmacol 2017;240:281–308. https://doi.org/10.1007/164_2017_3.

[4] Lakstygal AM, de Abreu MS, Lifanov DA, et al. Zebrafish models of diabetes-related CNS pathogenesis. Prog Neuro-Psychopharmacol Biol Psychiatry 2019;92:48–58. https://doi.org/10.1016/j.pnpbp.2018.11.016.

[5] Wong TY, Cheung CM, Larsen M, Sharma S, Simó R. Diabetic retinopathy. Nat Rev Dis Prim 2016;2:16012. https://doi.org/10.1038/nrdp.2016.12.

[6] Osowe OC, Adu OA, Oloruntola OD, Chineke CA, Atansuyi AJ, Olateju IS. The impact of breed, Ficus exasperata leaf powder and vitamin C on carcass traits, brain and meat oxidative enzymes of broiler chickens raised under the tropical condition. Trop Anim Health Prod 2022;54(6):404. https://doi.org/10.1007/s11250-022-03386-2.

[7] Dongfack MD, Lallemand MC, Kuete V, et al. A new sphingolipid and furanocoumarins with antimicrobial activity from Ficus exasperata. Chem Pharm Bull (Tokyo) 2012;60(8):1072–5. https://doi.org/10.1248/cpb.c12-00279.

[8] Taiwo BJ, Igbeneghu OA. Antioxidant and antibacterial activities of flavonoid glycosides from Ficus exasperata Vahl-Holl (moraceae) leaves. Afr J Tradit, Complementary Altern Med 2014;11(3):97–101. https://doi.org/10.4314/ajtcam.v11i3.14.

[9] Abotsi WM, Woode E, Ainooson GK, Amo-Barimah AK, Boakye-Gyasi E. Antiarthritic and antioxidant effects of the leaf extract of Ficus exasperata P. Beauv. (Moraceae). Pharmacogn Res 2010;2(2):89–97. https://doi.org/10.4103/0974-8490.62958.

[10] Avila J, Lucas JJ, Perez M, Hernandez F. Role of tau protein in both physiological and pathological conditions. Physiol Rev 2004;84(2):361–84. https://doi.org/10.1152/physrev.00024.2003.

[11] Mandour DA, Bendary MA, Alsemeh AE. Histological and imunohistochemical alterations of hippocampus and prefrontal cortex in a rat model of Alzheimer like-disease with a preferential role of the flavonoid "hesperidin". J Mol Histol 2021;52(5):1043–65. https://doi.org/10.1007/s10735-021-09998-6.

[12] Zakin E, Abrams R, Simpson DM. Diabetic neuropathy. Semin Neurol 2019;39(5):560–9. https://doi.org/10.1055/s-0039-1688978.

[13] Dekeryte R, Franklin Z, Hull C, et al. The BACE1 inhibitor LY2886721 improves diabetic phenotypes of BACE1 knock-in mice. Biochim Biophys Acta, Mol Basis Dis 2021;1867(7):166149. https://doi.org/10.1016/j.bbadis.2021.166149.

[14] Pugazhenthi S, Qin L, Reddy PH. Common neurodegenerative pathways in obesity, diabetes, and Alzheimer's disease. Biochim Biophys Acta, Mol Basis Dis 2017;1863(5):1037–45. https://doi.org/10.1016/j.bbadis.2016.04.017.

[15] Palumbo C, Nicolaci N, La Manna AA, Branek N, Pissano MN. Asociación de diabetes insípida central y diabetes mellitus tipo 2 [Association between central diabetes insipidus and type 2 diabetes mellitus]. Medicina (B Aires). 2018;78(2):127–30.

[16] Kapalavavi B, Doctor N, Zhang B, Yang Y. Subcritical water extraction of *Salvia miltiorrhiza*. Molecules 2021;26(6):1634. https://doi.org/10.3390/molecules26061634.

[17] World Medical Association. World Medical Association Declaration of Helsinki: ethical principles for medical research involving human subjects. JAMA 2013;310(20):2191–4. https://doi.org/10.1001/jama.2013.281053.

[18] Geetha DH, Rajeswari M, Jayashree I. Chemical profiling of Elaeocarpus serratus L. by GC-MS. Asian Pac J Trop Biomed 2013;3(12):985–7. https://doi.org/10.1016/S2221-1691(13)60190-2.

[19] Bolann BJ, Tangerås A, Ulvik RJ. Determination of manganese superoxide dismutase activity by direct spectrophotometry. Free Radic Res 1996;25(6):541–6. https://doi.org/10.3109/10715769609149075.

[20] Kryl'skii ED, Popova TN, Kirilova EM. Activity of glutathione antioxidant system and NADPH-generating enzymes in rats with experimental rheumatoid arthritis. Bull Exp Biol Med 2015;160(1):24–7. https://doi.org/10.1007/s10517-015-3089-0.

[21] Mueller S, Riedel HD, Stremmel W. Determination of catalase activity at physiological hydrogen peroxide concentrations. Anal Biochem 1997;245(1):55–60. https://doi.org/10.1006/abio.1996.9939.

[22] Mannervik B. Measurement of glutathione reductase activity. Curr Protoc Toxicol 2001:2. https://doi.org/10.1002/0471140856.tx0702s00 [Chapter 7]:Unit7.

[23] Tsikas D. Assessment of lipid peroxidation by measuring malondialdehyde (MDA) and relatives in biological samples: analytical and biological challenges. Anal Biochem 2017;524:13–30. https://doi.org/10.1016/j.ab.2016.10.021.

[24] Rossner P Jr, Orhan H, Koppen G, et al. Urinary 8-oxo-7,8-dihydro-2'-deoxyguanosine analysis by an improved ELISA: an inter-laboratory comparison study. Free Radic Biol Med 2016;95:169–79. https://doi.org/10.1016/j.freeradbiomed.2016.03.016.

[25] Rubio F, Kamp L, Carpino J, et al. Colorimetric microtiter plate receptor-binding assay for the detection of freshwater and marine neurotoxins targeting the nicotinic acetylcholine receptors. Toxicon 2014;91:45–56. https://doi.org/10.1016/j.toxicon.2014.08.073.

[26] Nishiyama T, Sulistyaningdyah WT, Ueda K, Kusakabe H. GABA enzymatic assay kit. Biosci Biotechnol Biochem 2020;84(1):118–25. https://doi.org/10.1080/09168451.2019.1661768.

[27] Hubbard KE, Wells A, Owens TS, Tagen M, Fraga CH, Stewart CF. Determination of dopamine, serotonin, and their metabolites in pediatric cerebrospinal fluid by isocratic high performance liquid chromatography coupled with electrochemical detection. Biomed Chromatogr 2010;24(6):626–31. https://doi.org/10.1002/bmc.1338.

[28] Olson BJ, Markwell J. Assays for determination of protein concentration. Curr Protoc Pharmacol 2007:3A. https://doi.org/10.1002/0471141755.pha03as38. Appendix 3.

[29] Rocha DJPG, Castro TLP, Aguiar ERGR, Pacheco LGC. Gene expression analysis in bacteria by RT-qPCR. Methods Mol Biol 2020;2065:119–37. https://doi.org/10.1007/978-1-4939-9833-3_10.

[30] Sakhawat A, Khan MU, Rehman R, et al. Natural compound targeting BDNF V66M variant: insights from in silico docking and molecular analysis. Amb Express 2023;13(1):134. https://doi.org/10.1186/s13568-023-01640-w.

[31] Adeyomoye OI, Adetunji JB, Olaniyan OT, Adetunji CO, Oluwafemi DY. TGR5, GLP-1, and GIP expression in diabetic Wistar rats in response to *Ficus exasperata* vahl leaf extract. Phytomedicine 2024;4(3):100594. https://doi.org/10.1016/j.phyplu.2024.100594.

[32] Becker A, Helmuth M, Trzeczak D, Chindo BA. Methanol extract of Ficus platyphylla decreases cerebral ischemia induced injury in mice. J Ethnopharmacol 2021;278:114219. https://doi.org/10.1016/j.jep.2021.114219.

[33] Bafor EE, Igbinuwen O. Acute toxicity studies of the leaf extract of Ficus exasperata on haematological parameters, body weight and body temperature. J Ethnopharmacol 2009;123(2):302–7. https://doi.org/10.1016/j.jep.2009.03.001.

[34] Adewole Stephen O, et al. Hypoglycaemic and hypotensive effects of Ficus exasperata vahl. (Moraceae) leaf aqueous extract in rats. Afr J Tradit, Complementary Altern Med : AJTCAM 2011;8(3):275–83. https://doi.org/10.4314/ajtcam.v8i3.65290.

[35] Agunloye OM, Oboh G. Effect of different processing methods on antihypertensive property and antioxidant activity of sandpaper leaf (Ficus exasperata) extracts. J Diet Suppl 2018;15(6):871–83. https://doi.org/10.1080/19390211.2017.1406423.

[36] Oboh G, Akinyemi AJ, Osanyinlusi FR, Ademiluyi AO, Boligon AA, Athayde ML. Phenolic compounds from sandpaper (ficus exasperata) leaf inhibits angiotensin 1 converting enzyme in high cholesterol diet fed rats. J Ethnopharmacol 2014;157:119–25. https://doi.org/10.1016/j.jep.2014.09.029.

[37] Akanni OO, Owumi SE, Adaramoye OA. In vitro studies to assess the antioxidative, radical scavenging and arginase inhibitory potentials of extracts from Artocarpus altilis, Ficus exasperate and Kigelia africana. Asian Pac J Trop Biomed 2014;4(Suppl 1):S492–9. https://doi.org/10.12980/APJTB.4.2014C581.

[38] Røikjer J, Mørch CD, Ejskjaer N. Diabetic peripheral neuropathy: diagnosis and treatment. Curr Drug Saf 2021;16(1):2–16. https://doi.org/10.2174/1574886315666200731173113.

[39] Warren RE, Frier BM. Hypoglycaemia and cognitive function. Diabetes Obes Metabol 2005;7(5):493–503. https://doi.org/10.1111/j.1463-1326.2004.00421.x.

[40] Neves D, Salazar IL, Almeida RD, Silva RM. Molecular mechanisms of ischemia and glutamate excitotoxicity. Life Sci 2023;328:121814. https://doi.org/10.1016/j.lfs.2023.121814.

[41] Lerner TN, Holloway AL, Seiler JL. Dopamine, updated: reward prediction error and beyond. Curr Opin Neurobiol 2021;67:123–30. https://doi.org/10.1016/j.conb.2020.10.012.

[42] Petroff OA. GABA and glutamate in the human brain. Neuroscientist 2002;8(6):562–73. https://doi.org/10.1177/1073858402238515.

[43] David DJ, Gardier AM. Les bases de pharmacologie fondamentale du système sérotoninergique : application à la réponse antidépressive [The pharmacological basis of the serotonin system: Application to antidepressant response]. Encephale 2016;42(3):255–63. https://doi.org/10.1016/j.encep.2016.03.012.

[44] Bafor EE, Omogbai EK, Ozolua RI. Oxytocin inhibiting effect of the aqueous leaf extract of Ficus exasperata (Moraceae) on the isolated rat uterus. Acta Pol Pharm 2011;68(4):541–7.

[45] Ananth MR, Rajebhosale P, Kim R, Talmage DA, Role LW. Basal forebrain cholinergic signalling: development, connectivity and roles in cognition. Nat Rev Neurosci 2023;24(4):233–51. https://doi.org/10.1038/s41583-023-00677-x.

[46] Adekeye AO, Irawo GJ, Fafure AA. *Ficus exasperata* Vahl leaves extract attenuates motor deficit in vanadium-induced parkinsonism mice. Anat Cell Biol 2020;53(2):183–93. https://doi.org/10.5115/acb.19.205.

[47] Adeyemi OE, Jaryum KH, Johnson TO. Elucidation and active ingredient identification of aqueous extract of *Ficus exasperata* Vahl leaf against bisphenol A-induced toxicity through in vivo and in silico assessments. Silico Pharmacol 2024;12(2):73. https://doi.org/10.1007/s40203-024-00248-7.

[48] Ajeigbe OF, Oboh G, Ademosun AO, Umar HI. Fig (Ficus exasperata and Ficus asperifolia)-Supplemented diet improves sexual function, endothelial nitric oxide synthase and suppresses tumour necrosis factor-alpha genes in hypertensive rats. Andrologia 2022;54(1):e14289. https://doi.org/10.1111/and.14289.

[49] Mandal SC, Maity TK, Das J, Saba BP, Pal M. Anti-inflammatory evaluation of Ficus racemosa Linn. leaf extract. J Ethnopharmacol 2000;72(1–2):87–92. https://doi.org/10.1016/s0378-8741(00)00210-5.
[50] Tao R, Auerbach SB. Regulation of serotonin release by GABA and excitatory amino acids. J Psychopharmacol 2000;14(2):100–13. https://doi.org/10.1177/026988110001400201.
[51] Rosa E, Mahendram S, Ke YD, Ittner LM, Ginsberg SD, Fahnestock M. Tau downregulates BDNF expression in animal and cellular models of Alzheimer's disease. Neurobiol Aging 2016;48:135–42. https://doi.org/10.1016/j.neurobiolaging.2016.08.020.
[52] Oreshko AS, Rodnyy AY, Bazovkina DV, Naumenko VS. Effects of central administration of the human Tau protein on the Bdnf, Trkb, p75, Mapt, Bax and Bcl-2 genes expression in the mouse brain. Vavilovskii Zhurnal Genet Selektsii. 2023;27(4):342–8. https://doi.org/10.18699/VJGB-23-41.
[53] Da Costa R, De Almeida S, Chevarin M, et al. Neutralization of HSF1 in cells from PIK3CA-related overgrowth spectrum patients blocks abnormal proliferation. Biochem Biophys Res Commun 2020;530(3):520–6. https://doi.org/10.1016/j.bbrc.2020.04.146.
[54] Kharas N, Whitt H, Reyes-Vasquez C, Dafny N. Methylphenidate modulates dorsal raphe neuronal activity: behavioral and neuronal recordings from adolescent rats. Brain Res Bull 2017;128:48–57. https://doi.org/10.1016/j.brainresbull.2016.10.011.
[55] Neumann U, Machauer R, Shimshek DR. The β-secretase (BACE) inhibitor NB-360 in preclinical models: from amyloid-β reduction to downstream disease-relevant effects. Br J Pharmacol 2019;176(18):3435–46. https://doi.org/10.1111/bph.14582.
[56] Mathys H, Peng Z, Boix CA, et al. Single-cell atlas reveals correlates of high cognitive function, dementia, and resilience to Alzheimer's disease pathology. Cell 2023;186(20):4365–4385.e27. https://doi.org/10.1016/j.cell.2023.08.039.
[57] Ramasamy S, Rajan AT. Unraveling the pharmaceutical benefits of freshly prepared amino acid-based schiff bases via DFT, in silico molecular docking and ADMET. J Fluoresc 2022;32(5):1873–88. https://doi.org/10.1007/s10895-022-02987-2.
[58] Wang J, Miao Y, Wicklein R, et al. RTN4/NoGo-receptor binding to Bai adhesion-GPCRs regulates neuronal development. [published correction appears in Cell 2022 Jan 6;185(1):218. https://doi.org/10.1016/j.cell.2021.12.017. Cell. 2021;184(24):5869-5885.e25. doi:10.1016/j.cell.2021.10.016.