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Original Article

Neocarya macrophylla ethyl acetate fraction attenuates oxidative stress and inflammation in high-fat diet and streptozotocin-induced diabetic rats

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ABSTRACT

Oxidative stress and inflammation drive diabetes. Conventional treatments inadequately target these. *Neocarya macrophylla* shows preliminary antioxidant promise. This study aims to investigate its antioxidant and anti-inflammatory effects in a diabetic rat model to address this research gap. Thirty-six male Wistar rats were randomised into four groups (n=6 per group): Normal Control, Diabetic Control, *N. macrophylla* Ethyl Acetate Fraction (120 mg/kg), and Metformin (200 mg/kg). Type 2 diabetes was induced using a 60% HFD for four weeks, followed by a single intraperitoneal injection of STZ (40 mg/kg). Treatment was administered orally for four weeks. Post-treatment, oxidative stress biomarkers—Superoxide Dismutase (SOD), Catalase (CAT), Glutathione Peroxidase (GPx), and Malondialdehyde (MDA)—were quantified using spectrophotometric assays. Pro-inflammatory cytokines—Interleukin-6 (IL-6) and Tumour Necrosis Factor-alpha (TNF-α)—were evaluated using ELISA, while Catalase mRNA expression was assessed via RT-qPCR. Statistical analysis was performed using GraphPad Prism 9, applying One-way ANOVA followed by Tukey's post hoc test, with $p<0.05$ considered significant.Treatment with the ethyl acetate fraction significantly increased SOD activity by 19% ($p=0.007$), GPx by 54% ($p=0.04$), and CAT by 136% ($p<0.001$) compared to diabetic controls, while reducing MDA levels by 41% ($p<0.001$), indicative of reduced lipid peroxidation. Pro-inflammatory cytokines IL-6 and TNF-α levels were reduced by 58% and 62%, respectively, in the treatment group compared to diabetic controls. Catalase mRNA expression showed a 1.4-fold increase ($p < 0.001$). Notably, the ethyl acetate fraction's effects were comparable to Metformin in modulating oxidative stress and inflammatory markers.This study demonstrates that the ethyl acetate fraction of *N. macrophylla* leaves exerts significant antioxidant and anti-inflammatory effects in HFD/STZ-induced diabetic rats, supporting its therapeutic potential as an adjunctive natural remedy in managing oxidative stress and inflammation associated with diabetes.

1. Introduction

Diabetes mellitus is a chronic metabolic disorder characterised by persistent hyperglycemia, which triggers a cascade of metabolic disturbances leading to severe microvascular and macrovascular complications, including diabetic

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nephropathy, neuropathy, retinopathy, and cardiovascular diseases [1, 2]. Prolonged hyperglycemia induces oxidative stress by enhancing the production of reactive oxygen species (ROS) through multiple biochemical pathways such as glucose auto-oxidation, polyol pathway flux, advanced glycation end-products (AGEs) formation, and activation of protein kinase C (PKC) [2]. The excessive generation of ROS overwhelms the body's endogenous antioxidant defenses, resulting in oxidative stress that activates pro-inflammatory pathways, notably the nuclear factor-kappa B (NF-κB) and NLRP3 inflammasome, contributing significantly to the progression of diabetic complications [3].

Among the various forms of diabetes, type 2 diabetes mellitus (T2DM) is predominantly associated with oxidative stress and chronic low-grade inflammation, which together mediate cellular and tissue damage, impairing organ function [4]. This pathophysiological interplay underpins the progression of diabetic complications and highlights oxidative stress as a pivotal therapeutic target in diabetes management [5].

Current pharmacological interventions, such as metformin, are effective in glycemic control and exhibit ancillary antioxidant and anti-inflammatory effects [6]. However, their use is often limited by adverse effects, including gastrointestinal discomfort and potential nephrotoxicity, especially in long-term therapy [7]. Additionally, newer antidiabetic agents like SGLT2 inhibitors and GLP-1 receptor agonists offer cardio-renal protective benefits but are often cost-prohibitive and associated with side effects such as genitourinary infections and gastrointestinal intolerance [8]. These challenges necessitate the exploration of alternative therapeutic strategies that target oxidative stress and inflammation with fewer adverse effects and lower costs, thus making plant-based therapies a promising adjunct or potential standalone treatment option [9].

The use of medicinal plants rich in bioactive compounds with antioxidant and anti-inflammatory properties has garnered significant interest in diabetes research [8]. *N. macrophylla* (Gingerbread plum), a medicinal plant native to West Africa, particularly northern Nigeria, has been traditionally employed to manage various ailments, including diabetes [10]. Phytochemical analyses have revealed that *N. macrophylla* leaves contain bioactive constituents such as flavonoids, phenolic acids, saponins, and alkaloids, which are known for their hypoglycemic, antioxidant, and anti-inflammatory effects [11]. Previous studies have demonstrated the hypoglycemic potential of *N. macrophylla* leaf extracts, with evidence of improved hematological profiles and modulation of oxidative stress biomarkers in diabetic animal models [3, 12].

Despite these promising findings, limited studies have elucidated the specific molecular mechanisms by which *N. macrophylla* exerts its protective effects, particularly regarding the modulation of oxidative stress and inflammatory pathways. Moreover, the ethyl acetate fraction, known for its enrichment of polyphenolic compounds, has been postulated to possess superior bioactivity compared to other fractions like n-hexane or chloroform, yet this remains underexplored [3]. Evaluating the antioxidant efficacy of this fraction through catalase mRNA expression profiling and other oxidative stress markers could provide mechanistic insights into its therapeutic potential.

In this context, the high-fat diet (HFD) and streptozotocin (STZ)-induced diabetic rat model is a well-established experimental platform that closely mimics the metabolic and inflammatory milieu of human T2DM, thereby providing a relevant system to investigate the efficacy of antioxidant-based interventions [13, 14]. Therefore, this study aimed to evaluate the antioxidant and anti-inflammatory effects of the ethyl acetate fraction of *N. macrophylla* leaf in HFD/STZ-induced diabetic rats by assessing its impact on oxidative stress markers, inflammatory mediators, and catalase gene expression. This investigation seeks to provide a mechanistic basis for the therapeutic use of *N. macrophylla* as a potential standalone intervention in the management of type 2 diabetes and its complications.

2. Materials and methods

2.1. Materials

Materials used include male Wistar rats, oral gavage cannula, dissecting kits, sample bottles, conical flasks, office pins, standard rat feed, ethanol, formaldehyde, xylene, centrifuge machine, methanol, burettes, cotton wool, glucometer (Accu-Chek, Roche Diagnostics), measuring cylinders, distilled water, custom-formulated high-fat diet (HFD), stopwatch, anaesthetizing chamber, digital weighing scale, phosphate-buffered saline (PBS), Streptozotocin (STZ; Sigma-Aldrich, USA), metformin 500 mg (Actavis, Chennai, India), 1-mL syringes with 23G and 25G needles.

2.2 Plant Collection and Preparation

The leaves of *N. macrophylla* (Gingerbread plum) were collected on 18th June 2022 from Jega Local Government Area, Kebbi State, Nigeria (Latitude: 12.2184° N; Longitude: 4.3795° E) during the wet season. The plant was taxonomically identified at the Herbarium Unit, Department of Pharmacognosy and Ethnopharmacy, Faculty of Pharmaceutical Sciences, Usmanu Danfodiyo University, Sokoto, by Mallam Musa Magaji**,** and a voucher specimen (PCG/UDUS/CHRY/0001) was deposited.

Leaves were air-dried under shade for 14 days, pulverised using a mechanical grinder, and stored in airtight containers. A total of 1961 g of powdered leaves was macerated in 90% methanol for 6 days with intermittent agitation**,** following protocols described by Forouzandeh et al. [15]. The extended maceration ensured exhaustive extraction of polyphenols and flavonoids, known to be abundant in *Nm* leaves.

The resultant filtrate was concentrated under reduced pressure using a rotary evaporator, yielding 288 g of crude Methanol Extract (MEL)**.** A portion (200 g) was re-suspended in distilled water, filtered, and sequentially partitioned with n-hexane (1 L), chloroform (1 L), ethyl acetate (2.5 L), and n-butanol (2.5 L) to obtain respective fractions. The ethyl acetate fraction (EAF) was selected for this study based on prior evidence of superior antioxidant activity [16].

The LD_{50} (565 mg/kg) was adopted from Yusuf et al. [17], derived from the leaf extract using oral administration in rats. For dosing, a stock solution of 24 mg/mL was prepared by dissolving the EAF in 0.1% dimethyl sulfoxide (DMSO) diluted with distilled water. This concentration was optimised based on solubility, preliminary tolerability tests, and dosing convenience for oral administration.

2.3. Experimental Animals

Thirty-six male Wistar rats (4 weeks old, 80–120 g) were procured from the Animal Breeding Unit, Department of Human Physiology, Faculty of Basic Medical Sciences, Ahmadu Bello University, Zaria. Rats were housed in standard plastic cages, maintained at 25°C ± 2°C with a 12-hour light-dark cycle, and had free access to feed and water. After a 14-day acclimatisation period, all experimental protocols were approved by the Research Ethics Committee of Usmanu Danfodiyo University, Sokoto (Ref: NHREC/UDUTH-HREC/30/03/202).

2.4, Preparation of High-Fat Diet

The 60% high-fat diet (HFD) was formulated by modifying the standard pellet diet (10 kg) by adding 3.6 kg (2.5 L) pig oil and 2.4 kg groundnut in a 3:2 ratio**.** This formulation was adapted from Gheibi et al. [18] and chosen for its proven efficacy in inducing insulin resistance mimicking human Type 2 diabetes.

2.5. Induction of HFD/STZ Diabetes

After acclimatization, rats were fed the HFD for 4 weeks**.** On day 29, rats were fasted for 8 hours and then administered a single intraperitoneal injection of STZ (40 mg/kg BW) dissolved in 0.1 M citrate buffer (pH 4.5)**.** This dose induces partial beta-cell cytotoxicity, effectively simulating Type 2 diabetes pathophysiology.

To prevent acute hypoglycemia, rats were provided with 5% glucose solution for 24 hours post-STZ injection**.** Fasting blood glucose levels were measured using an Accu-Chek glucometer at 72 hours post-STZ and again after 10 days of induction. Rats with fasting blood glucose ≥ 180 mg/dL were considered diabetic.

Body weights and naso-anal lengths were recorded at baseline, pre-STZ, and weekly thereafter using a sensitive digital beam balance to calculate BMI.

2.6. Experimental Design

Diabetic rats were randomized into four groups (n = 6 per group):

- ✓ **Group 1 (Normal Control)**: Non-diabetic rats receiving distilled water.
- ✓ **Group 2 (Diabetic Control)**: HFD/STZ rats receiving distilled water.
- ✓ **Group 3 (*Nm*-Treated)**: HFD/STZ rats treated with Ethyl acetate fraction (120 mg/kg BW) orally for 4 weeks.
- ✓ **Group 4 (Standard Drug Control)**: HFD/STZ rats treated with Metformin (200 mg/kg BW) orally for 4 weeks.

The sample size (n=6) per group was determined based on effect size estimates from prior *Nm* studies, aiming for a statistical power of 80% (β = 0.2) at a 95% confidence interval (α = 0.05).

2.7. Sample Collection and Preparation

At the study's conclusion, deep anesthesia was induced in rats via an intraperitoneal injection of ketamine (80 mg/kg) and xylazine (10 mg/kg). Following this, euthanasia was performed by cardiac puncture. Blood was collected into plain tubes, left to clot, and subsequently centrifuged at 3000 rpm for 10 minutes at 4°C. The resulting serum was isolated and stored for subsequent biochemical analysis.

Skeletal muscle was rinsed in ice-cold PBS, minced, and homogenized with protease inhibitors. The homogenate was centrifuged at 5000 × g for 15 minutes at 4°C. The resulting supernatant was collected for the subsequent analysis of oxidative stress markers and gene expression, preserving sample integrity for accurate assessment.

2.8. Biochemical Assays

Oxidative stress markers (SOD, CAT, MDA, GPx) were quantified using commercial assay kits (Beijing Solarbio Science and Technology Co., Ltd., China) following the manufacturer's protocols, with absorbance readings recorded using a microplate reader (450 nm for SOD & MDA; 540 nm for CAT; 412 nm for GPx).

2.9. Pro-Inflammatory Cytokine Assays

Serum levels of IL-6 and TNF-α were measured using sandwich ELISA kits (MyBioSource, USA), adhering to the manufacturer's instructions. Absorbance was measured at 450 nm, with a correction at 550 nm.

2.10. Gene Expression Analysis

Total RNA was extracted from muscle tissues using the Daan Gene Nucleic Acid Extraction Kit (Lifeover, China). RNA integrity was confirmed via agarose gel electrophoresis, and purity (A260/A280 ratio) was assessed using a NanoDrop spectrophotometer.

cDNA synthesis and qRT-PCR were performed using the TransScript® Green one-step qRT-PCR Supermix (TransGen Biotech, China). Primers (Table 1) were validated via BLAST (NCBI) to ensure specificity, and amplicon sizes were verified via melt curve analysis.

β-actin was used as a reference gene after stability verification via geNorm analysis, confirming minimal variability across experimental groups. Relative gene expression was calculated using the $2^{-\Delta\Delta Ct}$ method [19], and experiments were conducted in triplicate.

2.11. Statistical Analysis

Data distribution normality and variance homogeneity were evaluated using the Shapiro-Wilk and Levene's tests, respectively. All experimental values are reported as mean ± standard error of the mean (SEM). Group comparisons were conducted via one-way analysis of variance (ANOVA) with Tukey's honestly significant difference (HSD) post hoc test for multiple comparisons. A p-value less than 0.05 was deemed statistically significant. GraphPad Prism version 9.01 (GraphPad Software, Inc., USA) was used for all statistical analyses.

Table 1. The name and motif of the used primers

Gene **name**	**Strand**	**Motif** (5`-3`)	**Reference**
Il-6	Forward	AGGCAGAGTCATTCAGAGC	[20]
	Reverse	CATTGGTAGTTGGGGTAGGA	
CAT	Forward	CCTCAGAAACCCGATGTCCTG	[21]
	Reverse	GTCAAAGTGTGCCATCTCGTCG	
β-actin	Forward	AGAGGGAAATCGTGCGTGAC	[21]
	Reverse	CGATAGTGATGACCTGACCGT	

3. Results

3.1 Effect of Ethyl Acetate Fraction of Gingerbread Plum (*N. macrophylla*) Leaf on Oxidative Stress Markers in Type 2 Diabetic Wistar Rats

Table 2 shows the effects of the ethyl acetate fraction on oxidative stress parameters. Diabetic control rats exhibited a 39.6% decrease in SOD activity and a 1.85-fold increase in MDA levels compared to normal controls ($p < 0.001$). Treatment with the ethyl acetate fraction significantly improved SOD activity (+91.9% vs. diabetic control, $p = 0.007$) and GPx activity (+54.5%, $p = 0.04$), while reducing MDA levels by 40.6% ($p < 0.001$) relative to diabetic controls. Catalase activity was elevated in the ethyl acetate group (+135.5% vs. diabetic control, $p < 0.001$) and surpassed normal control levels by 15.7% ($p = 0.02$). The metformin group also demonstrated significant improvements across all oxidative stress markers, with SOD and catalase levels exceeding normal controls (+35.9% and +12.6%, respectively).

Table 2. Effect of ethyl acetate fraction of nm leaf on oxidative stress markers in type 2 diabetic wistar rats (n = 6, Mean ± SEM)

Variable	Normal Control	Diabetic Control	Metformin (200 mg/kg)	Ethyl acetate (120 mg/kg)
	Mean ± SEM	Mean ± SEM	Mean ± SEM	Mean ± SEM
SOD (u/ml)	15.62 ± 2.78	9.43 ± 0.26	21.22 ± 1.16	18.10 ± 0.22^{b**}
MDA (ng/ml)	88.15± 2.86	163.52 ± 2.27	99.51 ± 0.71	97.12 ± 1.34^{b***}
GPx (Miu/ml)	11.21 ± 2.86	7.52 ± 0.49	10.01 ± 1.47	11.62 ± 1.07^{b*}
Catalase (ng/ml)	17.72 ± 0.04	8.71 ± 0.49	19.95 ± 0.23	20.51 ± 0.26^{a*,b***}

SOD = Superoxide Dismutase, GPx = Glutathione Peroxidase, MDA = Malondialdehyde. Data were presented as the mean ± SEM, rats per group (n=6). SEM: Standard Error of Mean. 'a': p< 0.05 vs Normal Control. 'b': p< 0.05 vs Diabetic Control. 'c': p <0.05 vs Metformin. *: p<0.05, **: p < 0.01, ***: p< 0.001.

3.2. Effect of Ethyl Acetate Fraction of *N. macrophylla* Leaf on Pro-inflammatory Cytokines (TNF-α and IL-6)

As depicted in Figures 1 (left and right), a highly significant difference in the concentrations of the pro-inflammatory cytokines IL-6 and TNF-α was observed among the experimental groups ($p < 0.001$). Rats in the diabetic control group displayed a substantial inflammatory response, marked by a 2.8-fold elevation in IL-6 and a 2.5-fold rise in TNF-α relative to the normal controls. Therapeutic intervention with the ethyl acetate fraction produced a robust anti-inflammatory effect, significantly lowering IL-6 by 51.4% and TNF-α by 47.3% compared to the diabetic cohort ($p < 0.001$). Nonetheless, the suppression was not entirely complete, as IL-6 levels, while greatly improved, stayed slightly but significantly above those in the normal baseline group ($p = 0.043$). The response in the metformin-treated group mirrored this pattern of cytokine attenuation. Importantly, a direct statistical comparison between the ethyl acetate fraction and metformin revealed no significant difference in their efficacy for reducing either IL-6 ($p = 0.378$) or TNF-α ($p = 0.289$), suggesting the novel treatment performs comparably to the standard drug in mitigating inflammation.

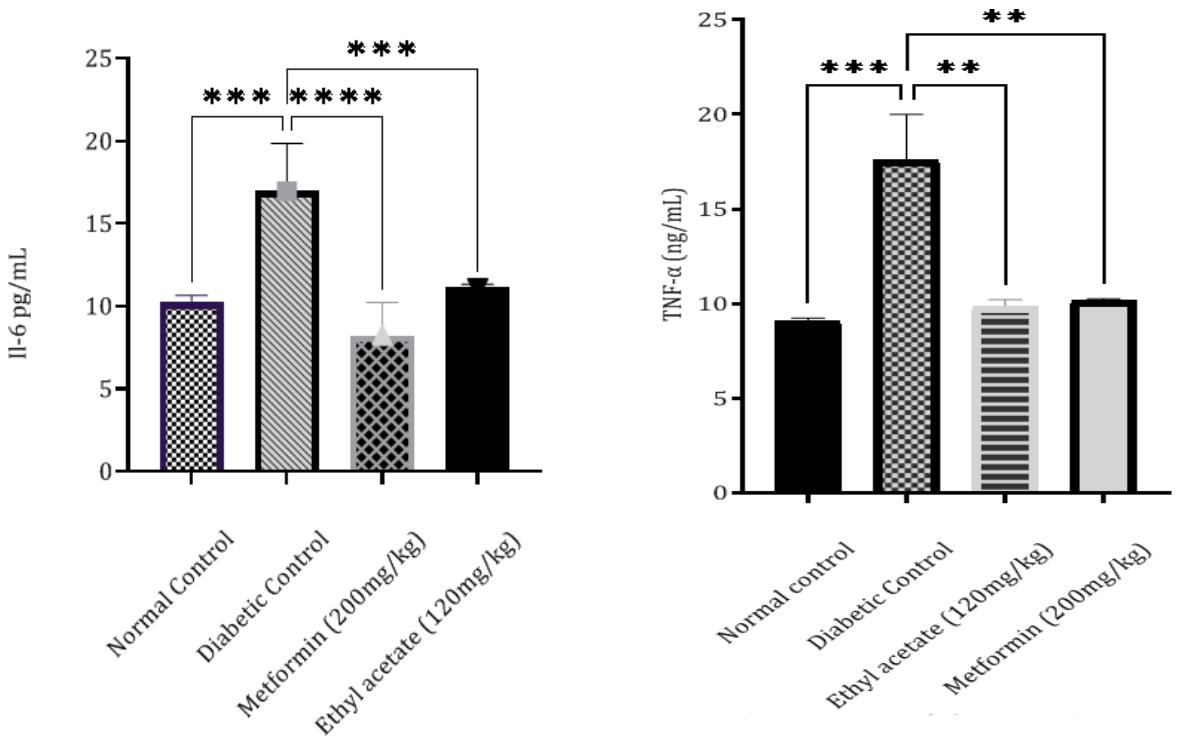


Fig. 1. Left) Effect of ethyl acetate fraction of nm leaf on interleukin-6 in type 2 diabetic wistar rats. *: p<0.05, **: p < 0.01, ***: p< 0.001. Right) Effect of ethyl acetate fraction of nm leaf on tumour necrosis factor-alpha in type 2 diabetic wistar rat. *: p<0.05, **: p < 0.01, ***: p< 0.001.

3.3 Effect of Ethyl Acetate Fraction on Relative Expression of Catalase mRNA

As shown in Figure 2 (left), catalase mRNA expression was severely reduced by 68.4% in diabetic controls versus normal animals. This deficit was powerfully counteracted by treatment with the ethyl acetate fraction, which induced a robust 2.2-fold upregulation in expression compared to the diabetic group ($p < 0.001$). This result demonstrates a potent restorative effect of the treatment on the compromised antioxidant gene expression characteristic of the diabetic state in the model, significantly reversing the pathological downregulation.

The standard drug metformin also showed efficacy, inducing a 1.9-fold increase. However, the restorative effects of both treatments were partial, as catalase mRNA levels, despite significant improvement, did not fully normalize and remained below the baseline of the healthy control group ($p = 0.034$). This indicates a substantial, yet incomplete, recovery of antioxidant gene expression. All data include error bars denoting the standard error of the mean (SEM).

3.4 Effect of Ethyl Acetate Fraction on Relative Expression of Interleukin-6 (IL-6) mRNA

As illustrated in Figure 2 (right), IL-6 mRNA expression was significantly upregulated, showing a 3.2-fold increase in diabetic control rats relative to normal controls. Administration of the ethyl acetate fraction effectively mitigated this elevation, reducing expression by 46.9% compared to the diabetic group ($p = 0.004$).Despite this strong anti-inflammatory effect, IL-6 mRNA levels in the treated animals remained elevated at 1.5-fold over the normal baseline.

The group treated with metformin exhibited a similar pattern of reduction, with no statistically significant difference in efficacy compared to the ethyl acetate treatment ($p = 0.061$). A notable divergence was observed between the mRNA expression data and the previously measured serum IL-6 protein levels. This discrepancy suggests that the regulation of IL-6 may involve substantial post-transcriptional mechanisms, a compelling area that merits deeper investigation to fully elucidate the pathway of inflammatory expression in this model.

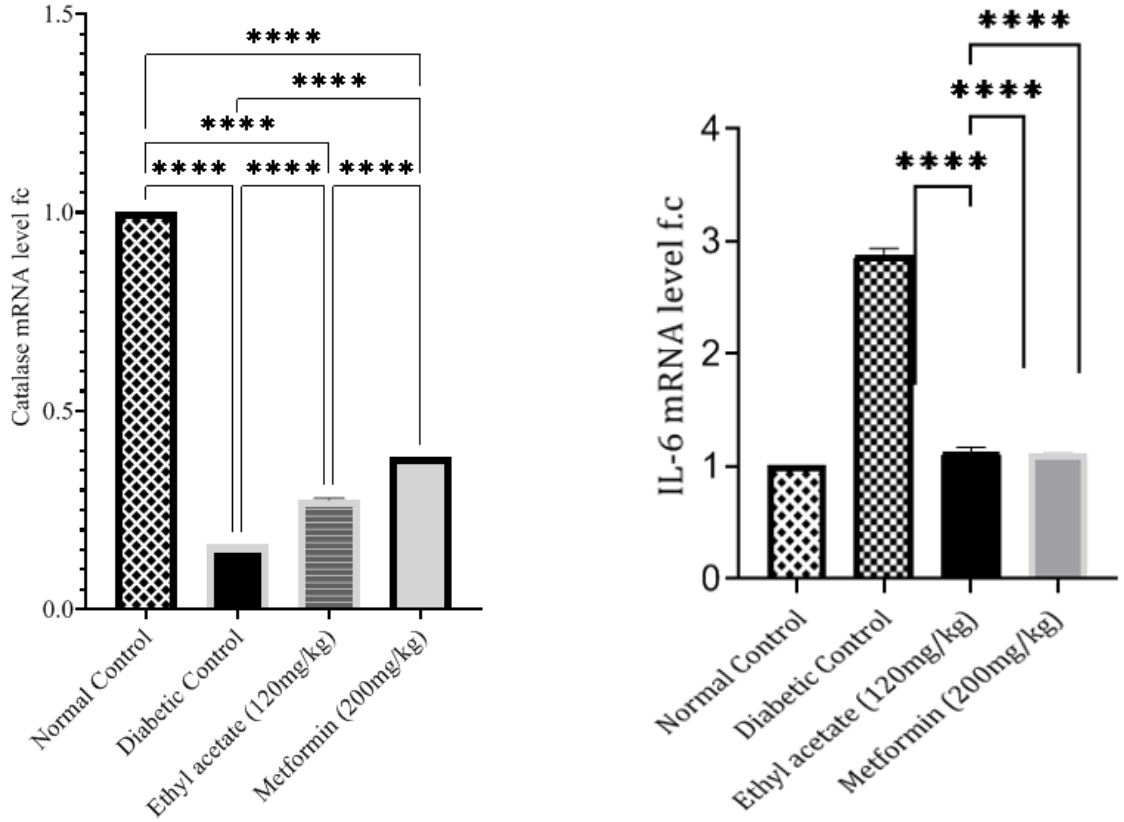


Fig. 2. Left) Relative Expression of Catalase mRNA (Fold-Change) in Type 2 Diabetic Wistar Rats. *: p<0.05, **: p < 0.01, ***: p< 0.001. Right) Relative expression of il-6 mrna in type 2 diabetic wistar rats. *: p<0.05, **: p < 0.01, ***: p< 0.001.

4. Discussion

This study investigated the antioxidant and anti-inflammatory properties of the ethyl acetate fraction of Gingerbread plum (*N. macrophylla*) leaves in high-fat diet and low-dose streptozotocin (HFD/STZ)-induced diabetic Wistar rats to elucidate its therapeutic potential in mitigating diabetes-associated oxidative stress and inflammation.

The results demonstrated a significant increase in antioxidant enzyme activities—superoxide dismutase (SOD), glutathione peroxidase (GPx), and catalase (CAT)—and a marked reduction in malondialdehyde (MDA) levels in both ethyl acetate-treated and metformin-treated groups compared to diabetic controls. The observed increase in SOD and GPx activity (~1.8-fold and ~1.5-fold, respectively) along with a decrease in MDA levels (a ~40.6% reduction compared to diabetic controls) underscores the antioxidant potential of the ethyl acetate fraction in ameliorating lipid peroxidation and oxidative stress-induced damage. Similar trends were reported in studies on *Solanum indicum*, which demonstrated a reduction in oxidative stress markers and enhanced enzymatic antioxidant defences in diabetic models [3, 12, 22].

The anti-inflammatory effects observed in this study, evidenced by significant reductions in interleukin-6 (IL-6) and tumour necrosis factor-alpha (TNF-α) levels, are consistent with prior research indicating that plant-derived bioactive compounds such as flavonoids, phenolics, and alkaloids possess anti-inflammatory and immunomodulatory activities [23, 24]. While the current study attributes these effects to phytochemicals present in *N. macrophylla*, the absence of HPLC/MS profiling data for the specific ethyl acetate fraction represents a limitation. Therefore, future studies should perform detailed phytochemical characterisation to identify active constituents responsible for these bioactivities.

Gene expression analysis revealed a significant upregulation of catalase (CAT)

mRNA in the ethyl acetate-treated group compared to diabetic controls, aligning with the increased catalase enzyme activity measured. However, the expression did not fully reach normal control levels, suggesting a partial restoration of antioxidant gene expression. This could reflect a compensatory response to chronic hyperglycemia-induced oxidative stress, a phenomenon similarly noted in metformin-treated groups. The crucial role of catalase in detoxifying hydrogen peroxide and mitigating oxidative stress-induced apoptosis has been emphasized in previous studies focusing on diabetic nephropathy and cellular protection mechanisms [25-27].

Regarding IL-6 mRNA expression, a 1.6-fold increase was observed in the ethyl acetate-treated group compared to normal controls, which is notably lower than the ~3.2-fold increase in the diabetic control group. Although this suggests partial modulation of IL-6 expression, the modest upregulation compared to normal rats indicates that post-transcriptional regulation mechanisms may influence protein-level outcomes, as evidenced by the significant reduction in serum IL-6 protein levels. This discrepancy between mRNA expression and cytokine protein levels may be attributed to translational control pathways or feedback mechanisms involved in inflammatory signaling cascades [28-30].

5. Conclusion

In conclusion, the ethyl acetate fraction of Gingerbread plum (*N. macrophylla*) leaves demonstrated significant antioxidant and anti-inflammatory activities in HFD/STZ-induced diabetic Wistar rats by enhancing key antioxidant enzymes (SOD, GPx, and catalase) and attenuating lipid peroxidation (MDA levels). Additionally, the fraction effectively reduced pro-inflammatory cytokines (IL-6 and TNF-α), indicating its potential in modulating oxidative stress and inflammatory responses associated with diabetes mellitus. Despite these promising findings, further investigations involving dose-response studies, phytochemical profiling (HPLC/MS), toxicity assessments, and functional metabolic evaluations are warranted to substantiate the therapeutic potential and safety of *N. macrophylla* leaf extracts. This study adds to the growing body of evidence supporting the development of plant-based therapies as adjuncts or alternatives to conventional antidiabetic medications in managing diabetic complications.

Conflict of Interests

The authors declare no conflict of interest.

Ethics approval and consent to participate

The research followed the tenets of the Declaration of Helsinki. The Ethics Committee of Usmanu Danfodiyo University, Sokoto approved this study (Ref: NHREC/UDUTH-HREC/30/03/202). Accordingly, written informed consent was taken from all participants before any intervention. Ethical issues (including plagiarism, data fabrication, double publication) have been completely observed by the authors.

Consent for publication

The authors read and approved the final manuscript for publication.

Informed Consent

The authors declare not used any patients in this research.

Availability of data and material

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Authors' contributions

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Investigation: Buhari Aliyu, Sadiq Muazu Maifata, and Zayyanu Umar Usman.
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Project administration: Abdullahi Adamu Ja'e, and Chinedu Onwuchekwa.
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Resources: All authors.
Supervision: Zayyanu Umar Usman.

Validation: Buhari Aliyu, Sadiq Muazu Maifata, and Zayyanu Umar Usman.
Visualization: Buhari Aliyu, Sadiq Muazu Maifata, and Zayyanu Umar Usman.
Writing–original draft: Sadiq Muazu Maifata, and Aminat Suleman-Alabi.
Writing–reviewing & editing: All authors.

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References

1. Forbes JM, Cooper ME (2013) Mechanisms of diabetic complications. Physiological reviews 93 (1): 137-188. doi: https://doi.org/10.1152/physrev.00045.2011
2. Nandi A, Yan L-J, Jana CK, Das N (2019) Role of catalase in oxidative stress-and age-associated degenerative diseases. Oxidative medicine and cellular longevity 2019 (1): 9613090. doi: https://doi.org/10.1155/2019/9613090
3. Gadewar MM, GK P, Mishra PC, Ashraf GM, Almashjary MN, Harakeh S, Upadhye V, Dey A, Singh P, Jha NK (2023) Evaluation of antidiabetic, antioxidant and anti-hyperlipidemic effects of *Solanum indicum* fruit extract in streptozotocin-induced diabetic rats. Current issues in molecular biology 45 (2): 903-917. doi: https://doi.org/10.3390/cimb45020058
4. Maritim A, Sanders a, Watkins Iii J (2003) Diabetes, oxidative stress, and antioxidants: a review. Journal of biochemical and molecular toxicology 17 (1): 24-38. doi: https://doi.org/10.1002/jbt.10058
5. Teodoro JS, Nunes S, Rolo AP, Reis F, Palmeira CM (2019) Therapeutic options targeting oxidative stress, mitochondrial dysfunction and inflammation to hinder the progression of vascular complications of diabetes. Frontiers in physiology 9: 1857. doi: https://doi.org/10.3389/fphys.2018.01857
6. Tauheed AM, Mamman M, Ahmed A, Sani NiA, Suleiman MM, Sulaiman MH, Balogun EO (2021) Acute, sub-acute, sub-chronic and chronic toxicity studies of four important Nigerian ethnomedicinal plants in rats. Clinical Phytoscience 7 (1): 1. doi: https://doi.org/10.1186/s40816-020-00244-2
7. Toppazzini MA, Wiener KKK (2017) Making workplaces safer: The influence of organisational climate and individual differences on safety behaviour. Heliyon 3 (6). doi: https://doi.org/10.1016/j.heliyon.2017.e00334
8. Furman BL (2015) Streptozotocin-induced diabetic models in mice and rats. Current protocols in pharmacology 70 (1): 5.47. 41-45.47. 20. doi: https://doi.org/10.1002/0471141755.ph0547s70
9. King AJ (2012) The use of animal models in diabetes research. British journal of pharmacology 166 (3): 877-894. doi: https://doi.org/10.1111/j.1476-5381.2012.01911.x
10. Bala A, Igwe E, Bamalli Z, Zubairu I (2022) A review on an underutilized wild edible plant: Gingerbread plum (*Neocarya macrophylla*). FUDMA Journal of Agriculture and Agricultural Technology 8 (2): 200-209. doi: https://doi.org/10.33003/jaat.2023.0802.27
11. Fatema K, Sharmin AA, Sharna JF, Haque MA, Rahman MM, Sarker S, Kazi M, Rahman MR, Namakka M, Uzzaman M (2024) Antioxidant and antidiabetic effects of *Flemingia macrophylla* leaf extract and fractions: in vitro, molecular docking, dynamic simulation, pharmacokinetics, and biological activity studies. BioResources 19 (3): 4960. doi: https://doi.org/10.15376/biores.19.3.4960-4983
12. Andargie Y, Sisay W, Molla M, Tessema G (2022) Evaluation of antidiabetic and antihyperlipidemic activity of 80% methanolic extract of the root of *Solanum incanum* linnaeus (solanaceae) in mice. Evidence-Based Complementary and Alternative Medicine 2022 (1): 4454881.

doi: https://doi.org/10.1155/2022/4454881
13. Bangar SP, Chaudhary V, Sharma N, Bansal V, Ozogul F, Lorenzo JM (2023) Kaempferol: A flavonoid with wider biological activities and its applications. Critical Reviews in Food science and nutrition 63 (28): 9580-9604. doi: https://doi.org/10.1080/10408398.2022.2067121
14. Yang Y, Chen Z, Zhao X, Xie H, Du L, Gao H, Xie C (2022) Mechanisms of Kaempferol in the treatment of diabetes: A comprehensive and latest review. Frontiers in endocrinology 13: 990299. doi: https://doi.org/10.3389/fendo.2022.990299
15. Forouzandeh M, Mohkami z, Fazeli-Nasab B (2019) Evaluation of Biotic Elicitors Foliar Application on Functional Changes, Physiological and Biochemical Parameters of Fennel (*Foeniculum vulgare*). Int J Plant Prod 25 (4): 49-65. doi: https://doi.org/10.22069/jopp.2018.14077.2262
16. Rodríguez-Yoldi MJ (2021) Anti-Inflammatory and Antioxidant Properties of Plant Extracts. Antioxidants 10 (6): 921. doi: https://doi.org/10.3390/antiox10060921
17. Bhatt ID, Rawat S, Rawal RS (2013) Antioxidants in Medicinal Plants. In: Chandra S, Lata H, Varma A (eds) Biotechnology for Medicinal Plants: Micropropagation and Improvement. Springer Berlin Heidelberg, Berlin, Heidelberg, pp 295-326. doi:https://doi.org/10.1007/978-3-642-29974-2_13
18. Gheibi S, Kashfi K, Ghasemi A (2017) A practical guide for induction of type-2 diabetes in rat: Incorporating a high-fat diet and streptozotocin. Biomedicine & Pharmacotherapy 95: 605-613. doi: https://doi.org/10.1016/j.biopha.2017.08.098
19. Yusuf AJ, Abdullahi MI, Muhammad AA, Ibrahim KG, Wasagu HA, Bräuning FZ, Bangay G, Rijo P (2024) Neocarya macrophylla (Chrysobalanaceae): Review of taxonomy, ethnobotany, phytochemistry and biological activities. Biomedical and biopharmaceutical research: jornal de investigação biomédica e biofarmacêutica 21 (1): 1-22. doi: http://dx.doi.org/10.19277/bbr.21.1.331
20. Shirvani H, Mirnejad R, Soleimani M, Arabzadeh E (2021) Swimming exercise improves gene expression of PPAR-γ and downregulates the overexpression of TLR4, MyD88, IL-6, and TNF-α after high-fat diet in rat skeletal muscle cells. Gene 775: 145441. doi: https://doi.org/10.1016/j.gene.2021.145441
21. Francini F, Castro MC, Schinella G, García ME, Maiztegui B, Raschia MA, Gagliardino JJ, Massa ML (2010) Changes induced by a fructose-rich diet on hepatic metabolism and the antioxidant system. Life Sciences 86 (25): 965-971. doi: https://doi.org/10.1016/j.lfs.2010.05.005
22. Chirumamilla P, Taduri S (2023) Assessment of in vitro anti-inflammatory, antioxidant and antidiabetic activities of *Solanum khasianum* Clarke. Vegetos 36 (2): 575-582. doi: https://doi.org/10.1007/s42535-022-00410-6
23. Pathak S, Fialho J, Nandi D (2022) Plant-based immunomodulators and their potential therapeutic actions. Journal of Exploratory Research in Pharmacology 7 (4): 243-256. doi: http://dx.doi.org/10.14218/JERP.2022.00033
24. Cuevas-Cianca SI, Romero-Castillo C, Gálvez-Romero JL, Juárez ZN, Hernández LR (2023) Antioxidant and anti-inflammatory compounds from edible plants with anti-cancer activity and their potential use as drugs. Molecules 28 (3): 1488. doi: https://doi.org/10.3390/molecules28031488
25. Albaqami NM, Monier MN (2025) Impacts of dietary α-tocopherol acetate on physiological response, antioxidant activity, innate immunity, and histopathological status of Nile tilapia, Oreochromis niloticus under heat and salinity stress. Fish Physiology and Biochemistry 51 (1): 46. doi: https://doi.org/10.1007/s10695-025-01460-8
26. Sindhu RK, Koo JR, Roberts CK, Vaziri ND (2004) Dysregulation of hepatic superoxide dismutase, catalase and

glutathione peroxidase in diabetes: response to insulin and antioxidant therapies. Clinical and experimental hypertension 26 (1): 43-53. doi: https://doi.org/10.1081/CEH-120027330

27. Sözmen EY, Sözmen B, Delen Y, Onat T (2001) Catalase/superoxide dismutase (SOD) and catalase/paraoxonase (PON) ratios may implicate poor glycemic control. Archives of medical research 32 (4): 283-287. doi: https://doi.org/10.1016/S0188-4409(01)00285-5
28. Rains JL, Jain SK (2011) Oxidative stress, insulin signaling, and diabetes. Free radical biology and medicine 50 (5): 567-575. doi: https://doi.org/10.1016/j.freeradbiomed.2010.12.006
29. Singh A, Kukreti R, Saso L, Kukreti S (2022) Mechanistic insight into oxidative stress-triggered signaling pathways and type 2 diabetes. Molecules 27 (3): 950. doi: https://doi.org/10.3390/molecules27030950
30. Andreadi A, Bellia A, Di Daniele N, Meloni M, Lauro R, Della-Morte D, Lauro D (2022) The molecular link between oxidative stress, insulin resistance, and type 2 diabetes: A target for new therapies against cardiovascular diseases. Current opinion in pharmacology 62: 85-96. doi: https://doi.org/10.1016/j.coph.2021.11.010

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