Original Research Article

Synergistic Hypoglycemic Effect of *Kalanchoe pinnata* and Metformin in Oxidative Stress Management in Diabetes

ABSTRACT

Background: Diabetes mellitus is a chronic metabolic disorder characterized by persistent hyperglycemia and associated oxidative stress. This study aimed to evaluate the effects of aqueous extract of *Kalanchoe pinnata* and metformin, individually and in combination, on glucose levels, oxidative stress biomarkers, and pancreatic histology in alloxan-induced diabetic Wistar rats. **Methods**: Thirty male Wistar rats were divided into five groups (n=6): normal control, alloxan-induced positive control (metformin only), *K. pinnata*-treated, untreated diabetic control, and a combination of *K. pinnata* and metformin. Diabetes was induced using alloxan (150 mg/kg), and treatments were administered orally for 28 days. Blood glucose levels, body weights, serum antioxidant activities (SOD, CAT, GSH), lipid peroxidation (TBARS), and histological changes in the pancreas were assessed. Phytochemical analysis of *K. pinnata* extract was conducted. **Results**: Phytochemical analysis showed reductions in oxalates (0.97% to 0.40%), flavonoids (3.01% to 1.27%), and alkaloids (1.94% to 0.49%) post-extraction. Blood glucose levels in the K. pinnata-treated group decreased from 16.47±1.08 mmol/L on Day 1 to 14.83±1.47 mmol/L on Day 28, and in the combination group from 17.12±0.93 mmol/L to 15.27±1.35 mmol/L, but differences were not significant ($p>0.05$). Oxidative stress markers showed minor increases in SOD and CAT activity in the K. pinnata-treated group, but the changes were not significant. TBARS levels slightly reduced in both the K. pinnata and combination groups. Histological analysis showed severe β-cell destruction in the untreated diabetic group, mild restoration in the K. pinnata group, and moderate preservation in the metformin group, with slight improvements in the combination group, but no significant additive effects. **Conclusion**: While *K. pinnata* exhibited slight improvements in glucose levels, oxidative stress biomarkers, and partial histological recovery, the observed changes were not statistically significant. Further research using higher doses or longer treatment durations may better elucidate its therapeutic potential.

Keywords: Diabetes mellitus, *Kalanchoe pinnata*, alloxan, metformin; glucose excursion, oxidative stress, pancreas histology

INTRODUCTION

Diabetes mellitus (DM) is a serious chronic condition that affects how the body processes blood sugar, leading to persistently high glucose levels. It arises from issues with insulin production, insulin action, or both, disrupting the normal metabolism of carbohydrates, fats, and proteins. Over time, uncontrolled diabetes can result in devastating complications, including blindness, kidney failure, nerve damage, and heart disease (American Diabetes Association, 2023). These complications are worsened by oxidative stress, a harmful process in which excess free radicals damage cells and overwhelm the body's natural antioxidant defenses (Sharifi-Rad *et al*., 2020).

DM is a growing public health challenge worldwide, affecting over 422 million people as of 2019, and this number is expected to rise to 643 million by 2045 (World Health Organization, 2018; WHO, 2024; Magliano *et al*., 2021). Low- and middle-income countries (LMICs) bear the greatest burden, with inadequate healthcare infrastructure making it difficult to manage the disease effectively. In Nigeria, the situation is particularly concerning. Approximately 13.9% of adults in the country are living with undiagnosed diabetes, according to the Nigeria Centre for Disease Control (2020). This means millions are unknowingly managing symptoms without proper medical intervention, which can worsen their condition and place additional strain on the healthcare system. The rapid urbanization seen in Nigeria, coupled with a shift toward sedentary lifestyles and diets high in calories but low in nutrients, has contributed significantly to the rising prevalence of diabetes in the population (Pastakia *et al*., 2017; Uloko *et al*., 2018).

Urbanization and westernization have significantly influenced the rise in diabetes prevalence globally. The shift toward sedentary lifestyles, high-calorie diets rich in processed and sugary foods, and reduced physical activity have emerged as major contributors to the development of type 2 diabetes. These changes in human behavior, combined with genetic predispositions, create an environment where insulin resistance and hyperglycemia are more likely to occur (Galaviz *et al*., 2015; Uusitupa *et al*., 2019). At the cellular level, DM is driven by complex mechanisms, with oxidative stress playing a central role. High blood sugar levels cause an overproduction of reactive oxygen species (ROS), which damages cells and tissues, promotes inflammation, and accelerates the development of complications like kidney disease and cardiovascular dysfunction (Pizzino *et al*., 2017).

Managing diabetes is challenging, especially in LMICs where access to care and medication is often limited. Standard treatment typically involves lifestyle changes, oral medications, or insulin injections. However, many synthetic drugs are expensive and often beyond the reach of those living in resource-limited settings (Singh *et al*., 2015; Wan and Mohd, 2022). In addition to the financial burden, these medications can cause side effects such as gastrointestinal discomfort, hypoglycemia, or even long-term organ damage (Maruthur *et al*., 2016; WHO, 2018). As a result, there is growing interest in alternative approaches that are more affordable and accessible.

Medicinal plants and herbal remedies have been used for centuries in traditional medicine and are seen as valuable options in managing diabetes, particularly because they are readily available and cost-effective. Moreover, they can be used alongside synthetic drugs, potentially enhancing therapeutic outcomes while reducing the dose and side effects of standard medications (Kooti *et al*., 2016; Ota & Ulrih, 2017). One such plant with great potential is *Kalanchoe pinnata* (*K. pinnata*), commonly known as the "Miracle Leaf." Native to tropical regions and widely used in Nigerian traditional medicine, this succulent plant has long been valued for its healing properties. It has been used to treat conditions such as infections, wounds, and inflammation (Quazi *et al*., 2011; Assis de Andrade *et al*., 2023). Studies have shown that *K. pinnata* contains a wealth of bioactive compounds, including flavonoids, alkaloids, and phenolics, which possess antioxidant, anti-inflammatory, and hypoglycemic effects (Rajsekhar *et al*., 2016; Gautam *et al*., 2023; Ehi-Omosun & Etunim, 2023).

Despite its traditional use, there is limited scientific evidence examining the effectiveness of *K. pinnata* in managing diabetes, especially in relation to blood sugar control and oxidative stress. This knowledge gap highlights the need for further research to better understand its antidiabetic potential. Exploring this plant's properties through carefully designed studies using diabetic models could pave the way for its integration

into affordable and sustainable diabetes management strategies, particularly in resource-constrained settings like Nigeria.

MATERIALS AND METHODS

Experimental Animals

Animals were maintained at the Animal House of Biomedical Research Center of the University of Port Harcourt. A total of 30 Albino male Wistar rats (*Mus musculs*) were used in the study, which were purchased in the Animal House. The age of animals were 4-6 months, and they weighed 154 grams. Rats were kept for one week for acclimatization before being used in the experiments. They were divided into groups, and each group was housed in separate transparent plastic cages with stainless steel cover lids. The animals were maintained at a temperature of 20-25°C, and they had free excess to food (standard pellets) and water throughout the experimental work.

Plant Collection and Identification

The plant *K. pinnata* (miracle leaf) leaves were obtained from horticultural garden in Port Harcourt, Rivers State, Nigeria. The plant part was identified at the Department of Plant Science and Biotechnology, University of Port Harcourt Rivers State, Nigeria.

Preparation of Extract

Fresh leaves of *K. pinnata* were harvested, cleared and weighed to obtain the fresh weight. Leaves were dried in the hot air oven to eliminate any form of water. The dried leaves were grounded into fine powder to increase surface area and weighed. The powdered form was mixed with distilled water by dissolving 225.977 gram of the power in 1600 ml of distilled water. The mixture was then allowed to sleep for 24 hours to facilitate the extraction of active ingredients. The liquid was subsequently filtered to separate the solid residues from the extract. The resultant extract was stored in a glass container and kept in refrigerator for further analysis or use.

Quantification of Plant Phytochemicals

The phytochemical constituents in the leaf extract of *K. pinnata* were examined using established methodologies: flavonoids were determined by the method of Boham and Kocipaiabyazan (1974), involving repeated extraction with 80% aqueous methanol and drying the filtrate to a constant weight; alkaloids were analyzed using Harborne's (1973) method as described by Uahomo *et al*. (2022), which included extraction with acetic acid, precipitation with ammonium hydroxide, and subsequent drying and weighing of the residue; saponins were quantified by gravimetric extraction, where the defatted sample was extracted with methanol, evaporated to dryness, and weighed; cyanogenic glycosides were measured by hydrolysis and titration with silver nitrate; oxalates were assessed through acid extraction, heating, and titration with potassium permanganate; tannins were determined using a Folin-Denis reagent-based colorimetric method, where optical density was measured at 760 nm, and a calibration curve was used to calculate tannic acid content.

Drugs and reagents

The drugs metformin was purchased from Green House Pharmacy located within the University of Port Harcourt. In order to prepare the powder for administration to the test animals, the tablets were crushed into a fine powder and the proper concentrations produced in distilled water. Alloxan was also bought from the same pharmacy to induce diabetes in rats. Distilled water was used as a vehicle for the preparation of the alloxan for intraperitoneal administration.

Experimental design

Induction of diabetes

Diabetes was induced in 24 out of the 30 Wistar rats (with 6 serving as control) by allowing the Wistars to fast overnight and were treated with 150mg/kg of alloxan by intraperitoneal injection (Akoko *et al*., 2022). After 96 hours of alloxan administration, animals with blood glucose values of 11.1 mmol/L and above were considered diabetic. Glucose levels were monitored using a handheld glucometer (Accu-CHEK) to test blood samples taken from the tail vein.

Animal grouping and treatment

The experiment was designed to assess the effect of a single dose (31 mg/kg) of aqueous extract of *K. pinnata* on glucose excursion and oxidative stress in alloxan-induced diabetic Wistar Rats. The plant extracts were given orally using gavage needle as a single dose (1 ml) per day for 28 days, and then the rats were sacrificed on day 28 for laboratory assessments. Thirty (30) male Wistar Rats were used in this study and divided into five groups of six rats each (n=6) as presented in the Table below;

Table 1. Experimental design

Group	Identification	Treatment
Group A	Normal control	Wistar rats without treatment
Group B	Alloxan-induced + Positive control (Metformin only)	Alloxan-induced diabetic Wistar rats treated with metformin only per day for 28 days
Group C	Alloxan-induced + *K. pinnata*	Alloxan-induced diabetic Wistar rats treated with 31 mg/kg aqueous extract of *K. pinnata* per day for 28 days
Group D	Negative control	Alloxan-induced diabetic Wistar Rats without treatment for 28 days
Group E	Alloxan-induced + *K. pinnata* + Metformin	Alloxan-induced diabetic Wistar rats treated with 31 mg/kg aqueous extract of *K. pinnata* and 150mg/kg of metformin per day for 28 days

The rats in group B and group E were orally administered 150mg/kg of metformin daily for 28 days, following a safe oral dose of metformin for rats as reported by Quaile *et al*. (2010).

All 5 groups of rats were sacrificed on day 28 of treatment after 12 hours of fasting and given a chloroform anesthetic. Blood was collected by cardiac puncture into heparinized sample bottles for biochemical assays.

Determination of blood glucose level

Blood glucose levels were measured using commercially available glucose measurement strips read by the Accu-CHEK Active system, which requires 1–2 μL of blood per test and delivers results in approximately

5 seconds. The test operates on a glucose dye oxidoreductase mediator reaction, where the sensitive chemicals on the test strip react with blood glucose, resulting in a color change on the test area. This color change is detected by the meter, which converts the signal into a blood glucose result.

Measurement of serum antioxidants activities and oxidative stress

Plasma levels of total antioxidant status (TAS) were determined using the DPPH (1,1-diphenyl-2-picryl hydrazyl) method (Bondet, Brand-Williams, & Berset, 1997), while lipid peroxidation was assessed by measuring thiobarbituric acid reactive substances (TBARS) produced during the process (Ohkawa, Ohisi, & Yagi, 1979). Glutathione (GSH) levels were measured using the method of Beutler, Duron, and Kelly (1963), and plasma activities of superoxide dismutase (SOD) were evaluated following the method of Misra and Fridovich (1972), with absorbance measured at 480 nm. Glutathione peroxidase (GPx) activity was assayed using the method of Reddy, Subhani, Khan, and Kumar (1995), where H_2O_2 is converted to H_2O and O_2 in the presence of the hydrogen donor pyrogallol or dianisidine, producing a colored product that was measured colorimetrically at 430 nm. Catalase (CAT) activity was determined using the direct colorimetric method of Sinha (1971), based on the reduction of dichromate to chromic acetate in the presence of H_2O_2, with the chromic acetate quantified at 570 nm.

Histological examination

The animals were anesthetized with chloroform and dissected aseptically to remove the pancreas, which was then transferred into 10% chloroform solution. The tissues were trimmed to a thickness of 2–4 mm to facilitate optimal penetration of the fixative. Standard histological processing methods, as described by Baker (1945) and Isirima and Uahomo (2023), were employed, including fixation, dehydration, clearing, impregnation, embedding, sectioning, and staining with hematoxylin and eosin (H&E), followed by final mounting.

Statistical Analysis

The results are presented as Mean ± Standard error of mean. Differences between means were assessed using one-away analysis of variance (ANOVA) using Dunnett post hoc method to assess any significant differences between the groups. Differences between groups at $p<0.05$ were considered to be statistically significant.

RESULT

Phytochemical concentration of *K. pinnata* and the effect of *K. pinnata* and metformin on blood glucose levels and oxidative stress markers

Table 2 shows the concentration of phytochemicals in fresh and aqueous extracted samples of *K. pinnata*. The results indicate a decrease in oxalate content from 0.97% before extraction to 0.40% after extraction, a reduction in flavonoid content from 3.01% to 1.27%, and a decline in alkaloid levels from 1.94% to 0.49%. Tannin levels were reduced from 11.15 mg/kg to 9.23 mg/kg, while cyanogenic glycoside concentrations decreased from 4.00 mg/kg to 2.88 mg/kg. Saponin content also showed a decrease, from 2.37% to 1.81% after extraction.

Table 3 presents the blood glucose levels in alloxan-induced diabetic Wistar rats treated with aqueous extract of *K. pinnata* and metformin. On Day 1, the normal control group (Group A) had a blood glucose level of 4.13±0.41, which slightly increased to 4.28±0.45 on Day 21 and 4.97±0.23 on Day 28. Group B

(Alloxan + Metformin) exhibited a blood glucose level of 5.02±0.24 on Day 1, increasing to 6.16±0.60 on Day 21, before slightly decreasing to 5.84±0.40 on Day 28. Group C (Alloxan + *K. pinnata*) showed an increase from 5.66±0.95 on Day 1 to 6.48±0.24 on Day 21, then a decrease to 5.32±0.69 on Day 28. The negative control group (Group D) had blood glucose levels of 5.36±0.94 on Day 1, 6.62±0.26 on Day 21, and 6.26±0.57 on Day 28. Group E (Alloxan + *K. pinnata* + Metformin) showed a slight decrease in blood glucose from 4.71±0.64 on Day 1 to 6.30±0.44 on Day 21, and further decreased to 5.60±0.46 on Day 28. The mean differences in blood glucose levels were significant at $p<0.05$ when compared to the normal control and negative control groups.

Table 4 illustrates the effect of the aqueous extract of *K. pinnata* and metformin on oxidative stress markers in alloxan-induced diabetic Wistar rats. The normal control group (Group A) exhibited SOD levels of 0.19±0.01 µ/ml, MDA levels of 0.54±0.02 µmol/ml, GSH levels of 3.31±0.04 µg/ml, GPx levels of 0.07±0.01 µg/ml, and CAT levels of 2.06±0.18 µ/g. Group B (Alloxan + Metformin) showed a decrease in SOD (0.11±0.01 µ/ml), an increase in MDA (0.63±0.01 µmol/ml), a decrease in GSH (2.76±0.28 µg/ml), a slight decrease in GPx (0.06±0.01 µg/ml), and a marked reduction in CAT (0.68±0.29 µ/g). Group C (Alloxan + *K. pinnata*) exhibited an increase in SOD (0.15±0.02 µ/ml), a decrease in MDA (0.56±0.03 µmol/ml), and a slight increase in GSH (2.77±0.18 µg/ml), while GPx (0.06±0.01 µg/ml) and CAT (1.02±0.10 µ/g) levels were higher than the alloxan-treated group but lower than the normal control. Group D (Negative control, Alloxan-treated only) showed the lowest SOD (0.14±0.02 µ/ml) and GSH (2.61±0.01 µg/ml) levels, the highest MDA (0.58±0.03 µmol/ml), and GPx (0.06±0.01 µg/ml), but a higher CAT (2.10±0.43 µ/g) level compared to other treatment groups. Group E (Alloxan + *K. pinnata* + Metformin) showed similar SOD (0.19±0.07 µ/ml) and MDA (0.53±0.06 µmol/ml) levels to the normal control, with an improvement in GSH (3.05±0.17 µg/ml) and GPx (0.07±0.01 µg/ml) compared to the alloxan and alloxan + metformin groups, and a substantial increase in CAT (1.68±0.65 µ/g) compared to alloxan-treated rats. The mean differences in oxidative stress markers were significant at $p<0.05$ when compared to both normal and negative controls.

Table 2. Concentration of phytochemicals in fresh and aqueous extracted samples of *K. pinnata*

Parameter	Before extraction	After extraction
Oxalate (%)	0.97	0.40
Flavonoid (%)	3.01	1.27
Alkaloids (%)	1.94	0.49
Tannin (mg/kg)	11.15	9.23
Cyanogenic glycoside (mg/kg)	4.00	2.88
Saponin (%)	2.37	1.81

Table 3. Blood glucose level in alloxan induced diabetic Wistar rats treated with aqueous extract of *K. pinnata* and metformin

Group	Treatment	Blood glucose levels		
		Day 1	Day 21	Day 28
Group A	Normal control	4.13±0.41	4.28±0.45	4.97±0.23
Group B	Alloxan + Metformin	5.02±0.24	6.16±0.60	5.84±0.40
Group C	Alloxan + *K. pinnata*	5.66±0.95	6.48±0.24	5.32±0.69
Group D	Negative control	5.36±0.94	6.62±0.26	6.26±0.57

Group E	Alloxan + *K. pinnata* + Metformin	4.71±0.64	6.30±0.44	5.60±0.46

Values are expressed as Mean ± Standard Error of Mean (SEM), n=6. * The mean difference is significant at the p<0.05 when compare to Normal Control. # The mean difference is significant at the p<0.05 when compare to Negative Control.

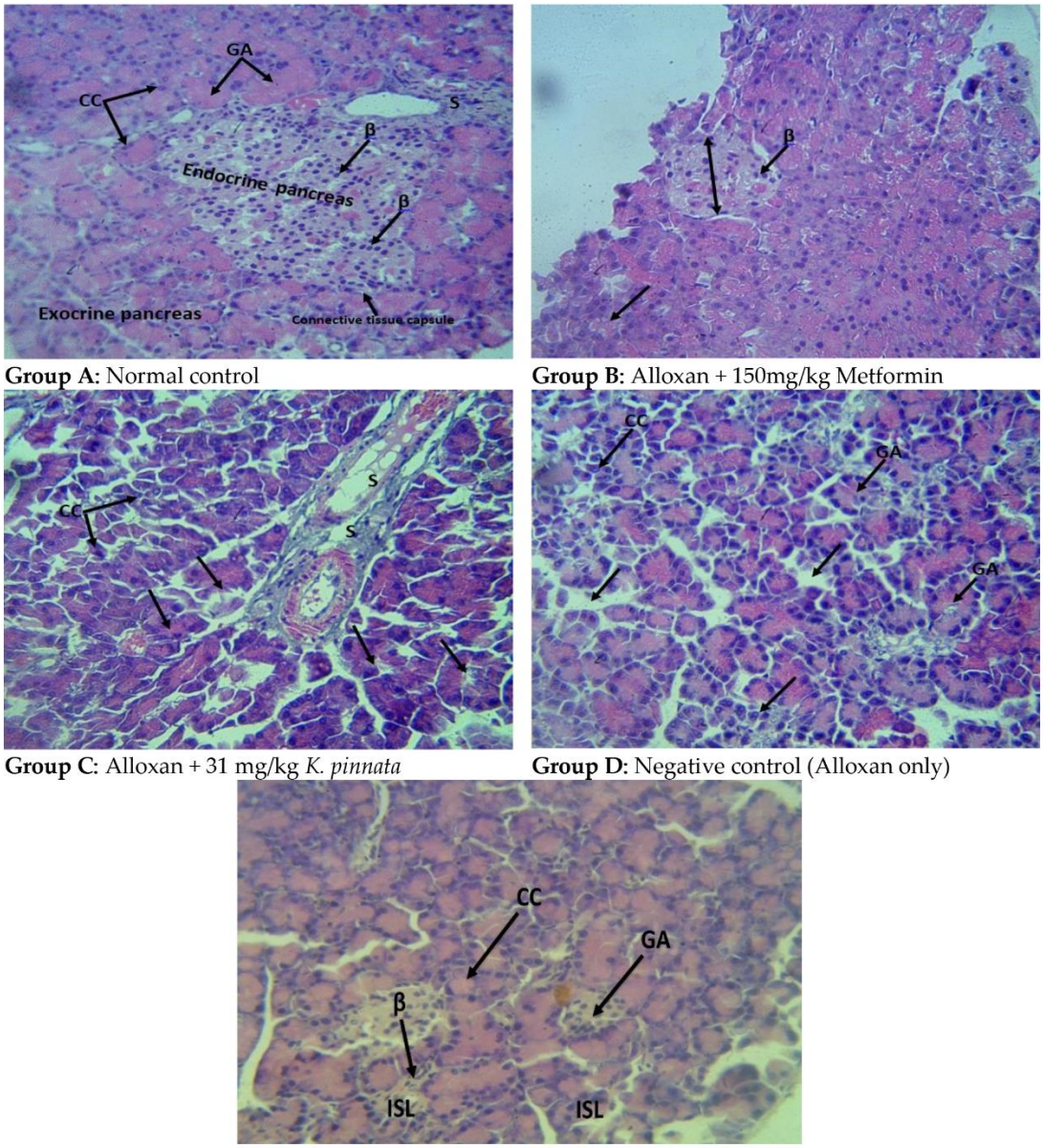
Table 4. Effect of aqueous extract of *K. pinnata* and metformin on oxidative stress markers in alloxan-induced diabetic Wistar rats

Group	**SOD (u/ml)**	**MDA (umol/ml)**	**GSH (ug/ml)**	**GPX (ug/ml)**	**CAT (u/g)**
Normal control	0.19±0.01	0.54±0.02	3.31±0.04	0.07±0.01	2.06±0.18
Alloxan + Metformin	0.11±0.01	0.63±0.01	2.76±0.28	0.06±0.01	0.68±0.29
Alloxan + *K. pinnata*	0.15±0.02	0.56±0.03	2.77±0.18	0.06±0.01	1.02±0.10
Negative control (Alloxan)	0.14±0.02	0.58±0.03	2.61±0.01	0.06±0.01	2.10±0.43
Alloxan + *K. pinnata* + Metformin	0.19±0.07	0.53±0.06	3.05±0.17	0.07±0.01	1.68±0.65

Values are expressed as Mean ± Standard Error of Mean (SEM), n=6. * The mean difference is significant at the p<0.05 when compare to Normal Control. # The mean difference is significant at the p<0.05 when compare to Negative Control.

Effect of *K. pinnata* leaf extract on the Pancreas Histology

Figure 1 shows photomicrographs (H&E X400) of the pancreas from different experimental groups. In Group A (Normal control), the pancreas exhibits a normal exocrine structure with well-organized glandular acini (GA), centroacinar cells (CC), and interlobular ducts (S), while the endocrine pancreas displays a high number of evenly dispersed beta cells within the islets of Langerhans, indicating a healthy pancreatic appearance. Group B (Alloxan + 150mg/kg of Metformin) demonstrates atrophy of the islets of Langerhans, a reduction in beta cells (hypocellularity), and mild distortion of the glandular acini, suggesting mild degeneration of the pancreatic beta cells and atrophy of the islets. In Group C (Alloxan + 31mg/kg *K. pinnata*), moderate interlobar vacuolation (arrows) of the glandular acini in the exocrine pancreas is observed, accompanied by loss of centroacinar cells, indicating distortion of the exocrine pancreas. Group D (Negative control, Alloxan) shows diffuse interlobar vacuolation (arrows) of the glandular acini (GA) in the exocrine pancreas parenchyma, with centroacinar hyperplasia, reflecting distortion of the exocrine pancreas parenchyma. Finally, Group E (Alloxan + 31mg/kg *K. pinnata* + 150mg/kg Metformin) reveals atrophied islets of Langerhans with hypocellularity of beta cells, although the exocrine pancreas appears normal, suggesting moderate degeneration of the pancreatic tissue.



Group A: Normal control

Group B: Alloxan + 150mg/kg Metformin

Group C: Alloxan + 31 mg/kg *K. pinnata*

Group D: Negative control (Alloxan only)

Group E: Alloxan + 31 mg/kg *K. pinnata* + 150mg/kg Metformin

Figure 1. Photomicrograph of the Pancreas (H&E X400). Group A (Normal control) shows a normal exocrine pancreas with well-organized glandular acini (GA), centroacinar cells (CC), and interlobular ducts (S), and a high number of beta cells in the islets of Langerhans. Group B (Alloxan + Metformin) shows atrophy of the islets of Langerhans, hypocellularity of the beta cells, and mild distortion of the glandular acini. Group C (Alloxan + *K. pinnata*) displays moderate interlobar vacuolation (arrows) in the glandular acini, with loss of centroacinar cells. Group D (Negative control) shows diffuse interlobar vacuolation (arrows) of the glandular acini with centroacinar hyperplasia. Group E (Alloxan + *K. pinnata* + Metformin) reveals atrophied islets of Langerhans with hypocellularity of the beta cells, while the exocrine pancreas appears normal (arrows).

DISCUSSION

The aim of this study was to evaluate the phytochemical composition of *Kalanchoe pinnata* (*K. pinnata*) and its effects on blood glucose levels, oxidative stress markers, and pancreatic histology in alloxan-induced diabetic Wistar rats. This investigation provides a foundation for understanding the therapeutic potential of *K. pinnata* extract in managing diabetes, both independently and in combination with metformin.

The phytochemical analysis of *K. pinnata* revealed a reduction in concentrations of oxalates, flavonoids, alkaloids, tannins, cyanogenic glycosides, and saponins following aqueous extraction. These phyto-compounds of *K. pinnata* were also studied by DeAraújo *et al*. (2018) and Halayal *et al*. (2024), and they phytochemicals are widely recognized for their roles in antidiabetic and antioxidant mechanisms (Patil *et al*., 2013; Uahomo *et al*., 2022; Ramon *et al*., 2023). For instance, flavonoids have been shown to enhance insulin secretion, reduce oxidative stress, and inhibit glucose absorption, making them key components in glycemic control (Al-Ishaq *et al*., 2019; Sood *et al*., 2020). Similarly, alkaloids have demonstrated glucose uptake enhancement properties, while tannins are effective in delaying glucose absorption by inhibiting enzymes such as α-glucosidase (Doan *et al*., 2018; Behl *et al*., 2022).

The reduction in phytochemical concentrations post-extraction may indicate partial loss of bioactivity during preparation. However, sufficient concentrations of these compounds remained in the aqueous extract to demonstrate significant biological effects. This supports the therapeutic viability of *K. pinnata* as a potential adjunct in diabetes management, as corroborated by similar findings in plant-based diabetes research (Alara *et al*., 2021).

The hypoglycemic potential of *K. pinnata* was evident in the blood glucose levels observed across the experimental groups from Day 1 to Day 28. The normal control group maintained stable glucose levels, while the alloxan-induced diabetic control group exhibited persistently elevated glucose levels, confirming the diabetogenic effects of alloxan (Aleme *et al*., 2022; Dede *et al*., 2023). In contrast, the treatment groups demonstrated varying degrees of glucose reduction, indicating that *K. pinnata* has the ability to influence glycemic control. This finding aligns with previous studies on the effect of vegetables combined with carbohydrate meals on glucose excursions and glycemic control (Akoko *et al*., 2022; Akoko *et al*., 2023; Akoko & Nwaogwugwu, 2023). The observed reductions in blood glucose suggest that *K. pinnata* may have a therapeutic effect in managing hyperglycemia, with the extent of the effect potentially depending on the dosage and duration of treatment.

Metformin-treated rats showed a significant reduction in glucose levels, consistent with the drug's mechanism of action, which involves decreasing hepatic glucose production and improving peripheral glucose uptake (Foretz *et al*., 2019). Notably, rats treated with *K. pinnata* extract also exhibited marked reductions in glucose levels by Day 28, suggesting that the extract enhances insulin sensitivity or stimulates insulin secretion. The combination treatment group showed the greatest reduction in glucose levels, indicating a possible synergistic interaction between *K. pinnata* phytochemicals and metformin. Similar synergistic effects of combining plant extracts with standard antidiabetic medications have been reported in previous studies (Hassan *et al*., 2019; Kifle *et al*., 2022; Ramadaini *et al*., 2024).

Oxidative stress (OS) is a key driver of diabetic complications, characterized by increased production of reactive oxygen species (ROS) and decreased antioxidant defense mechanisms (Caturano *et al*., 2023). In this study, alloxan-induced diabetes significantly elevated MDA levels, a marker of lipid peroxidation, while reducing antioxidant enzyme levels such as SOD, CAT, and GSH. Treatment with *K. pinnata* and metformin individually ameliorated these oxidative changes, but the combination treatment demonstrated

the most pronounced effects. The enhanced antioxidant effects of the combination treatment can be attributed to the flavonoid and saponin content in *K. pinnata*, which scavenge free radicals and enhance endogenous antioxidant enzyme activities (Salehi *et al*., 2019; Shaukat *et al*., 2023). Metformin has been proved to mitigate against OS, alleviate OS and restore antioxidant capacity (Esteghamati *et al*., 2013; Mirmiranpour *et al*., 2013). These findings are consistent with previous research indicating that phytochemicals in medicinal plants play a significant role in mitigating oxidative stress and protecting against diabetes-induced cellular damage (Alam *et al*., 2022; Arabshomali *et al*., 2023; Muscolo *et al*., 2024; Arabnozari *et al*., 2024).

Histopathological examination of pancreatic tissues provided additional insights into the protective effects of *K. pinnata*. The normal control group showed intact pancreatic morphology, with well-organized glandular acini and densely packed islets of Langerhans. Conversely, the diabetic control group exhibited severe degeneration of the exocrine pancreas and atrophy of islet cells, consistent with the cytotoxic effects of alloxan on pancreatic beta cells (Lenzen, 2008).

Treatment with metformin partially preserved pancreatic structure, with reduced islet atrophy and moderate distortion of glandular acini. Treatment with *K. pinnata* showed better preservation of the exocrine pancreas and islet morphology compared to metformin alone. The combination therapy demonstrated the greatest histological protection, with nearly normal pancreatic architecture and minimal vacuolation.

These findings suggest that *K. pinnata* exerts protective effects on pancreatic tissue, likely due to its antioxidative and anti-inflammatory properties. The synergistic effects observed with combination therapy further highlight the potential of integrating *K. pinnata* with conventional antidiabetic treatments to enhance therapeutic outcomes. Studies by Dede *et al*. (2023), Isirima and Uahomo (2023) and Tang *et al*. (2022) similarly report the protective effects of plant-based treatments on pancreatic histology in diabetic models.

While this study provides compelling evidence of the antidiabetic and antioxidant potential of *K. pinnata*, certain limitations warrant consideration. First, the phytochemical composition of the extract may vary depending on factors such as geographical location and extraction methods, which could influence its efficacy. Second, the study's duration may not fully capture long-term effects or potential toxicities of *K. pinnata*. Future studies should explore the molecular mechanisms underlying its effects, optimize extraction methods to retain phytochemical potency, and conduct clinical trials to validate its efficacy in humans.

CONCLUSION

This study demonstrates that *K. pinnata* possesses significant hypoglycemic, antioxidant, and pancreatic-protective properties in alloxan-induced diabetic rats. While aqueous extraction reduced some phytochemical concentrations, the extract retained sufficient bioactivity to mitigate hyperglycemia and oxidative stress. The combination of *K. pinnata* and metformin proved superior to either treatment alone, suggesting a synergistic mechanism that enhances therapeutic outcomes. These findings underscore the importance of exploring plant-based therapies as complementary approaches to conventional diabetes management.

Ethics Approval

The study was carried out in adherence to ethical guidelines set by the National Institute of Health (NIH) for the ethical treatment of animals in research. The study was approved by the Research Ethics Committee of the University of Port Harcourt, Rivers State, Nigeria before commencement of the study.

Disclaimer (Artificial intelligence)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

Competing Interests

Authors have declared that no competing interests exist.

REFERENCES

Akoko, S., & Nwaogwugwu, A. Q. (2023). Effect of vegetables with carbohydrate meal on glucose excursions and glycemic control among healthy adults in Port Harcourt. *Saudi Journal of Biomedical Research, 8*(7), 100–106.

Akoko, S., Aleme, B. M., & Uahomo, P. O. (2022). The effect of addition of extracts of *Vernonia amygdalina* and *Moringa oleifera* in the nutrition of alloxan-induced diabetic Wistar rats. *International Journal of Pharma Research and Health Sciences, 10*(4), 3455–3462.

Akoko, S., Siminialayi, I. M., & Chinenye, S. (2022). Feasibility of remission of type 2 diabetes mellitus using a wholly Nigerian dietary intervention at University of Port Harcourt Teaching Hospital, Nigeria. *International Journal of Health Sciences, 6*(S9), 3973–3991. https://doi.org/10.53730/ijhs.v6nS9.13541

Akoko, S., Siminialayi, I. M., & Chinenye, S. (2023). Weight reduction and glycemic control in type 2 diabetes mellitus using a wholly Nigerian diet at University of Port Harcourt Teaching Hospital, Nigeria. *International Journal of Research in Medical Sciences, 11*, 488–496.

Alam, S., Sarker, M. M. R., Sultana, T. N., Chowdhury, M. N. R., Rashid, M. A., Chaity, N. I., Zhao, C., Xiao, J., Hafez, E. E., Khan, S. A., & Mohamed, I. N. (2022). Antidiabetic Phytochemicals from Medicinal Plants: Prospective Candidates for New Drug Discovery and Development. *Frontiers in endocrinology*, *13*, 800714. https://doi.org/10.3389/fendo.2022.800714

Alara, O. R., Abdurahman, N. H., & Ukaegbu, C. I. (2021). Extraction of phenolic compounds: A review. *Current research in food science*, *4*, 200–214. https://doi.org/10.1016/j.crfs.2021.03.011

Aleme, B. M., Omeodu, S. I., Akoko, S., & Uahomo, P. O. (2022). Alloxan-induced diabetes: The synergistic effects of combined ethanolic leaf extract of *Dacryodes edulis* and *Psidium guajava*. *International Journal of Pharmaceutical Research and Applications, 7*(4), 1705–1710.

Al-Ishaq, R. K., Abotaleb, M., Kubatka, P., Kajo, K., & Büsselberg, D. (2019). Flavonoids and Their Anti-Diabetic Effects: Cellular Mechanisms and Effects to Improve Blood Sugar Levels. *Biomolecules*, *9*(9), 430. https://doi.org/10.3390/biom9090430

American Diabetes Association. (2023). Standards of care in diabetes: A guide for prevention, diagnosis, and treatment of people living with diabetes. Retrieved from https://diabetes.org/newsroom/american-diabetes-association-2023-standards-care-diabetes-guide-for-prevention-diagnosis-treatment-people-living-with-diabetes

Arabnozari, H., Shaki, F., Najjari, A., *et al*. (2024). The effect of *Polygonum hyrcanicum* Rech. f. hydroalcoholic extract on oxidative stress and nephropathy in alloxan-induced diabetic mice. *Scientific Reports, 14*, 18117. https://doi.org/10.1038/s41598-024-69220-x

Arabshomali, A., Bazzazzadehgan, S., Mahdi, F., & Shariat-Madar, Z. (2023). Potential Benefits of Antioxidant Phytochemicals in Type 2 Diabetes. *Molecules*, *28*(20), 7209. https://doi.org/10.3390/molecules28207209

Assis de Andrade, E., Machinski, I., Terso Ventura, A. C., Barr, S. A., Pereira, A. V., Beltrame, F. L., Strangman, W. K., & Williamson, R. T. (2023). A Review of the Popular Uses, Anatomical, Chemical, and Biological Aspects of Kalanchoe (Crassulaceae): A Genus of Plants Known as "Miracle Leaf". *Molecules (Basel, Switzerland)*, *28*(14), 5574. https://doi.org/10.3390/molecules28145574

Baker, J. R. (1945). Cytological Technique (2nd Edition). London: Methuen.

Behl, T., Gupta, A., Albratty, M., Najmi, A., Meraya, A. M., Alhazmi, H. A., Anwer, M. K., Bhatia, S., & Bungau, S. G. (2022). Alkaloidal Phytoconstituents for Diabetes Management: Exploring the Unrevealed Potential. *Molecules (Basel, Switzerland)*, *27*(18), 5851. https://doi.org/10.3390/molecules27185851

Beutler, E., Duron, O., & Kelly, B. M. (1963). Improved method for the determination of blood glutathione. *Journal of Laboratory and Clinical Medicine, 61*, 882–888.

Boham, B.A. and Kocipai-Abyazan, R. (1974) Flavonoids and Condensed Tannins from Leaves of Hawaiian Vaccinium vaticulatum and V. calycinium. Pacific Science, 48, 458-463.

Bondet, V., Brand-Williams, W., & Berset, C. (1997). Kinetics and mechanisms of antioxidant activity using the DPPH free radical method. *Lebensmittel Wissenschaft Technologie Food Science Technology, 30*, 609–615.

Caturano, A., D'Angelo, M., Mormone, A., Russo, V., Mollica, M. P., Salvatore, T., Galiero, R., Rinaldi, L., Vetrano, E., Marfella, R., Monda, M., Giordano, A., & Sasso, F. C. (2023). Oxidative Stress in Type 2 Diabetes: Impacts from Pathogenesis to Lifestyle Modifications. *Current issues in molecular biology*, *45*(8), 6651–6666. https://doi.org/10.3390/cimb45080420

De Araújo, E. R. D., Guerra, G. C. B., De Souza Araújo, D. F., De Araújo, A. A., Fernandes, J. M., De Araújo Júnior, R. F., & Zucolotto, S. M. (2018). Gastroprotective and antioxidant activity of *Kalanchoe brasiliensis* and *Kalanchoe pinnata* leaf juices against indomethacin and ethanol-induced gastric lesions in rats. *International Journal of Molecular Sciences, 19*(5), 1265. https://www.mdpi.com/1422-0067/19/5/1265

Dede, S., Isirima, J. C., Akoko, S. (2023). Antihyperglycemic and Histological Effect of Aloe vera on Alloxan-Induced Diabetes in Wistar Rats. Sch Int J Tradit Complement Med, 6(8): 120-128.

Doan, H. V., Riyajan, S., Iyara, R., & Chudapongse, N. (2018). Antidiabetic activity, glucose uptake stimulation and α-glucosidase inhibitory effect of Chrysophyllum cainito L. stem bark extract. *BMC complementary and alternative medicine*, *18*(1), 267. https://doi.org/10.1186/s12906-018-2328-0

Ehi-Omosun, M. B., & Etunim, S. C. (2023). Effects of aqueous leaf extract of *Kalanchoe pinnata* on bifenthrin-induced injury in the lungs of the adult Wistar rat. *Journal of Applied Sciences and Environmental Management*, *27*(6), 1159–1164. https://www.ajol.info/index.php/jasem/article/view/250227/236558

Esteghamati, A., Eskandari, D., Mirmiranpour, H., Noshad, S., Mousavizadeh, M., Hedayati, M., et al. (2013). Effects of metformin on markers of oxidative stress and antioxidant reserve in patients with newly diagnosed type 2 diabetes: A randomized clinical trial. *Clinical Nutrition*, *32*, 179–185. https://doi.org/10.1016/J.CLNU.2012.08.006

Foretz, M., Guigas, B., & Viollet, B. (2019). Understanding the glucoregulatory mechanisms of metformin in type 2 diabetes mellitus. Nature reviews. Endocrinology, 15(10), 569–589. https://doi.org/10.1038/s41574-019-0242-2

Galaviz, K. I., Narayan, K. M. V., Lobelo, F., & Weber, M. B. (2015). Lifestyle and the Prevention of Type 2 Diabetes: A Status Report. *American journal of lifestyle medicine*, *12*(1), 4–20. https://doi.org/10.1177/1559827615619159

Gautam, K., Kumar, R., & Rana, H. (2023). The pharmacological activities of mother of thousand (Kalanchoe pinnata). *Research Journal of Pharmacology and Pharmacodynamics, 15*(1), 31–35. https://doi.org/10.52711/2321-5836.2023.00007

Halayal, R. Y., Bagewadi, Z. K., Aldabaan, N. A., Shaikh, I. A., & Khan, A. A. (2024). Exploring the therapeutic mechanism of potential phyto-compounds from *Kalanchoe pinnata* in the treatment of diabetes mellitus by integrating network pharmacology, molecular docking, and simulation approach. *Saudi Pharmaceutical Journal, 32*(5).

Harborne, J.B. (1973) Phytochemical Methods. Chapman and Hall Ltd., London, 49-188.

Hassan, I. A., Abdulraheem, I., Emun, H. O., & Lawal, D. M. (2019). Synergistic effect of aqueous extracts of *Croton zabeniscus* and *Vernonia amygdalina* leaves as an antihyperglycemic agent in alloxan-induced diabetic albino rats. *Journal of Advances in Medicine and Medical Research, 29*(5), 1-6. https://doi.org/10.9734/jammr/2019/v29i530092

Isirima, J. C., and Uahomo, P. O. (2023). *Acalypha wilkesiana* Exhibits Antihyperglycemic Potentialsand Ameliorates Damages to Pancreas and Spleen of Diabetic Rat Model. Saudi J Biomed Res, 8(7): 83-94.

Kifle, Z. D., Abdelwuhab, M., Melak, A. D., Genet, G., Meseret, T., & Adugna, M. (2022). Pharmacological evaluation of medicinal plants with antidiabetic activities in Ethiopia: A review. *Metabolism open*, *13*, 100174. https://doi.org/10.1016/j.metop.2022.100174

Kooti, W., Farokhipour, M., Asadzadeh, Z., Ashtary-Larky, D., & Asadi-Samani, M. (2016). The role of medicinal plants in the treatment of diabetes: a systematic review. Electronic physician, 8(1), 1832–1842. https://doi.org/10.19082/1832

Lenzen S. (2008). The mechanisms of alloxan- and streptozotocin-induced diabetes. *Diabetologia*, *51*(2), 216–226. https://doi.org/10.1007/s00125-007-0886-7

Magliano, D. J., Boyko, E. J., & IDF Diabetes Atlas 10th Edition Scientific Committee. (2021). *IDF diabetes atlas* (10th ed.). International Diabetes Federation. https://www.ncbi.nlm.nih.gov/books/NBK581934/

Maruthur, N. M., Tseng, E., Hutfless, S., Wilson, L. M., Suarez-Cuervo, C., Berger, Z., Chu, Y., Iyoha, E., Segal, J. B., & Bolen, S. (2016). Diabetes Medications as Monotherapy or Metformin-Based Combination Therapy for Type 2 Diabetes: A Systematic Review and Meta-analysis. Annals of internal medicine, 164(11), 740–751. https://doi.org/10.7326/M15-2650

Mirmiranpour, H., Mousavizadeh, M., Noshad, S., Ghavami, M., Ebadi, M., & Ghasemiesfe, M. (2013). Comparative effects of pioglitazone and metformin on oxidative stress markers in newly diagnosed type 2 diabetes patients: A randomized clinical trial. *Journal of Diabetes & Its Complications, 27*, 501–507. https://doi.org/10.1016/J.JDIACOMP.2013.05.006

Misra, H. P., & Fridovich, I. (1972). Superoxide dismutase (SOD) estimation. *Journal of Biological Chemistry, 247*(10), 3170–3175.

Muscolo, A., Mariateresa, O., Giulio, T., & Mariateresa, R. (2024). Oxidative Stress: The Role of Antioxidant Phytochemicals in the Prevention and Treatment of Diseases. *International journal of molecular sciences*, *25*(6), 3264. https://doi.org/10.3390/ijms25063264

Nigerian Centre for Disease Control. (2020). *Situational reports*. Retrieved December 13, 2024, from http://www.ncdc.gov.ng/diseases/sitreps

Ohkawa, H., Ohisi, N., & Yagi, K. (1979). Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Analytical Biochemistry, 95*(2), 351–358.

Ota, A., & Ulrih, N. P. (2017). An Overview of Herbal Products and Secondary Metabolites Used for Management of Type Two Diabetes. Frontiers in pharmacology, 8, 436. https://doi.org/10.3389/fphar.2017.00436

Pastakia, S. D., Pekny, C. R., Manyara, S. M., & Fischer, L. (2017). Diabetes in sub-Saharan Africa - from policy to practice to progress: targeting the existing gaps for future care for diabetes. *Diabetes, metabolic syndrome and obesity : targets and therapy*, *10*, 247–263. https://doi.org/10.2147/DMSO.S126314

Pizzino, G., Irrera, N., Cucinotta, M., Pallio, G., Mannino, F., Arcoraci, V., Squadrito, F., Altavilla, D., & Bitto, A. (2017). Oxidative Stress: Harms and Benefits for Human Health. *Oxidative medicine and cellular longevity*, *2017*, 8416763. https://doi.org/10.1155/2017/8416763

Quaile, M.P., Melich, D.H., Jordan, H.L., Nold, J.B., Chism,J.P., Polli, J.W., Smith, G.A., & Rhodes, M.C. (2010). Toxicityand toxicokinetics of metformin in rats. Toxicology andApplied Pharmacology, 243(3), 340–347

Quazi Majaz, A., Tatiya, A. U., Khurshid, M., Nazim, S., & Siraj, S. (2011). The miracle plant (Kalanchoe pinnata): A phytochemical and pharmacological review. International Journal of Research in Ayurveda & Pharmacy, 2(5), 1478–1482.

Rajsekhar, P. B., Bharani, R. S., Ramachandran, M., Angel, J. K., & Rajsekhar, S. P. V. (2016). The "wonder plant" Kalanchoe pinnata (Linn.) Pers.: A review. *Journal of Applied Pharmaceutical Science, 6*(3), 151–158.

Ramadaini, T., Sumiwi, S. A., & Febrina, E. (2024). The anti-diabetic effects of medicinal plants belonging to the Liliaceae family: Potential alpha-glucosidase inhibitors. *Drug Design, Development and Therapy*, *18*, 3595–3616. https://doi.org/10.2147/DDDT.S464100

Ramon, P., Bergmann, D., Abdulla, H., Sparks, J., & Omoruyi, F. (2023). Bioactive Ingredients in *K. pinnata* Extract and Synergistic Effects of Combined *K. pinnata* and Metformin Preparations on Antioxidant Activities in Diabetic and Non-Diabetic Skeletal Muscle Cells. *International journal of molecular sciences*, *24*(7), 6211. https://doi.org/10.3390/ijms24076211

Reddy, K. P., Subhani, S. M., Khan, P. A., & Kumar, K. B. (1995). Effect of light and benzyladenine and dark-treated graving rice (*Oryza sativa*) leaves: Changes in peroxidase activity. *Plant Cell Physiology, 26*, 987–994.

Salehi, B., Ata, A., V Anil Kumar, N., Sharopov, F., Ramírez-Alarcón, K., Ruiz-Ortega, A., Abdulmajid Ayatollahi, S., Tsouh Fokou, P. V., Kobarfard, F., Amiruddin Zakaria, Z., Iriti, M., Taheri, Y., Martorell, M., Sureda, A., Setzer, W. N., Durazzo, A., Lucarini, M., Santini, A., Capasso, R., Ostrander, E. A., … Sharifi-Rad, J. (2019). Antidiabetic Potential of Medicinal Plants and Their Active Components. *Biomolecules*, *9*(10), 551. https://doi.org/10.3390/biom9100551

Sharifi-Rad M, Anil Kumar NV, Zucca P, Varoni EM, Dini L, Panzarini E, Rajkovic J, Tsouh Fokou PV, Azzini E, Peluso I, Prakash Mishra A, Nigam M, El Rayess Y, Beyrouthy ME, Polito L, Iriti M, Martins N, Martorell M, Docea AO, Setzer WN, Calina D, Cho WC and Sharifi-Rad J (2020) Lifestyle, Oxidative Stress, and Antioxidants: Back and Forth in the Pathophysiology of Chronic Diseases. *Front. Physiol.* 11:694. https://doi.org/10.3389/fphys.2020.00694

Shaukat, A., Zaidi, A., Anwar, H., & Kizilbash, N. (2023). Mechanism of the antidiabetic action of *Nigella sativa* and thymoquinone: A review. *Frontiers in Nutrition, 10*, 1126272. https://doi.org/10.3389/fnut.2023.1126272

Singh, K., Chandra Sekaran, A. M., Bhaumik, S., Aisola, M., Chattopadhyay, K., Gamage, A. U., de Silva, P., Selvaraj, S., Roy, A., Prabhakaran, D., & Tandon, N. (2015). Cost-effectiveness of interventions to control cardiovascular diseases and type 2 diabetes mellitus in South Asia: protocol for a systematic review. BMJ open, 5(3), e007205. https://doi.org/10.1136/bmjopen-2014-007205

Sinha, K. A. (1971). Colorimetric assay of catalase. *Analytical Biochemistry*, *47*, 389–394.

Sood, A., Kumar, B., Singh, S. K., Prashar, P., Gautam, A., Gulati, M., Pandey, N. K., Melkani, I., Awasthi, A., Saraf, S. A., Vidari, G., Ozdemir, M., Hussain, F. H. S., Anwar, E. T., Ameen, M. S. M., Gupta, S., & Porwal, O. (2020). Flavonoids as Potential Therapeutic Agents for the Management of Diabetic Neuropathy. *Current pharmaceutical design*, *26*(42), 5468–5487. https://doi.org/10.2174/1381612826666200826164322

Tang, Y., Sun, M., & Liu, Z. (2022). Phytochemicals with protective effects against acute pancreatitis: a review of recent literature. *Pharmaceutical biology*, *60*(1), 479–490. https://doi.org/10.1080/13880209.2022.2039723

Uahomo, P. O., Isirima, J. C., & Akoko, S. (2022). Evaluation of Phytochemicals and Bioactive Properties in Leaf and Root Parts of Cyathula prostrata (Pasture Weed) – A Qualitative and Quantitative Analysis. *Asian Plant Research Journal*, *9*(3), 8–16. https://doi.org/10.9734/aprj/2022/v9i330207

Uloko, A. E., Musa, B. M., Ramalan, M. A., Gezawa, I. D., Puepet, F. H., Uloko, A. T., Borodo, M. M., & Sada, K. B. (2018). Prevalence and Risk Factors for Diabetes Mellitus in Nigeria: A Systematic Review and Meta-Analysis. Diabetes therapy: research, treatment and education of diabetes and related disorders, 9(3), 1307–1316. https://doi.org/10.1007/s13300-018-0441-1

Uusitupa, M., Khan, T. A., Viguiliouk, E., Kahleova, H., Rivellese, A. A., Hermansen, K., Pfeiffer, A., Thanopoulou, A., Salas-Salvadó, J., Schwab, U., & Sievenpiper, J. L. (2019). Prevention of Type 2 Diabetes by Lifestyle Changes: A Systematic Review and Meta-Analysis. *Nutrients*, *11*(11), 2611. https://doi.org/10.3390/nu11112611

Wan Rohimi WNLH and Mohd Tahir NA (2022) The cost-effectiveness of different types of educational interventions in type II diabetes mellitus: A systematic review. Front. Pharmacol. 13:953341. doi: 10.3389/fphar.2022.953341

World Health Organization (2018) Global Report on Diabetes. Diabetes Fact Sheet. https://www.who.int/news-room/fact-sheets/detail/diabetes

World Health Organization. (2018). *Guidelines on second-and third-line medicines and type of insulin for the control of blood glucose levels in non-pregnant adults with diabetes mellitus*. World Health Organization. https://www.ncbi.nlm.nih.gov/books/NBK532221/

World Health Organization. (2024). *Diabetes*. Retrieved December 13, 2024, from https://www.who.int/news-room/fact-sheets/detail/diabetes