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Molecular basis of cardioprotective effects of methanol extract of *Ficus exasperata* in diabetic Wistar rats

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ABSTRACT

Cardiovascular complications are a significant concern in diabetes mellitus. *Ficus exasperata* Vahl leaf has been traditionally used for diabetes management, yet its impact on cardiovascular biomarkers in diabetic conditions remains unexplored. This study evaluated the effects of methanol extract of *Ficus exasperata* (MEFE) on antioxidant defense, oxidative stress markers, ion transport enzymes, inflammatory mediators, and cardiovascular gene expression in diabetic Wistar rats. Twenty Wistar rats were divided into four groups (n = 5): control, diabetic untreated, diabetes + MEFE (200 mg/kg), and diabetes + insulin (0.3 IU). Diabetes was induced with alloxan monohydrate (150 mg/kg), and treatments were administered orally for 28 days. Antioxidant enzyme activities (Glutathione peroxidase (GPx), Glutathione reductase (GR), Superoxide dismutase (SOD), Catalase, malondialdehyde and 8-hydroxy-2′-deoxyguanosine), Cardiac biomarkers (Na^+/K^+ ATPase, Ca^{2+} ATPase, Creatinine kinase-myocardial band (CK-MB), Troponin I, Troponin T, and Lactatate dehydrogenase), and gene expression of CRP, ACE, P-Selectin, and eNOS were evaluated. Data were analyzed using one-way analysis of variance, expressed as mean ± SEM, and $p < 0.05$ was considered statistically significant. The diabetic group treated with MEFE (200 mg/kg) significantly increased Ca^{2+} ATPase, SOD, and glutathione reductase activities compared to diabetic untreated. However, malondialdehyde and 8-OHdG levels decreased significantly in diabetes+MEFE (200 mg/kg) compared to diabetes untreated. CK-MB levels increased significantly in diabetes+MEFE (200 mg/kg) compared to diabetic untreated. MEFE reduced ACE and P-selectin expression in diabetes+MEFE (200 mg/kg) compared to diabetic untreated, indicating potential antihypertensive and antithrombotic effects. However, it increased CRP levels compared to control, suggesting an inflammatory response. MEFE significantly reduced eNOS expression compared to diabetic untreated, suggesting impaired vascular function. These findings suggest that while *Ficus exasperata* has some beneficial effects, its impact on inflammatory and cardiac biomarkers necessitates further research to fully understand its therapeutic potential and safety.

Abbreviations: MEFE, Methanol extract of *Ficus exasperata*; SOD, Superoxide Dismutase; GPx, Catalase; GR, Glutathione Reductase; Ck-MB, Creatinine Kinase-Myocardial Band; CRP, C Reactive Protein; ACE, Angiotensin Converting Enzyme; ENOS, Endothelial Nitric Oxide Synthase; GC-MS, Gas Chromatography-Mass Spectrometry; Na^+/K^+ ATPase, Sodium-Potassium Adenosine Triphosphatase; RAAS, Renin-Angiotensin-Aldosterone System; CDNA, Complementary deoxyribonucleic acid; RNAlater, Ribonucleic Acid later; GAPDH, Glyceraldehyde-3-Phosphate Dehydrogenase; QRT-PCR, quantitative Reverse Transcription Polymerase Chain Reaction.

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1. Introduction

Diabetes mellitus, a metabolic disease marked by persistently high blood sugar, continues to be a significant worldwide health concern [1]. It is associated with various secondary complications, including nephropathy, retinopathy, and cardiomyopathy, which significantly contribute to morbidity and mortality in affected populations [2]. Many of these complications arise from inflammation, endothelial dysfunction, and alterations in vasoactive mediators. To lessen the financial and health burden of diabetes and its comorbidities, new therapeutic approaches are being developed on a constant basis. A common medicinal plant in tropical Africa is *Ficus exasperata*, which is a member of the Moraceae family (Fig. 1). Folkloric medicine has long utilized various elements of this plant to treat diabetes and its complications. Bioactive substances such as flavonoids, tannins, saponins, and phenolic acids are found in its leaves and help explain its hypoglycemic, anti-inflammatory, and antioxidant qualities [3]. Recent studies have further highlighted its cardioprotective potential, demonstrating its ability to modulate key pathways involved in cardiovascular health and diabetes-related complications. These properties support its potential use as an antidiabetic agent, particularly in mitigating cardiovascular and endothelial dysfunction [4]. *Ficus exasperata*'s antioxidant, anti-inflammatory, and vasoprotective qualities are some of its potential defense mechanisms; they could help lower the risk of complications from diabetes [5]. Additionally, rats with L-NAME-induced hypertension were used to test the effects of *Ficus exasperata* aqueous extract. The plant's utility as an adjuvant treatment for hypertension was confirmed by the considerable reductions in mean arterial blood pressure, diastolic blood pressure, and systolic blood pressure [6]. Due to their high residual polyphenolic contents, species variants of fig plants (*Ficus species*) have been shown by Ajeigbe et al. [7] to have aphrodisiac and cardio-protective qualities that can help manage a variety of ailments, including erectile dysfunction and hypertension. Elevated CRP levels have been linked to increased mortality and a higher incidence of cardiovascular complications in diabetes [8]. Therefore, continuous monitoring of CRP is crucial for early detection of inflammatory status and potential cardiovascular challenges in diabetic patients. In order to maintain healthy blood vessels and regulate blood pressure, the renin-angiotensin-aldosterone system (RAAS) relies on the angiotensin-converting enzyme (ACE), which speeds up the conversion of angiotensin I into the potent vasoconstrictor angiotensin II. Dysregulation of ACE in diabetes contributes to endothelial dysfunction, impaired vasodilation, and increased total peripheral resistance, ultimately leading to cardiovascular abnormalities such as atherosclerosis, hypertension, and stroke [9]. Increased ACE activity can further exacerbate cardiovascular pathologies, highlighting the need for novel therapeutic strategies to modulate ACE levels and reduce the risk of diabetes-related cardiovascular complications [10]. Endothelial cells produce P-selectin, a protein that facilitates platelet adhesion and aggregation at sites of vascular injury during inflammation and thrombosis [11]. The risk of serious cardiovascular events, including heart attacks and strokes, is increased by elevated P-selectin expression, which is linked to faster blood clot formation, poor circulation, and endothelial dysfunction [12]. Furthermore, the enzyme endothelial nitric oxide synthase (eNOS) is in charge of producing nitric oxide (NO), which is essential for preserving vascular homeostasis. Diabetes-related hypertension, atherosclerosis, and other cardiovascular diseases are exacerbated by endothelial dysfunction brought on by a decrease in eNOS activity, which also results in less NO being produced [13]. The effects of *Ficus exasperata* leaf extract on cardiac biomarkers and expression of ACE, P-selectin, eNOS, and CRP in diabetic Wistar rats are assessed in this study. Therefore, this study aims to provide insights into the therapeutic efficacy of *Ficus exasperata,* paving the way for innovative treatment approaches to improve diabetes management and cardiovascular health.

Fig. 1. *Ficus exasperata* Vahl leaves (White fig) [51].

2. Materials and methods

2.1. Plant extraction

The plant extraction was conducted once and used for both this study and our previous research [5,51]. The plants were gathered during March and April of 2022, when there was an increase in light (12–14 hours per day), which encouraged the synthesis of vital natural components in other plant parts such as sap, flowers, seeds, bark, leaves, and pods. Fresh *Ficus exasperata* leaves were gathered on Oka-Akoko Street in Ondo Town, Ondo State, Nigeria, and confirmed at the Department of Botany, University of Ibadan (voucher specimen number UIH: 240407). The leaves were soaked in 99.9 % methanol (Sigma-Aldrich Co., LLC, Missouri, US) after being air-dried and milled into a powder. Methanol was selected because it is water-miscible, polar, has a low boiling point that makes it easier to remove after extraction. The extraction procedure adheres to Kapalavavi et al.'s cold maceration approach [14]. About 21.3 kg of *Ficus exasperata* Vahl leaves were gathered, rinsed under running water, and allowed to air dry for four weeks at room temperature (25°C) [5,51]. After that, 5.1 kg of the dried leaves were used to make 3.4 kg of powder. This powder was steeped in five liters of methanol for 72 hours before being filtered (Whatman No. 1) [5,51]. The filtrate was concentrated at 50°C using a rotary evaporator (Union Laboratory, California, US). A pasty, dark-green extract (96 g) with a yield percentage of 2.82 % was obtained by further drying the extracted material in a water bath [5,51].

2.1.1. Experimental design

The experiment was designed based on our previous research [5,51]. A total of five groups of twenty (20) Wistar rats were used. Group I was used as a control group; group II consisted of rats that were diabetic and not receiving treatment; group III was a diabetic group that received 200 mg/kg of *Ficus exasperata*; and group IV was a diabetic group that received 0.3 IU of insulin, the common pharmaceutical treatment for diabetes. To induce diabetes in experimental animals, a dose of 150 mg/kg of alloxan monohydrate (*I.p*) was given to each rat [15]. For a total of 28 days, the rats were administered both insulin and MEFE. At 200 mg/kg, *Ficus exasperata* was found to have cardiovascular effects based on earlier research [16].

2.2. Ethical consideration

All animal treatments were authorized by the University of Medical Sciences Ethical Review Committee, guaranteeing that the study adhered to ethical standards and regulations. Additionally, all studies were conducted in accordance with the Declaration of Helsinki, which sets ethical standards for medical research with animals [17]. In this

study, male Wistar rats weighing 150 ± 10 g were given a standard chow diet. For a more precise assessment of the rats' physiological condition, they were starved for the whole night. They had cervical dislocations to lessen their pain and suffering after being humanely put to sleep with 0.5 mg/kg of ketamine. The mRNA expression of cardiovascular genes was examined using a 50 mg sample of heart and aortic tissue that had been excised and stored in an RNAlater at −20°C. The remaining heart sections were blended into a homogenous mixture for additional examination using phosphate-buffered saline (PBS). The homogenized materials were centrifuged at 5000 rpm for five minutes at 4°C to separate them into different cellular components. A micropipette was then used to remove the supernatant for further biochemical analysis.

2.2.1. Gas Chromatography-mass spectrometry (GC-MS) of Ficus Exasperata

The GC-MS was conducted once and used for both this study and our previous research [5,51]. The extract was redissolved in methanol (Merck, Germany). The Agilent 7890B Gas Chromatograph, fitted with an Agilent 5977 A Mass Selective Detector (Agilent Technologies, USA), was filled with the sample after it had been evaporated. Agilent Technologies, USA's HP-5MS fused silica capillary column (30 m × 0.25 mm, 0.25 μm film thickness) was used to separate the chemical ingredients. The helium (99.999 % pure, Linde Gas, Germany) used as the carrier gas was kept at a steady flow rate of 1.0 mL/min. At a rate of 10°C per minute, the oven temperature was programmed to rise from 50°C (held for two minutes) to 300°C, with a final hold period of ten minutes. With a scan range of 50–600 *m/z*, mass spectrometric detection was carried out in electron impact (EI) mode at 70 eV. For the purpose of identifying compounds, the acquired mass spectra were contrasted with reference spectra from the National Institute of Standards and Technology (NIST) library [18].

2.2.2. Measurement of antioxidant activities

The reaction mixture for SOD assay (Sigma-Aldrich, USA) consists of 800 μL phosphate buffer (with 50 mM sodium carbonate and 0.1 mM EDTA), 100 μL NBT solution (0.25 mM), 50 μL xanthine solution (0.1 mM), 10 μL xanthine oxidase (0.08 U/mL), and 40 μL of the supernatant from homogenized heart as the test sample. A blank was prepared by replacing the sample with buffer, while SOD standards are included for calibration. The reaction was incubated at 37°C for 5 minutes, and absorbance was measured at 560 nm before and after incubation. The percentage inhibition of NBT reduction by SOD is calculated using the formula: % Inhibition = [(Absorbance of blank – Absorbance of sample) / Absorbance of blank] × 100 [19]. The reaction mixture for glutathione peroxidase (Sigma-Aldrich, USA) consists of 50 μL of supernatant from homogenized heart, 700 μL of 50 mM phosphate buffer (pH 7.4), 100 μL of 5 mM EDTA, 100 μL of 10 mM GSH, 100 μL of 2 mM NADPH, and 100 μL of glutathione reductase (1 U/mL). The reaction was initiated by adding 100 μL of 1 mM H_2O_2. A blank was prepared by replacing the sample with buffer. The decrease in absorbance at 340 nm due to NADPH oxidation is measured for 3–5 minutes at 37°C. GPx activity is calculated using the formula: Activity (U/mL) = [(ΔAbs/min × Total Volume) / (Extinction coefficient of NADPH × Sample Volume)] [20]. The reaction mixture for catalase activity (Sigma-Aldrich, USA) consists of 50 μL of the supernatant, 950 μL of 50 mM phosphate buffer (pH 7.4), and 1.0 mL of freshly prepared 30 mM H_2O_2. A blank was prepared by replacing the sample with buffer. The decrease in absorbance of H_2O_2 at 240 nm was measured every 15 seconds for 1–2 minutes at 25°C. Catalase activity was calculated using the formula: Activity (U/mL) = (ΔAbs/min × Total Volume) / (Extinction coefficient of H_2O_2 × Sample Volume) [21]. The reaction mixture glutathione reductase (Sigma-Aldrich, USA) consists of 50 μL of the supernatant, 850 μL of 50 mM phosphate buffer (pH 7.4), 100 μL of 1 mM EDTA, 100 μL of 2 mM NADPH, and 50 μL of 10 mM GSSG. A blank was prepared by replacing the sample with buffer. The decrease in absorbance at 340 nm due to NADPH oxidation was measured every 15 seconds for 3 minutes at 25°C. Glutathione reductase activity is calculated using the formula: Activity (U/mL) = (ΔAbs/min × Total Volume) / (Extinction coefficient of NADPH × Sample Volume) [22]. The reaction mixture for malondialdehyde (MDA) (Sigma-Aldrich, USA) consists of 200 μL of the supernatant from homogenized heart, 600 μL of 10 % trichloroacetic acid (TCA) to precipitate proteins, and 600 μL of 0.67 % TBA. The mixture was vortexed and incubated in a boiling water bath at 95°C for 15 minutes, then cooled on ice for 5 minutes. The precipitate was removed by centrifugation at 3000 rpm for 10 minutes, and the absorbance of the clear pink supernatant was measured at 532 nm. The MDA concentration is calculated using the formula: MDA (nmol/mL) = (Absorbance at 532 nm) / (Extinction coefficient of MDA-TBA complex, 1.56×10^5 $M^{-1}cm^{-1}$) [23]. For 8-hydroxy-2′-deoxyguanosine (8-OHdG), homogenates from heart samples were used, and DNA extraction was carried out using a Qiagen DNA Isolation Kit (Qiagen, Germany), following the manufacturer's protocol. For the ELISA (Sigma-Aldrich, USA), 50 μL of the hydrolyzed DNA sample was added to wells pre-coated with an anti-8-OHdG antibody, followed by 50 μL of an enzyme-labeled secondary antibody. The plate was incubated at 37°C for 1 hour and then washed thoroughly with buffer to remove unbound components. Next, 100 μL of a substrate solution was added to each well, and the reaction was allowed to develop for 15 minutes at room temperature. The enzymatic reaction was then stopped by adding stop solution, and the absorbance was measured at 450 nm using a microplate reader [24]. The concentration of 8-OHdG was determined using a standard curve generated from known concentrations of 8-OHdG standards.

2.2.3. Measurement of cardiac biomakers

The Na^+/K^+-ATPase assay (Sigma-Aldrich, USA) reaction mixture contains 100 mM NaCl, 20 mM KCl, 3 mM $MgCl_2$, and 50 mM Tris-HCl (pH 7.4), with 100 μL of homogenized sample supernatant added before initiating the reaction with 3 mM ATP [25,26]. After incubation at 37°C for 30 minutes, the reaction was terminated with 10 % trichloroacetic acid (TCA) and centrifuged. Inorganic phosphate (Pi) was quantified via the Fiske-Subbarow method by mixing 1 mL of supernatant with an acidic molybdate reagent and ascorbic acid, with absorbance measured at 660 nm. Ouabain-sensitive Na^+/K^+-ATPase activity was determined by a parallel reaction with 1 mM ouabain, and enzyme activity was calculated as Na^+/K^+-ATPase activity (μmol Pi/mg protein/min) = (Pi released in μmol) ÷ (Protein concentration in mg × Reaction time in min). The Ca^{2+}-ATPase assay (Sigma-Aldrich, USA) follows a similar protocol, with 1 mM $CaCl_2$ included in the reaction mixture and the enzyme activity calculated as Activity (μmol Pi/mg protein/min) = (Pi released in μmol) ÷ (Protein concentration in mg × Reaction time in min). The CK-MB assay (Sigma-Aldrich, USA) follows an ELISA, where 50 μL of homogenized heart supernatant was added to an anti-CK-MB antibody-coated well, followed by 50 μL of enzyme-labeled detection antibody [27]. After a 1-hour incubation at 37°C and thorough washing, 100 μL of substrate solution was added, and absorbance was measured at 450 nm to determine CK-MB concentration using a standard curve. Troponin I and Troponin T (Abbott Laboratories, USA) are measured using a chemiluminescent microparticle immunoassay (CMIA), where 50 μL of homogenized heart sample was incubated with paramagnetic microparticles coated with anti-Troponin I antibodies, and Troponin T is detected using biotinylated and ruthenylated antibodies [28]. The chemiluminescent signal is read with a luminometer, and concentrations are determined from a standard curve. The LDH assay (Sigma-Aldrich, USA) reaction mixture consists of 1.0 mL of reaction buffer (50 mM Tris-HCl, 0.2 mM NADH, and 1 mM pyruvate) with 50 μL of homogenized heart supernatant [29]. The reaction was initiated with 100 μL of pyruvate solution, and the decrease in NADH absorbance at 340 nm is monitored for 3 minutes at 37°C. LDH activity is calculated as Activity (U/L) = (ΔAbs/min × Total Volume) ÷ (Extinction coefficient of NADH ×

Sample Volume).

2.2.4. Gene expression analysis

Using quantitative reverse transcription polymerase chain reaction (qRT-PCR), the expression of genes encoding CRP, ACE, P-selectin, and eNOS was analyzed in Wistar rats [30]. Using the Qiagen RNeasy Mini Kit (Cat. No. 74104), total RNA was first extracted from the tissues of the rat heart and aorta. The RNA was then quantified using a NanoDropTM 2000 Spectrophotometer (Thermo Fisher Scientific) and its integrity assessed using an Agilent 2100 Bioanalyzer (Agilent Technologies). The High-Capacity cDNA Reverse Transcription Kit (Thermo Fisher Scientific, Cat. No. 4368814) was used to reverse-transcribe the extracted RNA and create complementary DNA (cDNA). Integrated DNA Technologies (IDT, USA) provided the PowerUp™ SYBR™ Green Master Mix (Thermo Fisher Scientific, Cat. No. A25742) and specific forward and reverse primers for CRP, ACE, P-Selectin, and eNOS, which were used in the qRT-PCR amplification (Table 1). Under ideal cycling conditions, the reactions were conducted on a Thermo Fisher Scientific QuantStudioTM 5 Real-Time PCR System. Fluorescence intensity, corresponding to the accumulation of PCR products, was monitored using SYBR Green dye, allowing quantification of gene expression levels. Gel electrophoresis was utilized to confirm the amplified products, and the band intensity was evaluated using ImageJ software. The ΔΔCt technique was used to calculate relative gene expression, with GAPDH serving as the housekeeping gene for standardization. This ensured the validity of comparisons between diabetic and control rats as well as across various treatment groups.

2.3. In silico docking study

Target receptors' crystal structures (α1 adrenoceptor, α2 adrenoceptor, β1 adrenoceptor, and β2 adrenoceptor) were retrieved from the RCSB-PDB database, while ligands of interest were obtained in SDF format from the PubChem database. In order to prepare the receptor, Biovia Studio Visualizer was used to add missing hydrogen atoms and remove water molecules, inhibitors, and co-crystallized chemicals. Utilizing UCSF Chimera techniques, the receptors were further refined. The PDB and SDF formats of the proteins and ligands were loaded into PyRx (version 0.8), which was used to perform molecular docking studies. To find the highest-affinity contacts, binding affinities were noted, ranked, and examined [31]. The binding interactions of the most promising ligands were further examined and visualized using Discovery Studio Visualizer. Additionally, the receptor cavity method was applied to assess the binding interactions of the ligands and the standard drugs with the highest affinity.

2.3.1. Statistical analysis

One-way analysis of variance (ANOVA) and Tukey's multiple comparison post-hoc test were used for statistical analysis using GraphPad Prism version 10.2.3. The mean ± SEM was used to express the data. $p < 0.05$ was established as the threshold for statistical significance.

Table 1
Sequences of primers of target genes.

Gene		Primer Sequence (5′ → 3′)
C-Reactive protein (CRP)	F	TCAGGCTTTTGGTCATGAAGACAT
	R	AGTGGCTTCTTGGACTCTGC
Angiotensin Converting Enzyme (ACE)	F	CTGACCAAAAGCTGCGAAGG
	R	AGGATGTTGGTGAGCTCTGG
P-Selectin	F	GTATGGCACAGATCCCCCAG
	R	TGCTGGCCTTTCTGTATCCG
Endothelial Nitric Oxide Synthase	F	CGAGATATCTTCAGTCCCAAGC
	R	GTGGATTTGCTGCTCTCTAGG

3. Results

Table 3 shows the effect of methanol extract of *Ficus exasperata* on body weight, heart weight, and blood glucose concentration. A one-way ANOVA was used to express the data as mean ± SEM (n = 5, all groups), followed by a Tukey multiple comparisons post-hoc test. Significant values were defined as $p < 0.05$ * , $p < 0.01$ * *, $p < 0.001$ * ** , and $p < 0.0001$ * ** *. * denotes values that differ significantly from those of diabetic untreated.

3.1. Chemical constituents of methanol extract of Ficus exasperata and its effects on weight and glucose level

Table 2 shows the list of compounds identified from a single GC-MS analysis used in this study and our previous research [5,51]. New binding scores were analyzed in this study. While kaur-16-ene had the highest binding affinity with alpha 1 (-9.3 kcal), alpha 2 (-9.3 kcal), and beta 1 (-8.9 kcal) adrenoceptors, cycloisolongifolene 8,9-dehydro- has the highest affinity with the beta 2 receptor (-9.1 kcal). When compared to the diabetic untreated group, the diabetes+insulin (0.3 IU) group's body weight increased significantly ($p < 0.01$). When comparing the diabetic group treated with MEFE to the diabetic group not receiving treatment, there was no discernible difference in body weight (Table 3). The diabetic group receiving MEFE also showed a decrease in heart weight. Blood glucose levels decreased significantly ($p < 0.01$, $p < 0.001$) in the diabetic group treated with MEFE (200 mg/kg) and insulin (0.3 IU) when compared to the diabetic untreated group. The concentration of blood glucose in the diabetic group treated with insulin (0.3 IU) was comparable to that of the control group (Table 3).

3.1.1. Effects of methanol extract of Ficus exasperata on cardiac biomakers

To monitor the condition of the heart and assess potential damage to cardiac muscle cells following alloxan administration and treatment with MEFE, we evaluated the effect of MEFE on cardiac biomarkers. Rats in the diabetic untreated group, diabetes + MEFE (200 mg/kg), and diabetes + insulin (0.3 IU) groups showed a significant decrease ($p < 0.0001$) in Na^+-K^+ ATPase activity compared to the control group (Fig. 2a). However, there was no significant difference ($p > 0.05$) in Na^+-K^+ ATPase activity in diabetes treated with MEFE (200 mg/kg) and insulin (0.3 IU) groups compared to the diabetic untreated group (Fig. 2a). Additionally, there was no significant difference ($p > 0.05$) in Na^+-K^+ ATPase activity between the diabetes + MEFE (200 mg/kg) group and the diabetes treated with insulin (0.3 IU) group. Ca^{2+} ATPase activity increased significantly ($p < 0.0001$) in the diabetic group treated with MEFE (200 mg/kg) when compared to the diabetic untreated (Fig. 2b). The activity of Ca^{2+} ATPase in this group was comparable with the control group. Treatment with insulin (0.3 IU) also caused a significant increase ($p < 0.001$) in Ca^{2+} ATPase activity when compared to diabetic untreated. We also determined the effect of MEFE on CK-MB formation, a potential marker of myocardial infarction and heart damage. Surprisingly, treatment with MEFE showed a significant increase ($p < 0.05$) in CK-MB in diabetic rats treated with MEFE compared to the control group and the diabetes + insulin (0.3 IU) group (Fig. 2c). However, no significant difference ($p > 0.05$) was observed in diabetic rats treated with MEFE compared to the diabetic untreated group (Fig. 2c). Troponin I, troponin T, and lactate dehydrogenase decreased significantly ($p < 0.0001$) in diabetic groups treated with MEFE (200 mg/kg) and insulin (0.3 IU) when compared to diabetic untreated. The concentrations of troponin I, troponin T, and lactate dehydrogenase in these groups were comparable with those of their control groups (Fig. 2d, e & f).

3.1.2. Effect of methanol extract of Ficus exasperata on antioxidant enzymes

The effects of the methanol extract of *Ficus exasperata* on superoxide dismutase (SOD), glutathione peroxidase (GPx), catalase, glutathione

Table 2
GC-MS constituents of methanol extract of *Ficus exasperata*.

S/N	RT (min)	Compound	α I adrenoceptor Kcal/mol	α 2 adrenoceptor Kcal/mol	β 1 adrenoceptor Kcal/mol	β2 adrenoceptor Kcal/mol
1	11.550	**Cycloisolongifolene 8,9-dehydro-**	−7.4	−7.5	−7.7	−9.1
2	12.209	**Eudesma−4(15),7-dien−1.beta –ol**	−6.7	−7.7	−8.6	−8.6
3	13.714	**Neophytadiene**	−5.6	−6.9	−6.2	−5.7
4	14.143	**9-Eicosene, (E)**	−4.9	−5.9	−5.8	−5.0
5	14.604	**Pentadecanoic acid, 14-methyl-**	−4.9	−6.8	−6.2	−6.2
6	15.009	**n-Hexadecanoic acid**	−5.3	−6.0	−6.3	−5.7
7	15.266	**Hexadecanoic acid, ethyl ester**	−4.8	−6.2	−5.6	−6.3
8	15.598	**7-Tetradecyne**	−5.6	−4.8	−6.0	−6.2
9	15.857	**Kaur−16-ene**	−9.3	−9.3	−8.9	−7.5
10	16.217	**10,13-Octadecadienoic acid, methyl**	−5.4	−6.6	−5.8	−5.3
11	16.272	**9-Octadecenoic acid (*Z*)-**	−5.0	−5.3	−6.1	−6.7
12	16.327	**11-Octadecenoic acid, methyl ester**	−5.7	−5.2	−6.6	−6.4
13	16.382	**Oxirane, decyl-**	−5.1	−4.8	−5.2	−5.6
14	16.504	**Methyl stearate**	−5.2	−4.7	−5.9	−6.4
15	16.597	**Linoelaidic acid**	−5.9	−6.8	−6.2	−6.7
16	16.650	**cis-Vaccenic acid**	−5.9	−6.0	−6.8	−6.6
17	16.831	**Linoleic acid ethyl ester**	−5.9	−5.7	−5.9	−5.8
18	16.880	**Ethyl Oleate**	−5.4	−6.1	−5.9	−5.4
19	17.100	**Octadecanoic acid, ethyl ester**	−5.4	−6.1	−5.5	−6.6
20	17.633	**Alpha-Farnesene**	−6.6	−7.3	−7.5	−7.2
21	17.872	**Hexane, 1-chloro−5-methyl-**	4.5	−4.8	−5.1	−4.7
22	18.689	**Caryophyllene oxide**	−6.6	−6.9	−6.5	−6.6
23	19.217	**Bicyclo [5.1.0] octane, 8-methylene-**	−6.1	−6.0	−6.0	−6.3

Table 3
Shows the effects of *Ficus exasperata* on blood glucose, body and heart weights.

Groups	Body Weight (g)	Heart Weight (g)	Relative Weight (%)	Glucose (mg/dL)
Control	158.0 ± 6.7	0.68 ± 0.04	0.55	70.3 ± 3.1
Diabetes Untreated	127.4 ± 2.9	0.45 ± 0.1	0.50	203.6 ± 6.7
Diabetes+*Ficus exasperata* (200 mg/kg)	129.9 ± 9.0	0.49 ± 0.1	0.30	129.5 ± 5.3 * *
Diabetes+Insulin (0.3 IU)	194.3 ± 7.2 * *	0.67 ± 0.1	0.59	66.2 ± 3.7 * **

reductase, activities, malondialdehyde, and 8-hydroxy-2′-deoxyguanosine (8-OHdG) levels were determined at a dose of 200 mg/kg in diabetic rats. MEFE caused a significant increase ($p < 0.01$) in the diabetes+MEFE (200 mg/kg) treated group compared to the control, DU, and insulin (0.3 IU) treated groups (Fig. 3a). Following oral administration of MEFE, a significant increase ($p < 0.05$) in GPx (Fig. 3b) was observed in the insulin (0.3 IU) treated group compared to the MEFE (200 mg/kg), diabetic untreated (DU), and control groups. However, there was no significant difference ($p > 0.05$) in GPx activity in the diabetes+MEFE (200 mg/kg) group when compared to DU and the control. Catalase activity was determined in the control, diabetic untreated, and treated rats, with no significant difference observed across the groups (Fig. 3c). Glutathione reductase enzyme activity increased significantly ($p < 0.0001$) in diabetic groups treated with MEFE (200 mg/kg) and insulin (0.3 IU) when compared to diabetic untreated (Fig. 3d). Malondialdehyde and 8-hydroxy-2′-deoxyguanosine levels decreased significantly ($p < 0.0001$) in diabetic untreated groups treated with MEFE (200 mg/kg) and insulin (0.3 IU) when compared to diabetic untreated.

3.2. Effects of MEFE on mRNA expression of inflammatory and cardiovascular markers

Inflammatory and cardiovascular marker mRNA expression of CRP, ACE, P-Selectin, and eNOS provides insights into the molecular mechanisms underlying cardiovascular diseases and inflammatory responses. Monitoring the expression levels of these markers helps in understanding disease progression and the effectiveness of therapeutic interventions. We determine the effect of MEFE on CRP, ACE, P-selectin, and eNOS (Fig. 5). C-reactive protein (CRP), a significant biomarker of inflammation, is often used clinically to assess the presence and intensity of inflammatory processes. Its expression increased significantly in untreated diabetes, diabetes + MEFE (200 mg/kg), and diabetes + insulin (0.3 IU) when compared to control (Fig. 4a). The expression of angiotensin-converting enzyme (ACE) is significant because it directly influences the production of angiotensin II, a critical regulator of blood pressure and vascular health, thereby impacting the development and progression of hypertension and cardiovascular diseases. Angiotensin-converting enzyme expression decreased significantly ($p < 0.0001$) in diabetes + MEFE (200 mg/kg) when compared to diabetic untreated (Fig. 4b). Activated endothelial cells and platelets express cell adhesion protein called P-selectin, which is essential in the early phases of thrombosis and inflammation. Its expression decreased significantly in diabetes + MEFE (200 mg/kg) and diabetes + insulin (0.3 IU) when compared to diabetic untreated (Fig. 4c). The expression of endothelial nitric oxide synthase (eNOS) was significantly decreased in diabetes + MEFE (200 mg/kg) and diabetes + insulin (0.3 IU) when compared to diabetic untreated (Fig. 4d).

3.3. Molecular interaction of MEFE Constituents with cardiac receptors

We evaluated the binding affinity of each of the constituents MEFE with the receptors of the heart and vessels that control cardiac and vascular functions. We found that Kaur-16-ene had the highest binding scores with the alpha 1 receptor (-9.3 kcal/mol) (Fig. 6a), the alpha 2 receptor (-9.3 kcal/mol) (Fig. 6b), and the beta 1 receptor (-8.9 kcal/mol) (Fig. 6c). Cycloisolongifolene 8,9-dehydro has the highest binding affinity score (-9.1 kcal/mol) with beta 2 receptors (Fig. 6d). The molecular interaction of kuar-16-ene and cycloisolongifene 8,9-dehydro was revealed by the van der Waals and akyl forces with the amino acid residues of these receptors (Fig. 6).

3.4. Histological evaluation of the heart

Fig. 7 reveals histological changes in the photomicrograph of the heart. Photomicrograph appears normal in the control group. The myofibers of the cardiac cell appeared normal in untreated diabetes;

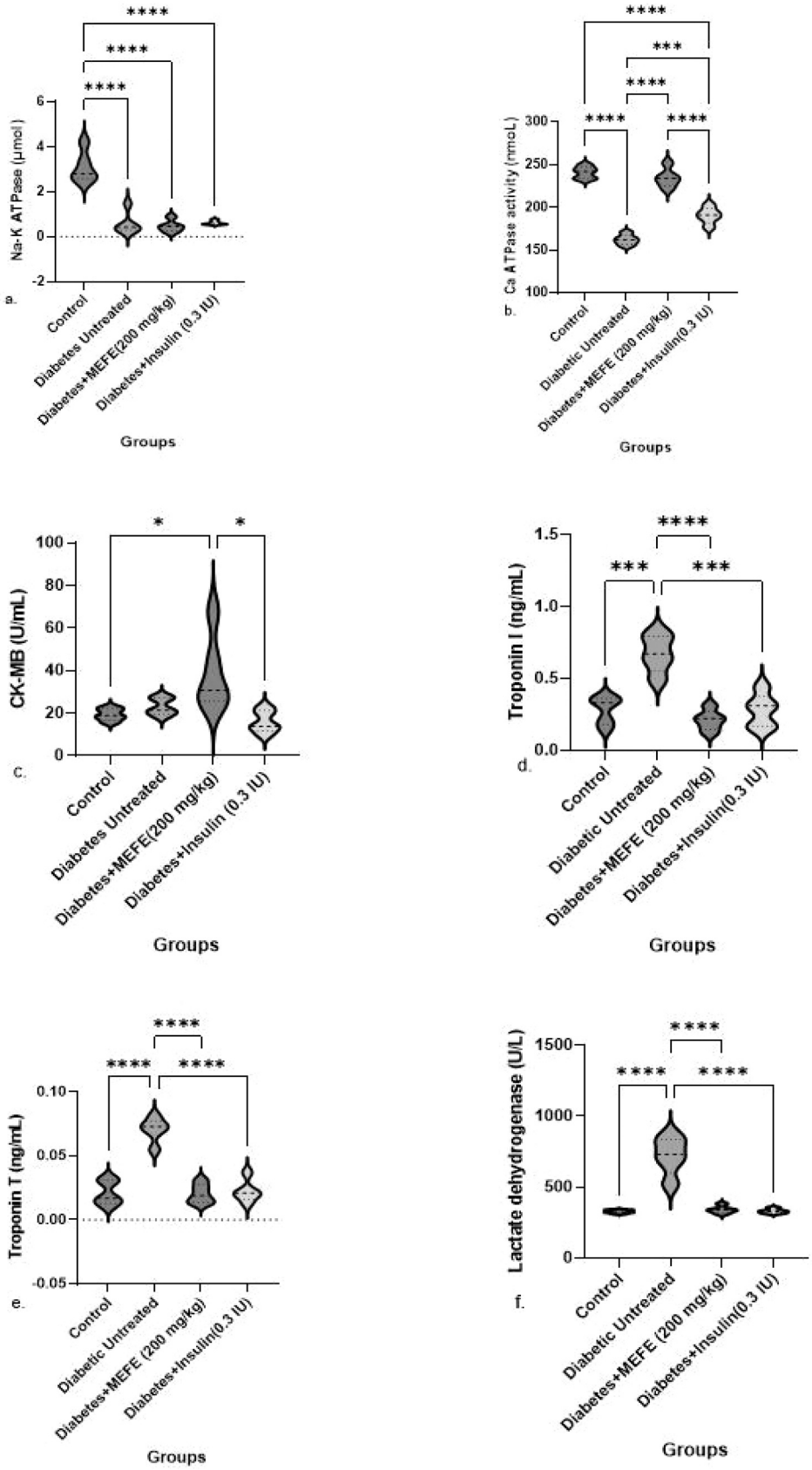

Fig. 2. shows the effect of methanol extract of *Ficus exasperata* on cardiac biomarkers (a) Na^{+}-K^{+} ATPase activity (b) Ca^{2+} ATPase activity (c) Creatinine Kinase-Myocardial Band (d) Troponin I, Troponin T, and Lactate dehydrogenase. A one-way ANOVA was used to express the data as mean ± SEM (n = 5, all groups), followed by a Tukey multiple comparisons post-hoc test. Significant values were defined as $p < 0.05$ * , $p < 0.01$ * *, $p < 0.001$ * ** , and $p < 0.0001$ * ** *. * denotes values that differ significantly from those of diabetic untreated.

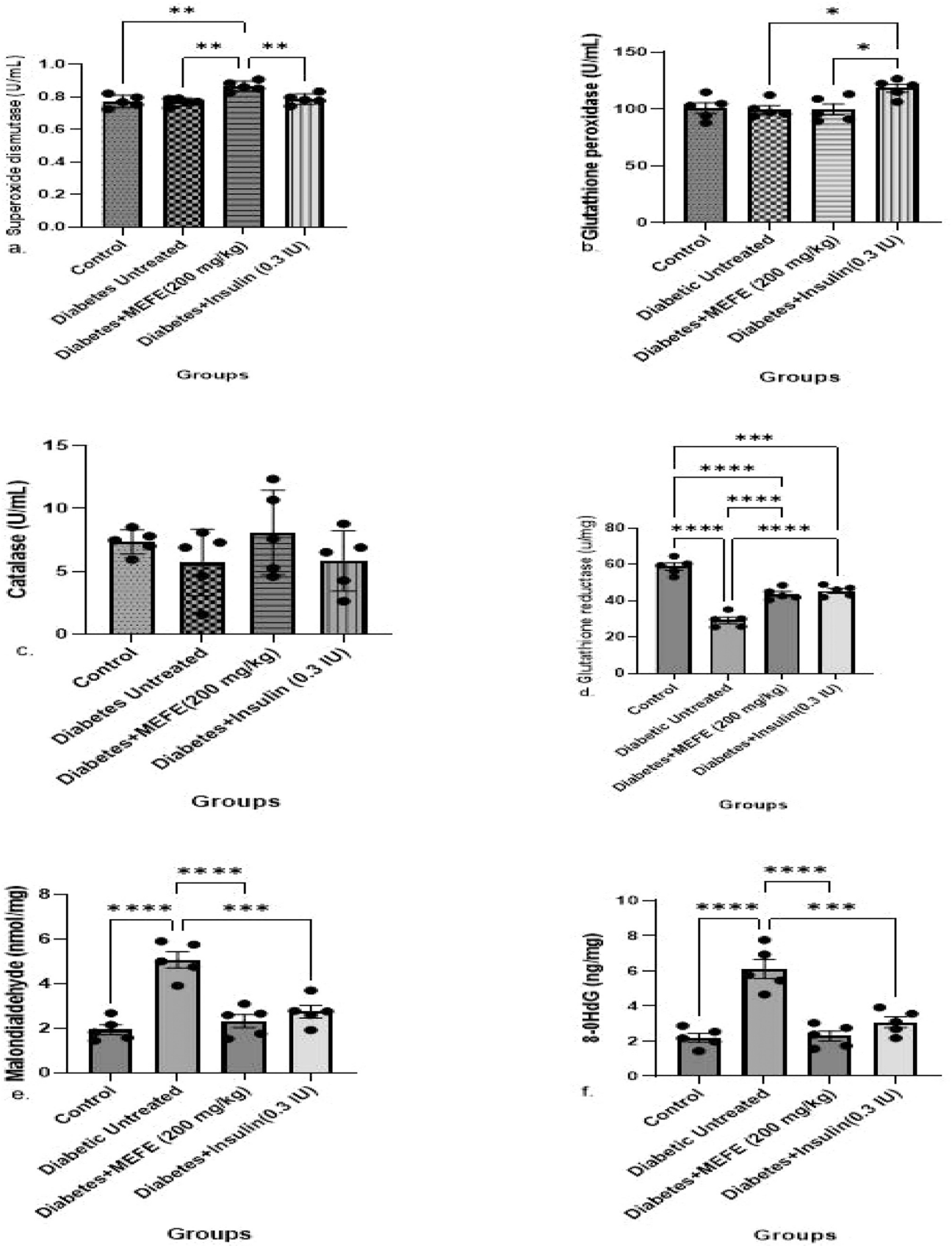

Fig. 3. shows the effect of methanol extract of *Ficus exasperata* on antioxidant activities (a). Superoxide dismutase (b) Glutathione peroxidase (c) Catalase (d) Glutathione Reductase (e) Malondialdehyde (f) 8-hydroxy-2′-deoxyguanosine. A one-way ANOVA was used to express the data as mean ± SEM (n = 5, all groups), followed by a Tukey multiple comparisons post-hoc test. Significant values were defined as $p < 0.05$ * , $p < 0.01$ * *, $p < 0.001$ * ** , and $p < 0.0001$ * ** *. * denotes values that differ significantly from those of diabetic untreated.

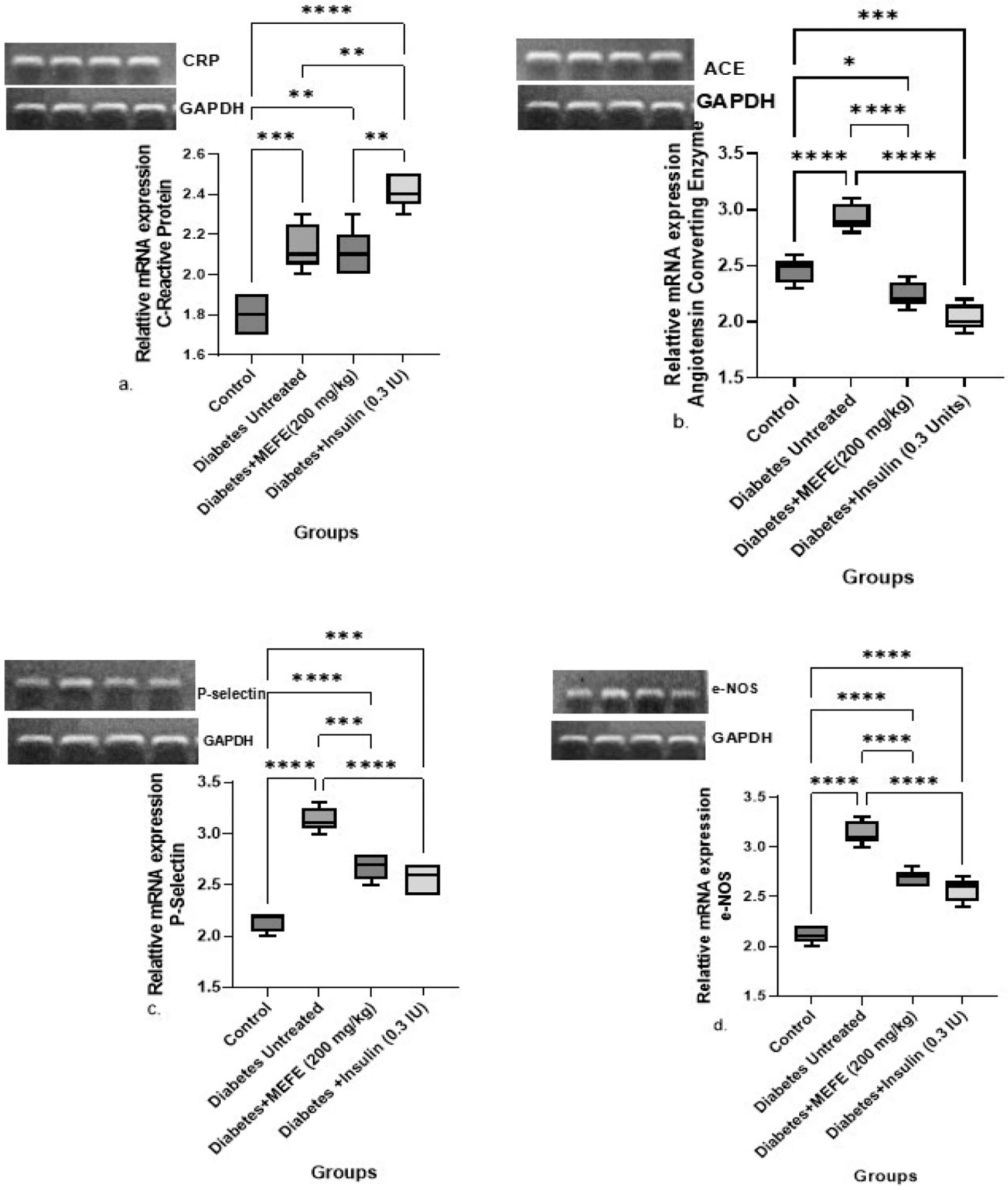

Fig. 4. shows the effect of the methanol extract of *Ficus exasperata* on the mRNA expression of inflammatory and cardiovascular markers: (a) C-reactive protein, (b) angiotensin-converting enzyme, (c) P-selectin, (d) endothelial nitric oxide synthase. A one-way ANOVA was used to express the data as mean ± SEM (n = 5, all groups), followed by a Tukey multiple comparisons post-hoc test. Significant values were defined as $p < 0.05$ * , $p < 0.01$ * *, $p < 0.001$ * ** , and $p < 0.0001$ * ** *. * denotes values that differ significantly from those of diabetic untreated.

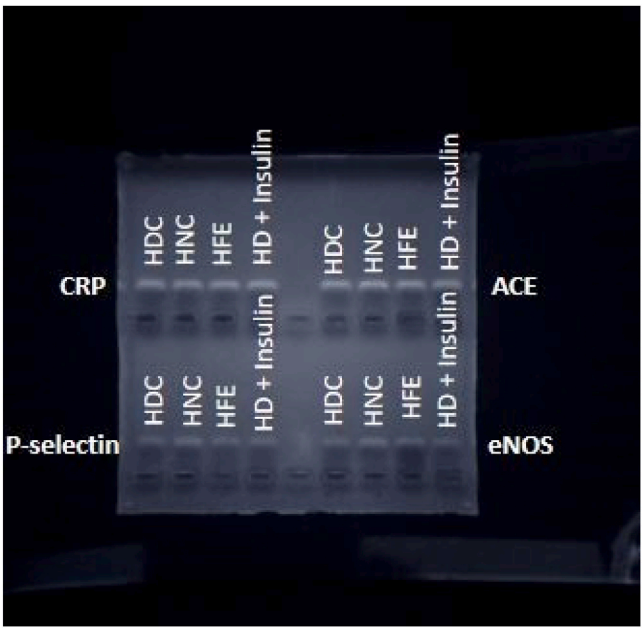

Fig. 5. shows full blots of mRNA expression of cardiovascular markers in rats treated with MEFE.

however, they were infiltrated with substantial layers of fat deposits. Diabetic rats treated with 200 mg/kg had normal structural architecture of the cardiac cell with good striations. In the diabetic treated with insulin (0.3 IU), the cardiac cells appeared distorted.

4. Discussion

Ficus exasperata contains various bioactive compounds, including terpenoids, fatty acids, esters, and alkanes, contributing to its pharmacological potential. Sesquiterpenoids such as cycloisolongifolene 8,9-dehydro-, eudesma-4(15),7-dien-1.beta-ol, and alpha-farnesene possess anti-inflammatory, antimicrobial, and antioxidant properties, while fatty acids and esters like n-hexadecanoic acid, linoleic acid ethyl ester, and ethyl oleate exhibit cardioprotective and lipid-lowering effects [31]. Most compounds, such as neophytadiene and 9-octadecenoic acid (Z-), have low toxicity, but Kaur-16-ene and certain alkanes may pose cytotoxic and hepatotoxic risks at high doses [32]. The observed decrease in body weight suggests MEFE does not prevent diabetes-associated weight loss but has hypoglycemic properties [33]. Adewole et al. [34] reported that an aqueous extract of *Ficus exasperata* causes hypotension and hypoglycemia in hypertensive and obese Zucker type 2 diabetic models. Similarly, Stephen Irudayaraj et al. [35] showed that Ficus carica, from the same Moraceae family, regulates glucose, lipid, and carbohydrate metabolism. The lack of significant difference in Na^{+}/K^{+} ATPase activity (Fig. 2a) following *Ficus exasperata* administration indicates no adverse effect on this enzyme in diabetes. This aligns with Vague et al. [36], who linked decreased Na+ /K+ ATPase activity in diabetes to genetic and environmental factors. Na+ /K+ ATPase maintains ion balance and membrane potential, and *Ficus exasperata* may exert its effects via alternative pathways. Spieker et al. [37] found no correlation between Ca^{2+} ATPase activity and diabetes, whereas Doğru Pekiner et al. [38] reported decreased brain Ca^{2+} ATPase activity in streptozotocin-induced diabetes. This study observed a decrease in Ca^{2+} ATPase activity in diabetes, but MEFE treatment significantly increased its activity, suggesting a role in calcium ion regulation and electrolyte balance (Fig. 2b) [38]. Creatine kinase myocardial band (CK-MB), a biomarker of myocardial damage, is predominantly found in cardiac muscle cells [39]. Elevated CK-MB levels indicate myocardial injury [40]. The increase in CK-MB following *Ficus exasperata* treatment raises concerns about potential cardiotoxic effects, despite observed benefits (Fig. 2c). This necessitates further investigation into its impact on myocardial cells. The rise in CK-MB could result from cytotoxic effects or inflammatory responses. Understanding these mechanisms is crucial to assessing whether its antioxidant properties outweigh potential cardiac risks. Cardiac troponin I, troponin T, and lactate dehydrogenase are markers of cardiac injury often elevated in diabetes [41,42]. These biomarkers were increased in diabetic untreated groups (Fig. 2d, e, & f), but MEFE treatment reduced them, suggesting cardioprotective properties. However, the concurrent increase in CK-MB raises concerns. The rise in SOD indicates that it may be able to protect cells from oxidative damage by converting superoxide radicals into hydrogen peroxide and oxygen [43,44]. This increase suggests enhanced cellular defense against oxidative stress, reinforcing its potential therapeutic value. Similar results were reported by Adetuyi et al. [3], who found that *Ficus exasperata* reduced acetaminophen-induced liver damage through NF-κB signaling. MEFE did not impact glutathione peroxidase but increased glutathione reductase activity (Fig. 3b & d). Glutathione reductase regulates redox homeostasis, suggesting MEFE reduces reactive oxygen species via oxidative stress modulation [45]. MEFE also reduced malondialdehyde and 8-hydroxy-2′-deoxyguanosine levels, biomarkers of oxidative stress capable of causing lipid peroxidation and DNA/protein modification (Fig. 3e & f) [46]. This suggests MEFE helps prevent macromolecular alterations linked to oxidative stress. The significant increase in C-reactive protein (CRP) levels in *Ficus exasperata*--treated rats indicates an enhanced inflammatory response (Fig. 4a). This increase suggests a possible pro-inflammatory effect of the extract, contrasting with its expected antioxidative properties [47]. This contradicts Nworu et al. [15], who reported that *Ficus exasperata* exhibited anti-inflammatory activity by inhibiting TNFα, IL-1β, and iNO production. The significant decrease in angiotensin-converting enzyme (ACE) levels compared to diabetic untreated rats suggests a modulatory effect on this enzyme (Fig. 4b).

Angiotensin I is converted to the vasoconstrictor angiotensin II by ACE, which is crucial to the renin-angiotensin-aldosterone system (RAAS) [48]. Reduced ACE levels result in vasodilation and lower blood pressure [49]. This suggests *Ficus exasperata* has antihypertensive potential, particularly beneficial for diabetics at risk for cardiovascular complications. According to Oboh et al. [50], in rats given a high-cholesterol diet, *Ficus exasperata*'s phenolic components were responsible for its ACE-inhibitory activity. The significant decrease in P-selectin levels in diabetic untreated rats indicates an anti-thrombotic effect of *Ficus exasperata* (Fig. 4c). P-selectin, a cell adhesion molecule expressed on activated endothelial cells and platelets, mediates leukocyte recruitment and thrombus formation [51]. Lower P-selectin levels suggest reduced leukocyte adhesion and platelet aggregation, potentially reducing thrombosis risk in diabetes. The decrease in endothelial nitric oxide synthase (eNOS) levels in *Ficus exasperata*-treated rats compared to diabetic untreated rats has cardiovascular implications (Fig. 4d). eNOS produces nitric oxide (NO), essential for vasodilation, blood pressure regulation, platelet inhibition, and inflammation prevention [52]. Reduced eNOS suggests diminished NO production, impairing vascular function, increasing vascular resistance, and heightening hypertension risk. This is concerning for diabetic individuals, already predisposed to cardiovascular complications [53]. Further research is needed to assess its safety and efficacy in cardiovascular health. The observed effects of *Ficus exasperata* on cardiovascular biomarkers are likely due to its bioactive compounds identified via GC-MS analysis (Table 2). These compounds may collectively influence physiological outcomes [54]. Simulated docking analysis of MEFE compounds with alpha-1, alpha-2, beta-1, and beta-2 adrenoceptors suggests that kuar-16-ene and cycloisolongifolene-8,9-dehydro play significant cardiovascular roles (Fig. 6a-d). These adrenoceptors regulate vasoconstriction, vasodilation, heart rate, and contractility [54]. Their molecular interactions suggest that hydrophobic interactions, hydrogen bonds, and van der Waals forces may contribute to binding

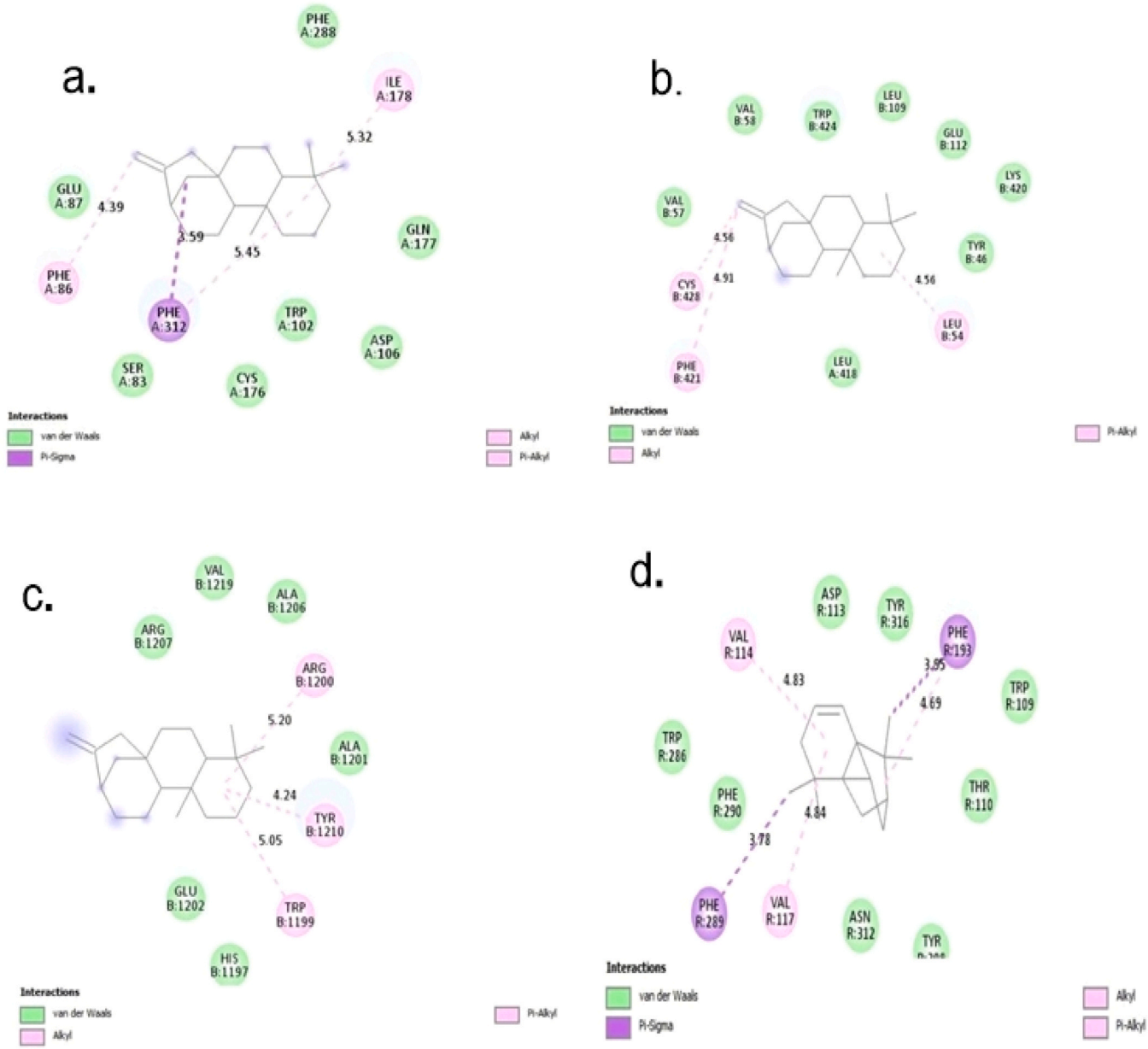

Fig. 6. Shows the molecular interaction between a. Kaur-16-ene and alpha 1 adrenoceptor b. Kaur-16-ene and alpha 2 adrenoceptor c. Kaur-16-ene and beta 1 receptor and d. Cycloisolongifene 8,9-dehydro- and beta 2 adrenoceptor.

stability [55]. The photomicrograph of the diabetic untreated group revealed adipocyte layers, indicative of metabolic derangement in diabetes (Fig. 7a-d). Excess adipocyte deposition promotes proinflammatory cytokines and free fatty acids, worsening hyperglycemia [56]. However, no fat deposits were observed following MEFE treatment, suggesting inhibition of preadipocyte differentiation and modulation of adipogenesis and lipid metabolism. To evaluate MEFE safety, future studies should include acute and chronic toxicity assessments involving single and repeated doses over varying durations. Hematological and biochemical analyses would assess key blood parameters, liver/kidney function markers, and oxidative stress indicators for systemic toxicity. Histopathological examinations of the liver, kidneys, heart, and pancreas would provide insights into structural or cellular alterations due to prolonged exposure. Dose-escalation studies are essential to establish the highest non-toxic dose, therapeutic window, and adverse effects. While MEFE demonstrated antioxidant properties, suggesting protection against oxidative stress, genotoxic studies should be conducted, as some bioactive compounds may exhibit pro-oxidant behavior at high concentrations or induce DNA damage through alternative mechanisms.

5. Conclusion

Methanol extract of *Ficus exasperata* exhibits antioxidant, antihypertensive, and anti-thrombotic properties, increasing SOD and glutathione reductase while reducing ACE and P-selectin. However, elevated CK-MB and CRP suggest myocardial damage and inflammation, raising safety concerns. Reduced eNOS levels further indicate potential vascular impairment with long-term cardiovascular risks. While MEFE shows therapeutic promise, its possible adverse effects necessitate thorough evaluation. Further studies are crucial to establish a comprehensive safety profile, ensuring its benefits outweigh potential cardiovascular risks.

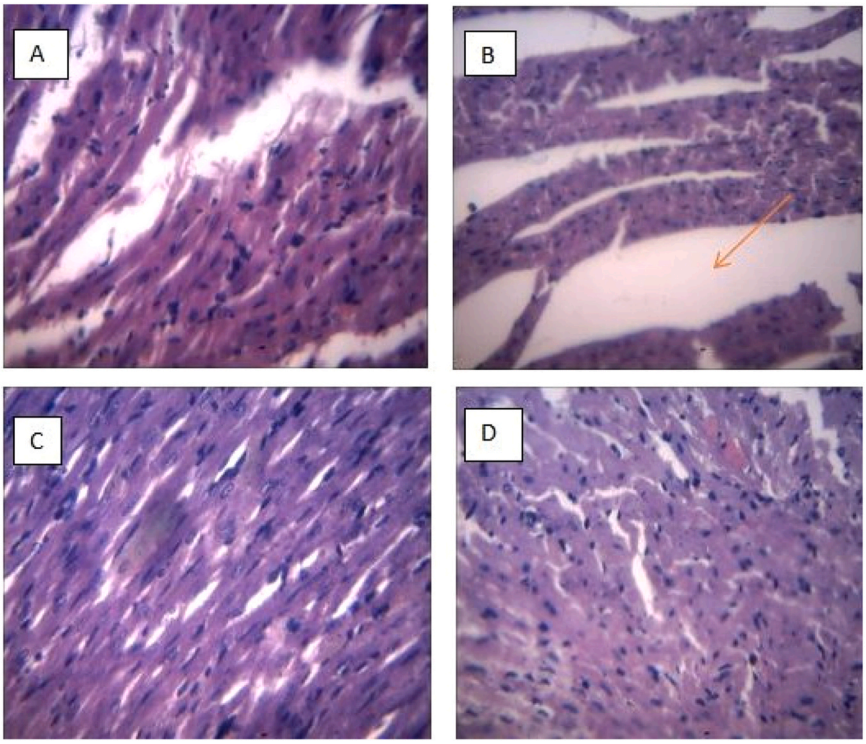

Fig. 7. The photomicrograph of the heart. A (Control) showed normal architecture of cardiac cells, while B (Diabetic untreated) showed the myofibers of the cardiac cells infiltrated with layers of adipose tissue (orange color). C (diabetes + 200 mg/kg of MEFE) showed a normal architecture and striation of the cardiac cells, and D (diabetes + 0.3 units of insulin) showed mild structural distortion and increased granules.

CRediT authorship contribution statement

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

Authors statement

The revised manuscript titled "**Molecular basis of cardioprotective effects of methanol extract of *Ficus exasperata* in diabetic Wistar rats** " has been carefully reviewed and accepted by all authors.

The revisions were made in response to the constructive feedback provided by the reviewers and editors. The authors reached a consensus on the changes implemented to enhance the clarity, scientific rigor, and overall quality of the manuscript.

The final version reflects a collaborative effort to address comments and concerns, ensuring the research findings are accurately and effectively communicated. The authors appreciate the valuable input from the reviewers and believe the revised manuscript significantly strengthens the contribution to the scientific community.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: The authors declare that there are no conflicts of interest related to this publication If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.toxrep.2025.102028.

Data Availability

Data will be made available on request.

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