

# Synergistic Hypoglycemic Effects 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  $\beta$ -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 musculus*) 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 Aqueous 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 steep for 24 hours to facilitate the extraction of flavors, nutrients, and 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 keep 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 + <i>K. pinnata</i>	Alloxan-induced diabetic Wistar rats treated with 31 mg/kg aqueous extract of <i>K. pinnata</i> per day for 28 days
Group D	Negative control	Alloxan-induced diabetic Wistar Rats without treatment for 28 days
Group E	Alloxan-induced + <i>K. pinnata</i> + Metformin	Alloxan-induced diabetic Wistar rats treated with 31 mg/kg aqueous extract of <i>K. pinnata</i> 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  $\mu$ L 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-picrylhydrazyl) 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  $\pm$  Standard error of mean. Differences between means were assessed using one-way 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.

### 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.

## RESULT

### Phytochemical concentration of *K. pinnata* and the effect of *K. pinnata* and metformin on 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  $4.13 \pm 0.41$ , which slightly increased to  $4.28 \pm 0.45$  on Day 21 and  $4.97 \pm 0.23$  on Day 28. Group B

(Alloxan + Metformin) exhibited a blood glucose level of  $5.02 \pm 0.24$  on Day 1, increasing to  $6.16 \pm 0.60$  on Day 21, before slightly decreasing to  $5.84 \pm 0.40$  on Day 28. Group C (Alloxan + *K. pinnata*) showed an increase from  $5.66 \pm 0.95$  on Day 1 to  $6.48 \pm 0.24$  on Day 21, then a decrease to  $5.32 \pm 0.69$  on Day 28. The negative control group (Group D) had blood glucose levels of  $5.36 \pm 0.94$  on Day 1,  $6.62 \pm 0.26$  on Day 21, and  $6.26 \pm 0.57$  on Day 28. Group E (Alloxan + *K. pinnata* + Metformin) showed a slight decrease in blood glucose from  $4.71 \pm 0.64$  on Day 1 to  $6.30 \pm 0.44$  on Day 21, and further decreased to  $5.60 \pm 0.46$  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  $0.19 \pm 0.01$   $\mu\text{ml}$ , MDA levels of  $0.54 \pm 0.02$   $\mu\text{mol/ml}$ , GSH levels of  $3.31 \pm 0.04$   $\mu\text{g/ml}$ , GPx levels of  $0.07 \pm 0.01$   $\mu\text{g/ml}$ , and CAT levels of  $2.06 \pm 0.18$   $\mu\text{g}$ . Group B (Alloxan + Metformin) showed a decrease in SOD ( $0.11 \pm 0.01$   $\mu\text{ml}$ ), an increase in MDA ( $0.63 \pm 0.01$   $\mu\text{mol/ml}$ ), a decrease in GSH ( $2.76 \pm 0.28$   $\mu\text{g/ml}$ ), a slight decrease in GPx ( $0.06 \pm 0.01$   $\mu\text{g/ml}$ ), and a marked reduction in CAT ( $0.68 \pm 0.29$   $\mu\text{g}$ ). Group C (Alloxan + *K. pinnata*) exhibited an increase in SOD ( $0.15 \pm 0.02$   $\mu\text{ml}$ ), a decrease in MDA ( $0.56 \pm 0.03$   $\mu\text{mol/ml}$ ), and a slight increase in GSH ( $2.77 \pm 0.18$   $\mu\text{g/ml}$ ), while GPx ( $0.06 \pm 0.01$   $\mu\text{g/ml}$ ) and CAT ( $1.02 \pm 0.10$   $\mu\text{g}$ ) 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 ( $0.14 \pm 0.02$   $\mu\text{ml}$ ) and GSH ( $2.61 \pm 0.01$   $\mu\text{g/ml}$ ) levels, the highest MDA ( $0.58 \pm 0.03$   $\mu\text{mol/ml}$ ), and GPx ( $0.06 \pm 0.01$   $\mu\text{g/ml}$ ), but a higher CAT ( $2.10 \pm 0.43$   $\mu\text{g}$ ) level compared to other treatment groups. Group E (Alloxan + *K. pinnata* + Metformin) showed similar SOD ( $0.19 \pm 0.07$   $\mu\text{ml}$ ) and MDA ( $0.53 \pm 0.06$   $\mu\text{mol/ml}$ ) levels to the normal control, with an improvement in GSH ( $3.05 \pm 0.17$   $\mu\text{g/ml}$ ) and GPx ( $0.07 \pm 0.01$   $\mu\text{g/ml}$ ) compared to the alloxan and alloxan + metformin groups, and a substantial increase in CAT ( $1.68 \pm 0.65$   $\mu\text{g}$ ) 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	$4.13 \pm 0.41$	$4.28 \pm 0.45$	$4.97 \pm 0.23$
Group B	Alloxan + Metformin	$5.02 \pm 0.24$	$6.16 \pm 0.60$	$5.84 \pm 0.40$
Group C	Alloxan + <i>K. pinnata</i>	$5.66 \pm 0.95$	$6.48 \pm 0.24$	$5.32 \pm 0.69$
Group D	Negative control	$5.36 \pm 0.94$	$6.62 \pm 0.26$	$6.26 \pm 0.57$

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

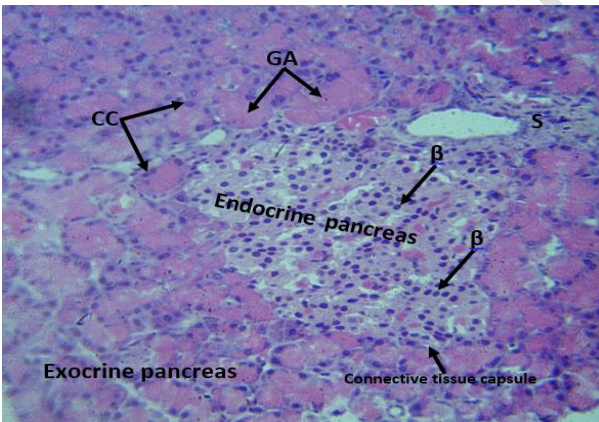
Group	SOD (u/ml)	MDA (umol/ml)	GSH (ug/ml)	GPX (ug/ml)	CAT (u/g)
Normal control	0.19±0.01	0.54±0.02	3.31±0.04	0.07±0.01	2.06±0.18
Alloxan + Metformin	0.11±0.01	0.63±0.01	2.76±0.28	0.06±0.01	0.68±0.29
Alloxan + <i>K. pinnata</i>	0.15±0.02	0.56±0.03	2.77±0.18	0.06±0.01	1.02±0.10
Negative control (Alloxan)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

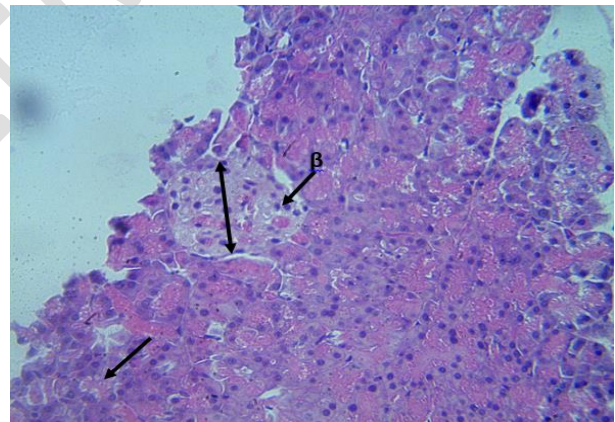
#### Effect of *K. pinnata* leaf extract on the Pancreas Histology

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.

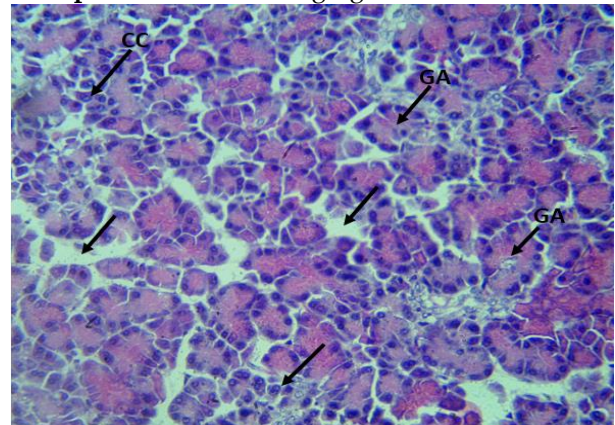
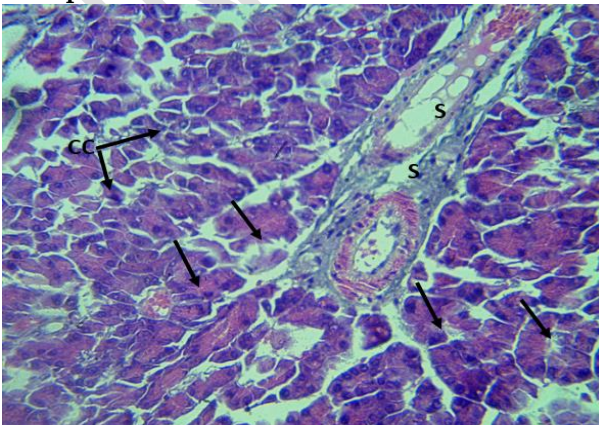




**Group A: Normal control**



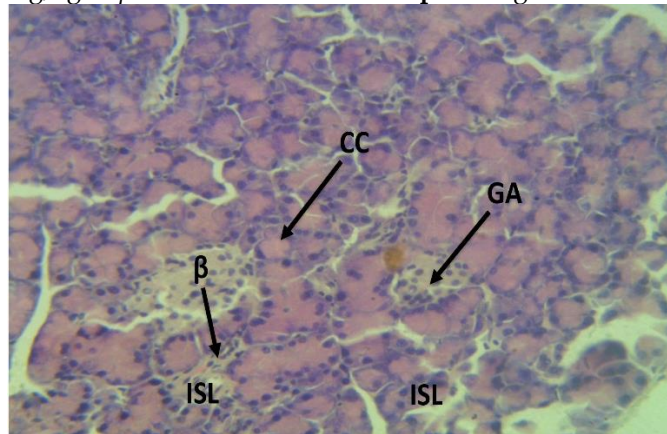
**Group B: Alloxan + 150mg/kg Metformin**





**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 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  $\alpha$ -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 *Kalanchoe pinnata* (*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 (Kifle *et al.*, 2022; Ramadaini *et al.*, 2024).

Oxidative stress 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). 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.

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