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(RESEARCH ARTICLE)

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Anti-oxidative stress effects of ethyl acetate fraction of *Diaphananthe bidens* Leaf in Streptozotocin-Induced Type-2 Diabetic (T2D) Rats

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Abstract

The research examined the anti-oxidative stress properties of the ethyl acetate fraction derived from *Diaphananthe bidens* leaves in rats with T2D by the analysis of blood glucose levels, lipid profile, lipid peroxidation (MDA), advanced oxidation protein products (AOPP), pro-inflammatory cytokine (tumour necrosis factor-alpha, TNF-α), and cardiovascular biomarkers. The extracts underwent fractionation, and the total phenolic content was quantified. The rodents were administered a high-fat diet over two weeks, followed by an I.P. injection of 50 mg/kg of nicotinamide before induction with 100 mg/kg of streptozotocin to prevent insulin degradation. Animals exhibiting fasting blood sugar levels exceeding 160 mg/dl were selected and categorized into five groups: uninduced (10mg/kg tween 20) and a control group (100mg/kg metformin). The ethyl acetate was administered orally to groups at dosages of 93, 186, and 372 mg/kg daily over 30 days. The measurement of Fasting Blood Sugar was conducted, and the percentage reduction was documented on the 15th and 30th days. Blood samples were obtained for the assessment of lipid profile, MDA, AOPP, TNF-α, and cardiovascular biomarkers, utilizing established methodologies. The high-fat diet leading to the induction of T2D resulted in a significant($P<0.05$) elevation in blood glucose levels, total cholesterol, LDL, oxidized LDL, MDA, AOPP, TNF-α, atherogenic risk index, and atherosclerosis index, while significantly diminishing HDL levels. The administration of the ethyl acetate fraction resulted in a notable significant ($P<0.05$) decreases in the measured parameters and a significant increase in HDL-c levels. The results substantiated the anti-oxidative stress, anti-cholesterolemia and cardioprotective effects of *D. bidens*.

Keywords: Anti-oxidative stress; *Diaphananthe bidens*; TNF-α; AOPP; MDA and cardiovascular biomarkers

1. Introduction

Oxidative stress, characterized by an imbalance between the creation of free radicals and the defense against them, offers a substantial risk to human health. It is responsible for the development and progression of several chronic diseases, including diabetes, neurodegenerative disorders, malignancies, and ageing [1]. Type 2 diabetics, on the other hand, are marked by chronic hyperglycemia, insulin resistance, and oxidative stress, which ultimately leads to cellular damage, inflammation, and problems. Despite standard treatment, patients with type 2 diabetes frequently undergo oxidative stress, which accelerates the course of the disease [2]. Oxidative stress affects an estimated 65 per cent of the world's population[3]. Because the current antioxidant therapies are insufficient, there is a pressing need for a targeted and efficient intervention that uses natural compounds derived from plants and herbs to alleviate the effects of oxidative stress that occur during therapy for type 2 diabetes.

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Eighty (80) percent of herbal remedies show significant anti-oxidant activity to mitigate the effects caused by type-2 diabetics and Scientists are exploring the plants' secondary metabolites to bridge the gap with conventional drugs which have shown deleterious effects [4]. *Diaphanathe bidens*, (*D. bidens*) a family of *Orchardiaceae* is an epiphyte with a tough wire stem that has little medicinal properties such as antiasthmatic, anti-inflammatory and antineoplastic among others [5], but its oxidative stress properties in streptozocin-induced type-2 diabetic Rats has not been well documented hence this research.

2. Material and methods

2.1. Chemicals and Reagents

Streptozotocin and nicotinamide were products of Sigma Aldrich, Germany. Multi-Analyte Perchloric acid (Cyaman chemical, Canada), sodium carbonate (Griffin & George, England), Folin-Ciocalteu's reagent (LobaChemie, India), Hydrogen peroxide (Avondale Laboratories, England), thiobarbituric acid (TBA) (Guangdong Guanghua Chemical Factory Co., Ltd, China). HCL, Potassium dichromate and Potassium ferricyanide were products of Hopkin and Williams Ltd, England. Glutathione peroxidase kit (Bioassay Technology Laboratory, China). Superoxide dismutase, Oxidized low-density lipoprotein, low-density lipoprotein and tumour necrosis factor alpha kits were products of Elabscience Biotechnology Co. Ltd. Freshly prepared distilled water was used when required.

2.2. Plant Material

Taxonomist, Alfred Ozioko collected *Diaphanathe bidens (D. bidens)* leaves in June 2023 from Nsukka, Nigeria. The leaves were cleaned, shade-dried, and pulverized for solvent extraction. The resulting 6.7 kg sample was used for further study.

2.3. Animals

Albino rats (100–150 g) used in the study were obtained from the Animal House of the Department of Pharmacology, Faculty of Pharmaceutical Science, Enugu State University of Science and Technology, Nigeria. The animals ate pelletized feed (Vital Feeds, Nigeria) and had free water. The Pharmacology Animal House housed the animals in standard cages. The animals acclimatized for 7 days before the study. All animal experiments followed the NIH Guide for the Care and Use of Laboratory Animals and were approved by the ESUT Animal Care and Ethics Committee **(ESUT/AEC/0728/AP643).**

2.4. Extraction

For 72 h with intermittent shaking, 6 kg of *D. bidens* leaf powder was cold macerated in 30 L of 70% aqueous ethanol. After filtering, the filtrate was pre-concentrated in vacuo using a rotary evaporator at 40°C and dried to a constant weight in an open water bath at 50°C to obtain the ethanol extract.

2.5. The liquid-liquid Chromatography Fractionation

The ethanol extract (100 g) was dissolved in distilled water and partitioned with 2.5 L of n-hexane, ethyl acetate, and butanol using a separating funnel to obtain the soluble fractions. Following partitioning, the water fraction was taken. These fractions were pre-concentrated by rotary evaporators at 40^{o}C and dried by water baths at 50^{oC}. The water fraction was freeze-dried at -50^{o}C using a Telstar LyoQuest dryer.

2.6. Phytochemical Analysis

The leaf extract and fractions were phytochemically analyzed using standard methods [6, 7].

2.7. Total Phenolic content

The total phenolic content of extracts and fractions was determined using Srivastava *et al* [6] method. One ml of samples (62.5 µg/ml) was mixed with 0.2 ml of Folin-Ciocalteu's phenol reagent. The mixture received 1 ml of 7.6% Na2CO3 solution and 2 ml of distilled water after 5 minutes. After incubating the mixture in duplicate at 40°C for 30 minutes, a *UV-VIS* spectrophotometer (Model 752, China) measured absorbance at 760 nm. Total phenolic content was estimated from the calibrated curve made by preparing the gallic acid solution and expressed as milligrams of gallic acid equivalent (GAE) per gramme of extracts.

2.8. Vacuum Liquid Chromatography of the most bioactive fractions (Ethyl acetate)

Ethyl acetate from D. bidens (1.5 g) was separated by VLC using a 5 L sintered column packed with Silica gel (200–400 mesh) to a 10 cm bed size. The column was eluted with 500 ml of hexane: ethyl acetate (10:0, 9:1, 8:2, 7:3, 6:4, 5:5, 4:6, 3:7, 2:8, 1:9, 0:10) and dichloromethane: Methanol. Total phenolic content was screened in VLC fractions.

2.9. Experimental design

2.9.1. Dosage selection

The doses used in this study were selected from the effective dose of the most bioactive fractions (ethyl acetate, 186 mg/kg), the dose halved (93 mg/kg) and doubled (372 mg/kg) as medium, small and high doses respectively.

2.9.2. High-fat diet feeding

The obesity-mediated insulin resistance of type two diabetes was induced in 60 albino rats pre-fed a high-fat diet. The naive rats (6) were fed a normal diet (ND) of 20% crude protein, 70% carbohydrate, and 10% fat (total caloric energy value = 4057 Kcal/kg: Animal Care feeds, Asaba, Nigeria), while the other 60 were fed a high-fat (HFD) diet of 20% crude protein, 35% carbohydrate, and 45% fat. For 30 days, the animals had free food and water and a Normal or High-fat diet.

2.9.3. Induction of diabetes Type-2

Streptozotocin (STZ) (Sigma Aldrich, Germany) was used to induce diabetes in animals fed a high-fat diet for 30 days, except for the naïve, the beta cells were partially protected from STZ-induced chemical pancreatectomy by injecting 50 mg/kg Nicotinamide (Sigma Aldrich, Germany) intraperitoneally before induction. Streptozotocin (100 mg/kg) was then given intraperitoneally within 15 min, as described by Furman [9]. Physiological saline was intraperitoneally given to non-diabetic rats (Henry Schein Animal Health, Dulin). In 100 ml of the aqueous solution, sodium chloride (0.9 g) and 154 mEq/L sodium and chloride ions were present, with a total osmolarity of 308 mEq/L. One Touch Glucometer (Lifeshield, Johnson & Johnson, California) measured fasting blood glucose concentrations from tail vein blood 72 h post-induction. Diabetes was diagnosed in rats with fasting blood glucose levels above 160 mg/dl. About 30 diabetic rats with blood glucose levels between 250 and 300 mg/dl were chosen for the study.

2.9.4. Animal grouping and administration

The rats were divided into 5 diabetic groups and one un-induced naïve group. Control groups of diabetics received 10 ml/kg 5% Tween 20 and 100 mg/kg metformin. Ethyl acetate was given to the remaining three groups at 93, 186, and 372 mg/kg. High-fat diets were maintained for diabetic animals, while a normal diet was maintained for the naïve control Oral treatment was given daily at 9:00 am WAT for 30 days. We measured fasting blood glucose (FBG) levels again on days 15 and 30 and calculated the percentage reduction for each treatment group.

2.9.5. Blood collection

At the end of the study (31st day), animals' blood was drawn through the retro-orbital plexus using EDTA-containing plasma tubes and plain serum tubes. After 30 minutes to cloth, plain samples were centrifuged for 10 minutes at 3000 rpm. Decanted serum was stored in Eppendorf tubes. EDTA tube samples were centrifuged and the plasma was stored in Eppendorf tubes.

2.9.6. Determination of lipid profile

The lipid profile (total cholesterol, and low-density lipoproteins) was assayed using standard methods as described by Kumari *et al* [10] and oxidized low-density lipoproteins were assayed using an ELISA kit procedure as Tsimikas *et al* [11].

2.9.7. Determination of Advanced Protein Oxidation products (APOP)

To measure APOP, plasma samples were diluted to 10% in PBS and 200 µL was placed in a 96-well microplate. Standards of chloramine T (200 µL; 0–100 µM) were added to the plate. KI (1.16 M, 10 µL) was added to each well, followed by a bolus addition of 20 µL glacial acetic acid 2 minutes later. The optical density (OD340) was measured immediately at 340 nm using a microplate reader. APOP concentrations were stated as µM/L Chloramine-T equivalents as Witko-Sarsat et al [12] described.

2.9.8. Determination of Tumour Necrotic factor-alpha (TNF-α)

The rat-specific TNF-α assay kit (Elabscience Biotechnology Co. Ltd., China) was used to estimate serum TNF-α activity using a quantitative sandwich enzyme immunoassay (ELISA) technique, as described by *Abdulaziz et al* [13].

2.9.9. Determination of Lipid peroxidation (Malondialdehyde. MDA)

The modified thiobarbituric acid method of Draper and Hadley as described by Anookpumar-Drukie *et al*[14] was used to estimate serum MDA using a malondialdehyde assay kit (Elabscience Biotechnology Co. Ltd., South Africa) and absorbance read at 532nm.

2.10. Determination of Cardiovascular biomarkers (Atherogenic risk index and Atherosclerosis index)

The atherogenic risk index and atherosclerosis index were estimated as described by Niroumad *et al* [15].

2.11. Statistical Analysis

The study, analyzed results using ANOVA, Turkey's test, and Microsoft Excel 2010, with statistical significance set at P values less than 0.05. ED_{50} concentrations were derived from the regression equation.

3. Results and discussion

3.1. Phytochemical analysis of *Diaphananthe bidens* (*D. bidens*) leaf

The phytochemical analysis of *Diaphananthe bidens* revealed the presence of different secondary metabolites as shown in Table 1.

Table 1 Phytochemical analysis and total phenolic content of the extract and fractions

Phytocompounds	Extract	N-Hexane Fraction	Ethyl acetate Fraction	Butanol Fraction	Water Fraction
Flavonoids	+	-	+	+	+
Saponins	+	+	-	+	+
Tannins	+	+	+	+	+
Reducing sugar	+	-	+	+	+
Steroids	+	+	-	+	-
Terpenoids	+	-	-	+	-
Alkaloids	+	-	+	+	+
Glycosides	+	-	+	+	+

+ Present, - absent.

3.2. The total phenolic content of *D. bidens* leaf

The total phenolic content of *D. bidens* leaf showed ethyl acetate fraction with the most phenolic content at 328.2 with 40.32% while N-hexane with the lowest yield as shown in Figure 1.

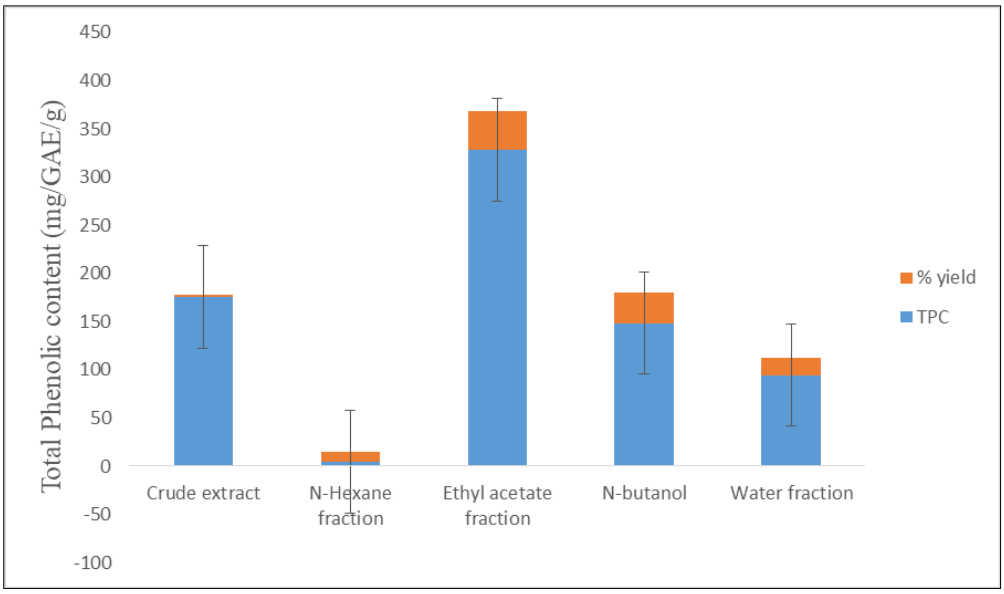
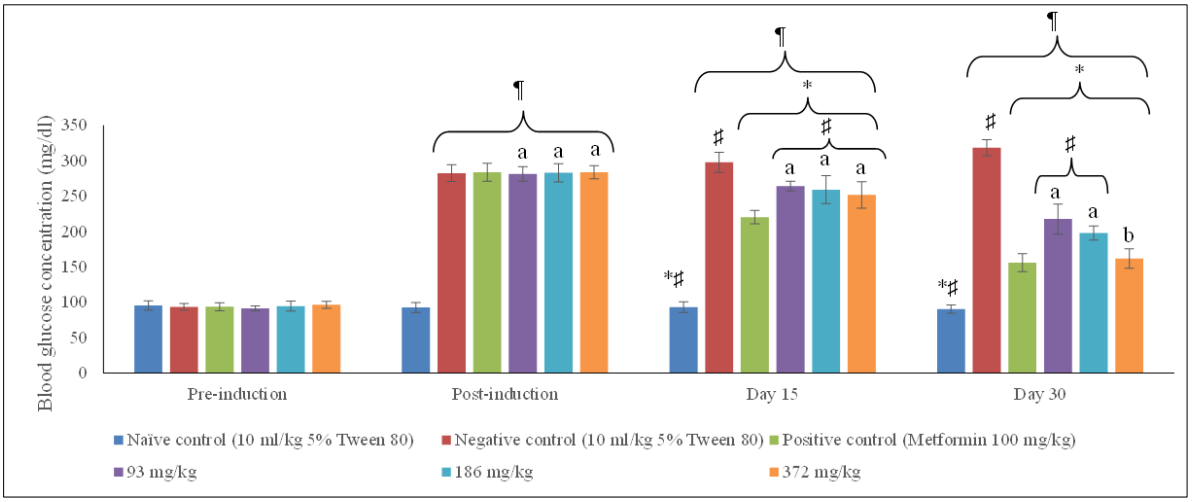


Figure 1 Total Phenolic content of *Diaphananthe bidens* Leaf extract and fractions

3.3. Effect of the ethyl acetate fraction on blood glucose in diabetes-induced chronic oxidative stress

High-fat diet streptozotocin (STZ) administration produced an increase in blood glucose concentration as seen from the significant (P<0.05) difference between the post-inductive and pre-inductive blood glucose concentration (figure 2). A similar level of blood glucose was maintained in all the groups before treatment. Ethyl acetate fraction at all tested treatment doses (93, 186 and 372 mg/kg) produced a significant (P<0.05) reduction in blood glucose concentration compared to the negative (vehicle) control group. No significant (P>0.05) difference was recorded in the effect produced by various doses of the ethyl acetate fraction when compared with each other on Day 15 of administration. However, the 372 mg/kg dose showed a significantly (P<0.05) higher effect compared to the other two lower doses on day 30 of treatment. Similarly, the effect produced by 372 mg/kg of the ethyl acetate fraction was comparable to that of the reference drug with no significant (P>0.05) difference between both groups on day 30.



Where: ¶ P<0.05 compared to naïve control; * P<0.05 compared to vehicle control group; # P<0.05 compared to Reference drug – Metformin 100 mg/kg; different letter alphabet (a,b, c) P<0.05 compared to the doses of the ethyl acetate

Figure 2 Effect of ethyl acetate fraction of *D. bidens* on blood glucose in diabetes-induced chronic oxidative stress

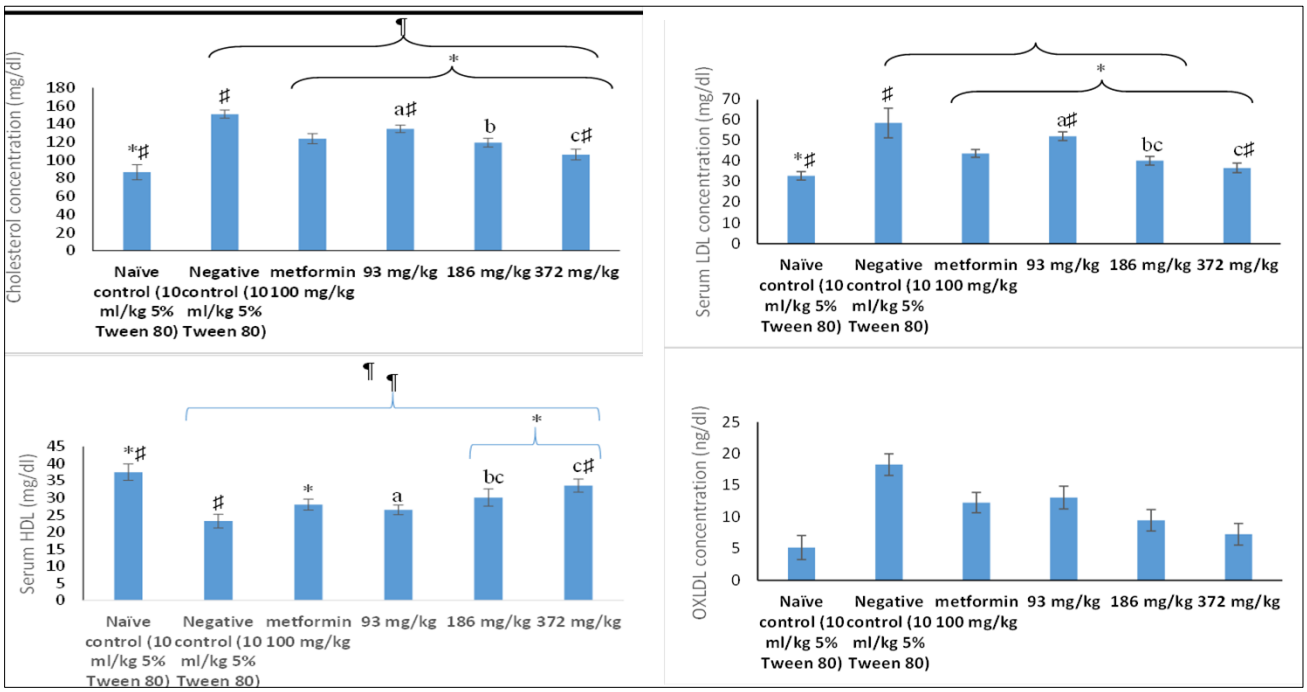
3.4. Effect of the ethyl acetate fraction on lipid profile in diabetes-induced chronic oxidative stress

High-fat diet STZ-induced diabetes produced an increase in serum total cholesterol, low-density lipoprotein (LDL) cholesterol, and oxidized LDL cholesterol with a reduction in high-density lipoprotein (HDL) cholesterol compared to the naïve control group. Treatment with the ethyl acetate fraction at all tested doses produced a significant (P<0.05)

reduction in total cholesterol concentration compared to the vehicle control group. A similar effect was seen between 186 mg/kg of the ethyl acetate fraction and the reference drug with no significant differences. However, at 372 mg/kg, a significantly (P<0.05) lower serum cholesterol concentration was recorded compared to the reference standard. Similarly, the ethyl acetate fraction at 186 and 372 mg/kg dose produced a significant (P<0.05) increase in serum HDL compared to the vehicle control group. Non-significant (P>0.05) difference existed between the effect produced by 186 mg/kg dose of the ethyl acetate fraction and the reference standard while 372 mg/kg dose produced a significantly (P<0.05) better effect compared to the reference drug.

The increase in serum LDL produced by high-fat diet STZ was significantly (P<0.05) lowered by all the tested doses of ethyl acetate fraction. Comparable effects were produced by the reference drug and 186 mg/kg dose of the ethyl acetate fraction while a significantly (P<0.05) higher reduction was achieved after treatment with 372 mg/kg dose of the ethyl acetate fraction compared to the reference drug. Of all the treatments, only 372 mg/kg dose of the ethyl acetate fraction was able to restore serum LDL concentration to a similar value obtained in the naïve uninduced control group.

Just as in LDL concentration, elevated oxidized LDL concentration was also significantly (P<0.05) reduced by the 3 tested doses of the ethyl acetate fraction. A similar effect was also produced by 186 mg/kg dose of the ethyl acetate fraction compared to the effect produced by the reference drug while 372 mg/kg dose maintained a significantly (P<0.05) better effect compared to the reference drug. Also similar value was recorded for the 372 mg/kg ethyl acetate treated group compared to the naïve control group.



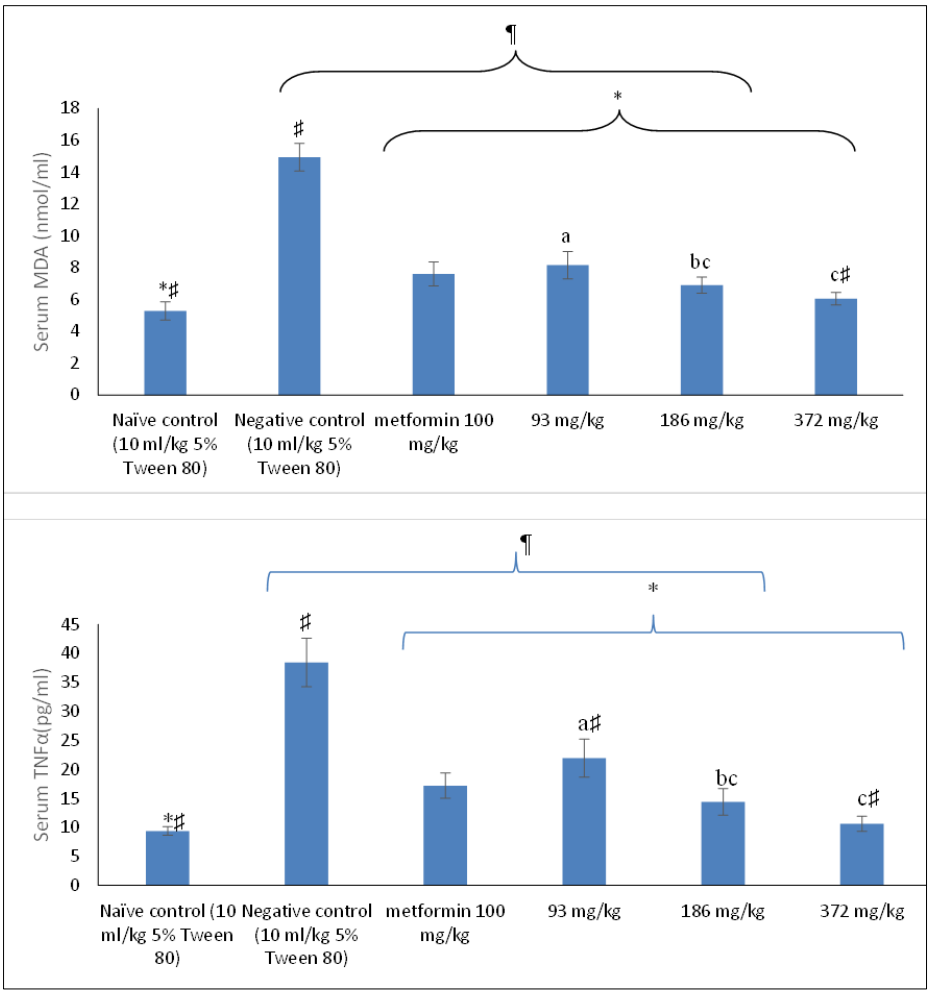
Where: ¶ P<0.05 compared to naïve control; * P<0.05 compared to vehicle control group; # P<0.05 compared to Reference drug – Metformin 100 mg/kg; different letter alphabet (a,b, c) P<0.05 compared to the doses of the ethyl acetate

Figure 3 Effect of ethyl acetate fraction of *D. bidens* on lipid profile in diabetes-induced chronic oxidative stress

3.5. Effect of the ethyl acetate fraction on lipid peroxidation and proinflammatory cytokine in type-2 diabetes-induced chronic oxidative stress

High-fat diet STZ-induced diabetes led to increased lipid peroxidation shown by a significant (P<0.05) increase in serum concentration of malondialdehyde (MDA) in negative control group animals compared to naïve uninduced control (figure 4). However, treatment with the ethyl acetate fraction of *D. bidens* produced a dose-related significant (P<0.05) reduction in serum MDA concentration compared to the vehicle control group. When compared to the reference drug (100 mg/kg metformin), 93 and 186 mg/kg doses of the ethyl acetate showed comparable effect with no significant difference. The higher dose of the ethyl acetate fraction (372 mg/kg) produced significantly (P<0.05) better effect compared to the reference standard. This dose was also able to revert MDA concentration to a similar value recorded in the naïve uninduced control group.

Diabetes induction also significantly (P<0.05) elevated serum concentration of inflammatory cytokine - tumour necrosis factor-alpha (TNF-α) (figure 4). The same trend of effect exhibited by the ethyl acetate fraction against lipid peroxidation was reproduced in serum TNF-α.

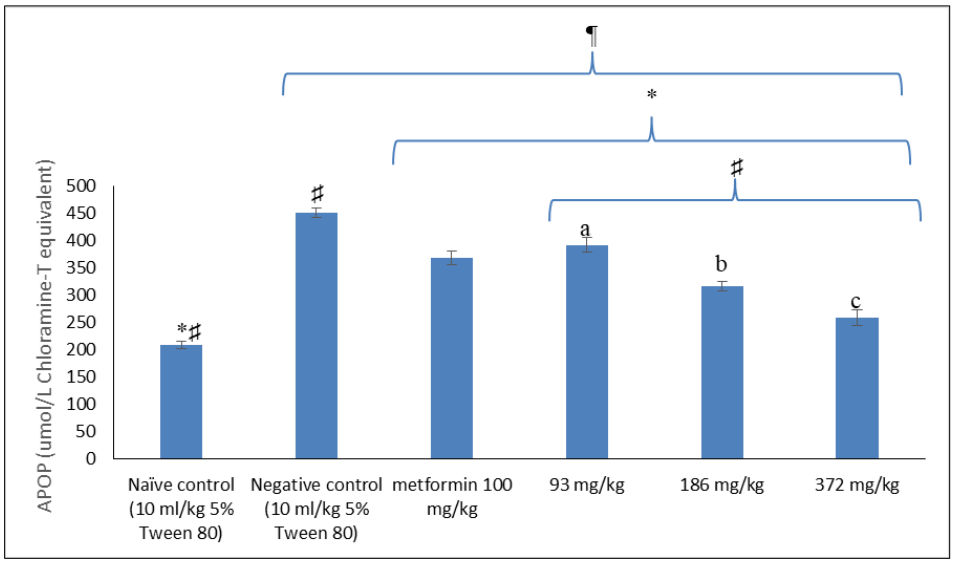


Where: ¶ P<0.05 compared to naïve control; * P<0.05 compared to a vehicle control group; # P<0.05 compared to Reference drug – Metformin 100 mg/kg; different letter alphabet (a,b, c) P<0.05 compared to the doses of the ethyl acetate.

Figure 4 Effect of ethyl acetate fraction of *D. bidens* on lipid peroxidation and serum tumour necrosis factor (TNF-α) in diabetes-induced chronic oxidative stress

3.6. Effect of the ethyl acetate fraction on advanced protein oxidative product (APOP) in diabetes-induced chronic oxidative stress

Advanced protein oxidative product was significantly (P<0.05) elevated by the high-fat diet STZ-induced oxidative stress (figure 5). Treatment with graded doses of the ethyl acetate fraction produced a significant (P<0.05) reduction in APOP compared to the negative (vehicle) control group. The effects produced by 186 and 372 mg/kg were significantly better when compared to the reference standard (metformin 100 mg/kg).



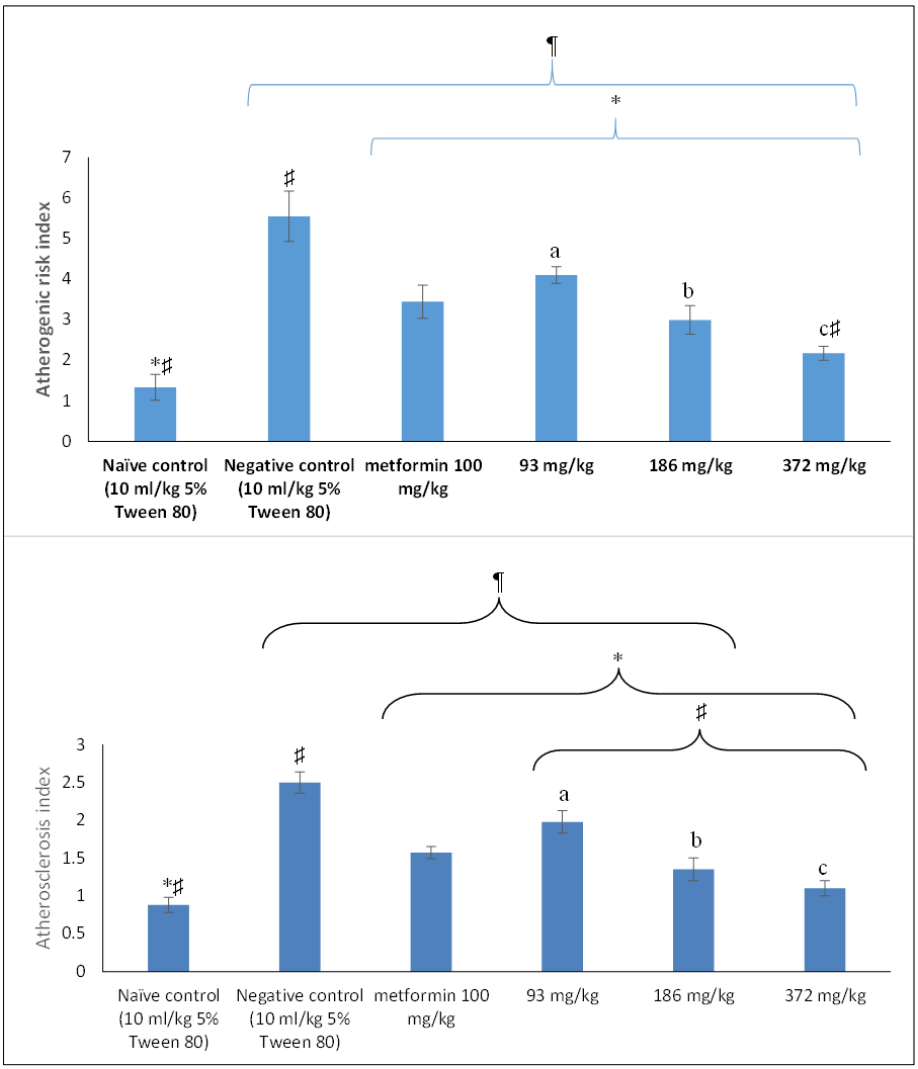
Where: ¶ P<0.05 compared to naïve control; * P<0.05 compared to a vehicle control group; # P<0.05 compared to Reference drug – Metformin 100 mg/kg; different letter alphabet (a,b, c) P<0.05 compared to the doses of the ethyl acetate

Figure 5 Effect of ethyl acetate fraction of *D. bidens* on Advanced Protein Oxidative Product in diabetes-induced chronic oxidative stress

3.7. Effect of the ethyl acetate fraction on atherogenic risk and atherosclerosis indices in diabetes-induced chronic oxidative stress

High fat diet STZ-induced diabetes was accompanied by a significant increase in atherogenic risk index (figure 6). Just like as metformin, treatment with 93 and 186 mg/kg doses of the ethyl acetate fraction produced a significant (P<0.05) decrease in the atherogenic risk index in a dose-related manner. The higher dose of the ethyl acetate fraction (372 mg/kg) produced a significantly (P<0.05) better reduction in the atherogenic risk index compared to the reference standard.

Similarly, a significant elevation of the atherosclerosis index was produced by high-fat diet STZ-induced diabetes (figure 6). Treatment with the ethyl acetate fraction produced a graded dose-related effect that was significant (P<0.05) compared to the negative (vehicle) control group. Higher doses of the ethyl acetate fraction 186 and 372 mg/kg produced a significantly better effect compared to the reference standard (metformin 100 mg/kg) while 372 mg/kg dose showed an atherosclerosis index similar to that of naïve uninduced control.



Where: ¶ P<0.05 compared to naïve control; * P<0.05 compared to a vehicle control group; # P<0.05 compared to Reference drug – Metformin 100 mg/kg; different letter alphabet (a,b, c) P<0.05 compared to the doses of the ethyl acetate.

Figure 6 Effect of ethyl acetate fraction of *D. bidens* on Atherogenic risk index and Atherosclerosis index in diabetes-induced chronic oxidative stress

4. Discussion

This investigation assessed the impact of oxidative stress induced by the ethyl acetate fraction of *Diaphananthe bidens* (*D. bidens*) in albino rats with type-2 diabetes. The evaluation involved measuring lipid peroxidation (MDA), lipid profile parameters, advanced protein oxidative products, and pro-inflammatory cytokines (specifically tumour necrosis factor-alpha), alongside the atherogenic risk index and atherosclerosis index following streptozotocin induction and the oral administration of a high-fat diet. The effective dose (ED50) of the fraction is determined to be 186 mg/kg, utilizing levamisole as the standard, which was the selected dose for the study. The percentage yield and total phenolic content of the fraction are 40.32% and 328.2, respectively, indicating that the ethyl acetate fraction of the plant encompasses non-polar secondary metabolites. The phytochemical analysis indicated the existence of tannins, saponins, terpenoids, glycosides, and alkaloids in varying proportions. The presence of these secondary metabolites is supported by the works of Aba *et al* [5] and Onyegbule *et al* [16].

The oral administration of high-fat diet-induced streptozotocin elevated the animals' blood glucose levels. The elevation causes hyperglycemia due to insulin resistance, which hinders the conversion of glucose into its stored form, glycogen. This, in turn, leads to polyuria, dehydration, fatigue, and blurred vision. The research indicated that the administration of streptozotocin in conjunction with a high-fat diet significantly ($P<0.05$) increased blood glucose concentrations. The administration of the ethyl acetate fraction at varying doses led to a noteworthy reduction ($P<0.05$) in blood glucose levels when contrasted with the control group. Nonetheless, the 372 mg/kg dosage exhibited a more pronounced effect

(P<0.05) by the 30th day, with its impact paralleling that of the reference drug (metformin). The reduction in blood glucose levels is supported by [17- 20].

Lipid peroxidation represents a complex chain reaction initiated when a free radical seizes an electron from a lipid molecule. This process generates a fatty acid radical, which subsequently engages with additional fatty acids, leading to the formation of lipid peroxides and an increase in fatty acid radicals [21]. Malondialdehyde (MDA) is widely recognized as an indicator of oxidative stress, representing a terminal product of the peroxidation of polyunsaturated fatty acids within cells [22]. An elevation in free radicals, stemming from both endogenous and exogenous stressors, leads to the excessive production of MDA [23]. High-fat diets causing diabetes through STZ resulted in higher lipid peroxidation and increased serum malondialdehyde levels (P<0.05). However, the ethyl acetate fraction of *D. bidens* reduced MDA levels significantly (P<0.05). Dosages of 93 and 186 mg/kg showed similar effects, with higher doses, 372 mg/kg having a stronger effect. The higher dose successfully restored MDA concentration to a naïve control group compared to the reference drug (metformin 100mg/kg). These findings are consistent with Abou-Khalil *et al*(2), Baffoe *et al* [24] and Al-Chalabi *et al* [25]. The decrease in lipid peroxidation biomarkers attributed to the ethyl acetate fraction of *D. bidens* may be due to the presence of secondary metabolites such as tannins, which have been documented to exhibit antioxidative effects on serum MDA levels[26, 27].

The elevation of oxidation processes affecting proteins, lipids, carbohydrates, and DNA occurs when the production of reactive oxygen species surpasses the local capacity for antioxidants. The oxidation of proteins is fundamentally significant in the development of numerous degenerative diseases[28]. Advanced oxidation protein product (AOPP) represents a novel biomarker for oxidative stress, comprising cross-linked products that arise from the oxidation of albumin and proteins [12]. Elevated concentrations of AOPP have been observed in conditions such as diabetes, cardiovascular diseases, hypertension, and atherosclerosis[9]. They exhibit structural similarities to advanced glycation end-products (AGE) and have also shown biological activity, as indicated by their capacity to trigger inflammatory cytokines and binding molecules [30]. AOPP levels were significantly (P<0.05) increased in mice obese groups (High-fat diet group). When treated with the ethyl acetate fraction, the *D. bidens* caused a significant (P<0.05) decrease in a like manner as malondialdehyde. This position is supported by Kalousova *et al* [31], Bagyura *et al* [32] and Kar [33]. The decrease in the serum levels of AOPP could be a result of secondary metabolites present in the plant extract. Polyphenols present in the ethyl acetate fraction have been shown to possess antioxidant properties, thus slowing the pathogenesis of degenerative diseases [34].

Cardiovascular indices are crucial for the early detection and management of atherosclerotic cardiovascular disorders in individuals with type-2 diabetes [35]. The Atherogenic Index of the blood and other lipid profiles showed that diabetics have significantly higher levels of total cholesterol, triglycerides, low-density lipoprotein cholesterol (LDL-c), and oxidized low-density lipoprotein cholesterol (ox-LDL-c), which can lead to atherosclerotic plaques and obstructing arteries. High-density lipoprotein cholesterol is lower. LDL-c is a type of lipid resulting from abnormal lipid metabolism, often linked to high-fat diets, sedentary lifestyles, and metabolic disorders like diabetes [20]. Hyperglycemia can lead to diabetic dyslipidemia and cardiovascular complications[36]. This leads to elevated TC, LDL-c, oxidized LDL-c levels and reduced HDL-c levels. Insulin resistance can also cause decreased hormone-sensitive lipase activity[37]. Treatment with the ethyl acetate fractions caused significant (P<0.05) reductions in all the cardiovascular and lipid profile parameters measured in a dose-dependent manner. These reduction effects on the atherogenic risk index and lipid profile could be the presence of phytochemical constituents in the fraction. This, in turn, results in an enhanced degradation of intracellular triglycerides and the subsequent liberation of free fatty acids into the bloodstream. A positive correlation exists between elevated free fatty acids and enhanced hepatic triglyceride synthesis, ultimately resulting in increased blood levels of very low-density lipoproteins. The remodeling of low-density lipoproteins and high-density lipoproteins by various lipases leads to enhanced renal clearance of HDL-c, which consequently results in reduced levels of HDL-c in the bloodstream [38].

The findings of this research indicate that the ethyl acetate fraction derived from *D. bidens* leaves offers advantages for animals subjected to a high-fat diet in the context of type-2 diabetes, particularly by enhancing lipid profiles and mitigating the atherogenic risks associated with diabetes. This is supported by previous findings [39-41].

Tumour necrosis factor-alpha (TNF-α) stands as a pivotal pro-inflammatory mediator. It is primarily produced by adipocytes and/or peripheral tissues, and it triggers inflammation in specific tissues through the generation of reactive oxygen species and the activation of various intermediate transcriptional pathways [13]. When TNF-α is consistently elevated, it activates insulin resistance in both peripheral tissues and adipocytes by obstructing insulin signalling through serine phosphorylation. This is a cytokine that is released by cells experiencing chronic inflammation. The immune system generates it [42]. The function of cytokines in facilitating the synthesis of acute-phase proteins is widely recognized. The elevation of inflammatory biomarkers can be attributed to the dual influences of obesity and

hyperglycemia [43]. Following the oral treatment of obese rats which had elevated pro-inflammatory cytokine TNF-α, the ethyl acetate fraction of *D .bidens* significantly (P<0.05) caused a reduction in TNF-α levels in a dose-dependent manner. This reduction caused enhanced glucose absorption in the peripheral blood stream evidenced in the reduction of blood glucose levels in treated groups suggesting that TNF-α is pivotal in the pathogenesis of insulin resistance and diabetes. This position is supported by Owumi *et al* [44]; Ren *et al* [45] and Xin *et al* [46]. The plant ethyl acetate secondary metabolites could be promising ingredients that modulate pro-inflammatory cytokines and enhance glucose uptake in high-fat diet mice.

5. Conclusion

The findings from the anti-oxidative stress of ethyl acetate fraction of *Diaphanathe bidens* leaves in T2D-induced indicate the plant possesses anti-oxidative stress biomarkers, anti-diabetic, anti-cholesterolemia and cardio-protection due to the presence of some secondary metabolites like Tannins and flavonoids.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest is to be disclosed.

Statement of ethical approval

Anti-oxidative stress effects of ethyl acetate fraction of *Diaphananthe bidens* leaf in type-2 diabetic-induced rats were approved by the Enugu State University of Science and Technology Animal Care and Ethics Committee (ESUT/AEC/0728/AP643)

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