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Histoprotective Effect of Aqueous Extract of Zobo (*Hibiscus sabdariffa*) Calyces on Alloxan-induced Pancreatic Damage in Adult Female Wistar Rats

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ABSTRACT

Diabetes mellitus represents a persistent metabolic condition defined by elevated blood sugar, stemming from either the loss of insulin-producing beta cells or a diminished response to insulin. While conventional treatment like metformin manages glucose levels, they often fail to reverse underlying pancreatic damage. Consequently, there is an increasing interest in phytomedicines like Zobo (*Hibiscus sabdariffa*), known for its antioxidant-rich phytochemical profile, to aid in tissue regeneration. The present research evaluated the regenerative effects of *H. sabdariffa* aqueous extract on the pancreases of Wistar rats after inducing type 1 diabetes using alloxan monohydrate. Ten female rats were categorized into five experimental sets: normal control, diabetic control (alloxan 200 mg/kg), two Zobo extract cohorts (200 and 500 mg/kg), and a metformin-treated group (150 mg/kg). Successful induction of type 1 diabetes was verified by fasting glucose readings exceeding 200 mg/dL after single intraperitoneal administration. After a 7-day treatment period, pancreatic tissue was harvested and prepared for H&E histological evaluation. Histological results from the diabetic control group indicated disrupted acini and no pancreatic islets. In contrast, rats treated with *H. sabdariffa* extract showed dose-dependent structural recovery. The 500 mg/kg dose demonstrated significant restoration of pancreatic histoarchitecture, characterized by the presence of healthy islets and acinar cells, comparable to the regeneration observed in the metformin-treated group although the metformin-treated group appeared slightly better. The findings suggest that *H. sabdariffa* aqueous extract possesses potent restorative properties. By protecting beta cells from oxidative stress-induced apoptosis, it facilitates pancreatic regeneration, offering a promising, affordable, and natural adjunct therapy for diabetes management.

Keywords: alloxan, diabetes mellitus, *Hibiscus sabdariffa*, pancreatic regeneration, phytomedicine

INTRODUCTION

The pancreas is a heterocrine organ, possessing both digestive (exocrine) and hormonal (endocrine) responsibilities [1]. In adult human, this lobulated, salmon-hued organ typically measures about 12 to 15 cm (4.7 to 5.9 in) in length [2]. Structurally, it is overwhelmingly exocrine, with 99% of its mass dedicated to producing digestive enzymes for the gastrointestinal tract while the remaining 1% consists of the endocrine component—the pancreatic islets—which are responsible for secreting insulin and other metabolic hormones essential for regulating systemic blood glucose levels [3, 4].

Diabetes mellitus comprises a group of metabolic disorders characterized by prolonged elevated glucose levels [5]. This condition stems from insufficient insulin synthesis by the pancreas or when the cells no long respond to insulin [6]. Diabetes mellitus may also arise from various specific factors, such as monogenic diabetes syndromes, pancreatic diseases like pancreatitis, or the administration of medications like glucocorticoids and post-transplant therapies [7]. Globally, the burden of this disease is shifting significantly toward the low- and the middle-income regions [8]. The depletion of pancreatic beta cells results in critical insulin deficits in type 1 and type 2 (late-stage) diabetes [9]. The long-term consequences are systemic, involving vascular damage that leads to cardiovascular disease, renal failure, retinopathy, and neuropathy—the latter of which frequently results in foot ulceration and potential limb loss [10-12].

Metformin is one of the most commonly prescribed medication in diabetes treatment [13]. Conventional

diabetes treatment, including insulin therapy and oral hypoglycemic agents, help manage blood glucose levels but often come with associated adverse pharmacological reactions and significant financial burdens, and the inability to reverse pancreatic damage [14]. As a result, researchers have turned to natural remedies, exploring the therapeutic potential of plant-derived compounds able to mitigate oxidative stress and reduce systemic inflammation.

Zobo (*Hibiscus sabdariffa*), widely regarded as a significant medicinal plant for its rich bioactive compounds, is also used as social drink, beverage, and tea in Nigeria, and is recognized as a herbal remedy, safe for diabetes mellitus [15,16]. *Hibiscus sabdariffa* has a rich phytochemical profile, which includes a variety of bioactive compounds that contribute to its health benefits. *Hibiscus sabdariffa* particularly is rich in antioxidants, including anthocyanins and flavonoids [17]. They contribute to the preservation of cellular integrity by neutralizing reactive oxygen species and reducing the risk of chronic disease [18]. Flavonoids are prominent in *Hibiscus sabdariffa*; they provide antioxidant properties and contribute to the beverage's overall health-promoting effects. These compounds protect the body from environmental toxins and may exert beneficial effects on neuronal health [19]. *Hibiscus sabdariffa* is also a source of phenolic compounds, including polyphenols and organic acids, which are associated with physiological benefits, like improved cardiovascular health and enhanced metabolic properties [20]. Flavonoids in *Hibiscus sabdariffa* together with anthocyanins scavenge free radicals, reducing oxidative damage to tissues exposed to diabetes [21]. *Hibiscus sabdariffa* enhances insulin secretion by protecting beta cells from oxidative stress-induced apoptosis [22].

Despite large and important advances in diabetes management, the search for a cure remains a global challenge. Currently, treatment strategies focus mainly on symptom control, instead of dealing with the pancreatic damage underlying the disease's perpetuation. The health conditions linked to diabetes, such as heart-related, nervous system, and kidney failure condition, make healthcare more expensive. For this reason, researchers and health practitioners are beginning to explore natural alternatives that might not only help control blood sugar but also encourage the healing of pancreatic tissues. This study has the potential to stimulate further research into plant-based therapies for diabetes, as well as to offer a large understanding within the field of phytomedicine. Furthermore, the exploration of natural remedies for diabetes is important in areas where costly pharmaceuticals remain inaccessible. Zobo calyx is affordable, widely available, and has an established ethnobotanical history. Consequently, this research seeks to evaluate the efficacy of aqueous zobo (*Hibiscus sabdariffa*) calyx extracts in supporting pancreatic regeneration in diabetic Wistar rats, offering a promising natural approach to diabetes management

MATERIALS AND METHODS

Plant procurement and extraction

The calyces utilized in this study were sourced from a local commercial outlet in Masaka, located in Nasarawa State, Nigeria. The preparation of zobo (*Hibiscus sabdariffa*) calyces was obtained by maceration procedure. The dried zobo calyces were rinsed thoroughly with clean water to remove dust and unwanted particles and air dried. After which they were soaked in distilled water for three days. During this time, the solution remained at room temperature and was regularly stirred [23]. This Maceration process intended to permeabilize and break down the cell walls, thereby releasing the plant's soluble constituents. The mixture was filtered, a damp solid, and the solvents were separated by filtration or decantation after standing [24].

Ethical oversight

Ethical oversight for animal handling was provided by the BHU Ethics Review Committee at Bingham University, ensuring that all experimental protocols adhered to established IACUC (Institutional Animal Care and Use Committee standards). The approval number is BHUCAUC/CMS/2025/008

Experimental design

The experiment consisted of ten (10) adult female Wistar rats (150 to 200 g), maintained within the animal facility at Bingham University. Prior to the commencement of the study, the rats underwent a 14-day acclimatization period under controlled environmental conditions, including a regulated 12-hour light/dark cycle and a constant ambient temperature of 25°C, with a standard daily diet of laboratory chow. Their cages were cushioned with sawdust and cleaned every three to five days before commencement of the experiment.

The Wistar rats were randomly selected after acclimatization and grouped into five (5), with each group containing two (2) rats. Experimental animals (Groups 2-5) were made diabetic by inducing type 1 diabetes with alloxan (200mg/kg body weight). To induce type 1 diabetes, the rats were first fasted for 12 hours and then administered a single intraperitoneal dose of alloxan monohydrate dissolved in 0.9% saline. After 2-3 days, the rats were confirmed diabetic when the level of their blood glucose using Accu-Check Active glucometer was found to be above 200 mg/dL [25]. After inducing diabetes, the rats were treated for 7 days, with aqueous extract of zobo and metformin. At the conclusion of the study, the rats were sacrificed under chloroform anesthesia. The pancreas was excised and fixed in 10% formalin. Table 1 shows the grouping and administration of the drug and extract of the Wistar rats.

Table 1: Animal Grouping and administration dosage of Wistar rats

Group	Treatment and Doses	Duration (Days)
1	Non-diabetic rats were given feed and distilled water only	10
2	Diabetic control-SIAMI (200 mg/kg bw)	10
3	SIAMI (200 mg/kg bw) + AZE (200mg/kg bw)	10
4	SIAMI (200 mg/kg bw) + AZE (500mg/kg bw)	10
5	SIAMI (200 mg/kg bw) + 150 mg/kg bw MTFM	10

SIAMI- Single intraperitoneal alloxan monohydrate injection; AZE- Aqueous zobo extract; MTFM-Metformin

Histological analysis

Histological examination of the pancreas was conducted following this procedure.

- The pancreas was fixed in 10% formalin with organ bottles to preserve the tissue component and to inhibit autolysis and putrefaction.
- The tissues then underwent dehydration through a graded series of ethanol to eliminate residual water and fixative
- The tissues were cleared in xylene to take out the dehydrating solution and make the tissue component more receptive to infiltration.
- Paraffin infiltration followed to fill up empty spaces resulting from alcohol and provide structural support
- Once embedded, the blocks were sectioned using a microtome into thinner blocks. After cutting into thinner sections, the sections were placed on slides and were stained using Hematoxylin and Eosin stains.

RESULTS

Histological analysis of the Pancreas was observed after staining with Hematoxylin and Eosin (H & E) stain. Figure 1 (A-E) show photomicrographs that were taken for all groups while viewing under the microscope following 7 days treatment after inducing with 200 mg/kg/bw of alloxan monohydrate.

Histological profile for normal control group 1, which received distilled water, only shows normal histoarchitecture of the pancreas with distinct pancreatic acinar cells, pancreatic islets (A1-A2) and alpha and beta cells (A2). Photomicrograph for group 2 (B1 and B2) which was induced with 200 mg/kg bw alloxan monohydrate shows absence of pancreatic islets with altered pancreatic acinar cells. Group 3, induced with 200 mg/kg bw alloxan monohydrate and administered 200 mg/kg bw of the aqueous extract of zobo calyces, shows regeneration of pancreatic islets and a slight alteration of pancreatic acinar cells (C1-C2). Group 4, (D1-D2) induced with 200 mg/kg bw alloxan monohydrate and administered 500 mg/kg bw of the aqueous extract of zobo calyces shows better regeneration because of the presence normal architecture of pancreas with pancreatic islets. Photomicrograph for group 5 (E1-E2), induced with alloxan monohydrate (200 mg/kg bw) and treated with 150 mg/kg bw of the standard drug metformin shows presence of normal architecture of the pancreas with several pancreatic acinar cells suggesting improved regeneration.

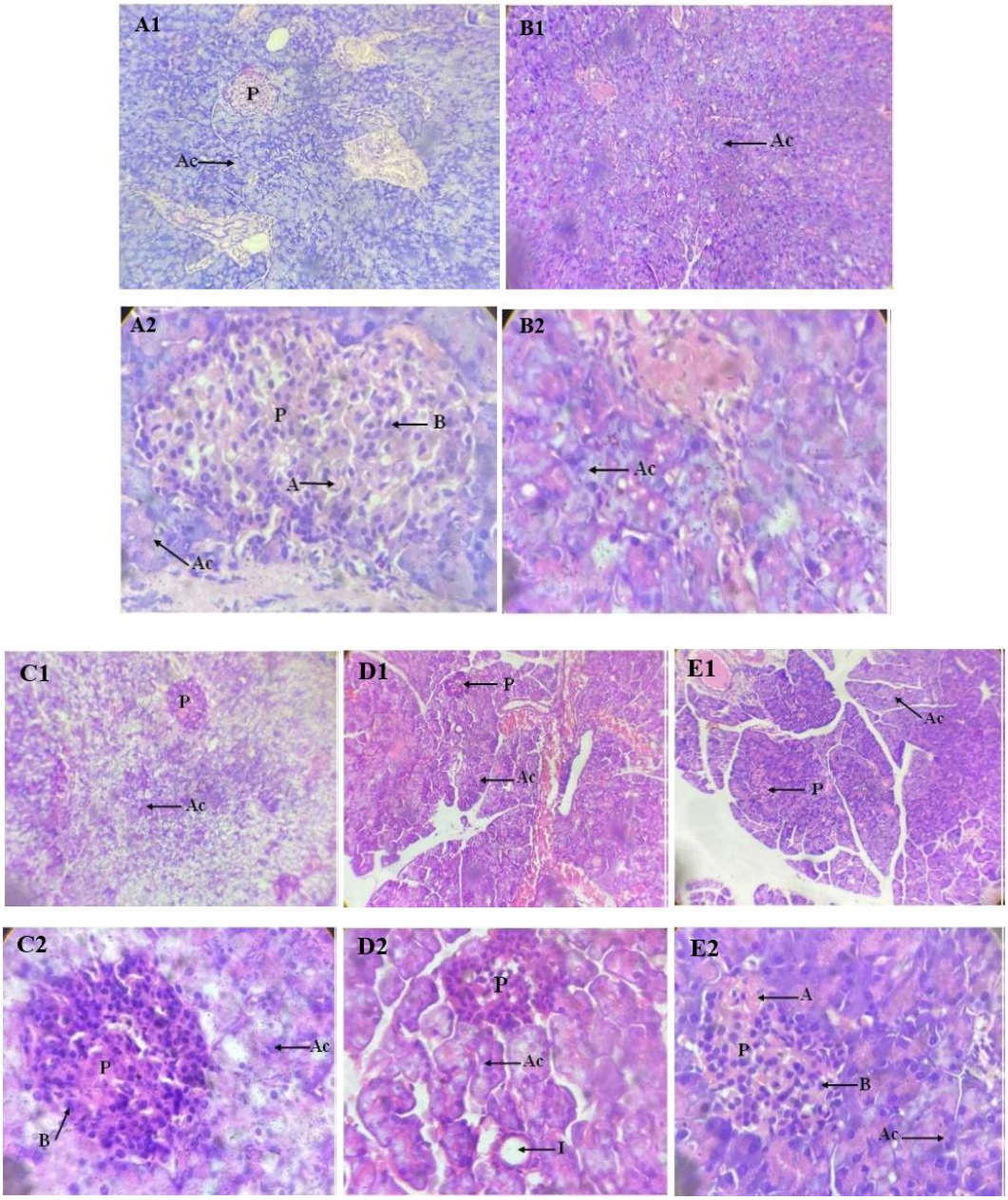

Figure 1. Photomicrographs of pancreas sections stained with Hematoxylin and Eosin (H&E) (A1-E1 Magnification 100X; A2-E2 Magnification 400X). (A1 & A2) The pancreas contains several distinct pancreatic acinar cells with pancreatic islets with alpha and beta cells. (B1 & B2) The 200 mg/kg bw alloxan monohydrate only shows the absence of pancreatic islets with altered pancreatic acinar cells. (C1 & C2) diabetic group treated with 200 mg/kg/bw of the aqueous extract of zobo calyces shows regeneration of pancreatic islets with slight alteration of pancreatic acinar cells. (D1 & D2) diabetic group treated with 500 mg/kg bw of the aqueous extract of zobo calyces shows normal architecture of the pancreas with pancreatic islets and several pancreatic acinar cells compared to C and D. (E1 & E2) diabetic group treated with 150 mg/kg bw metformin shows normal architecture of the pancreas with pancreatic islets and several pancreatic acinar cells suggesting regeneration. Alpha and beta cells are present in the islets of the pancreas. Ac= Acinar cells, P=Islets of the pancreas, A=Alpha pancreatic cells, B=Beta pancreatic cells, I= Interlobular duct

DISCUSSION

This research investigated the impact of aqueous Zobo (*Hibiscus sabdariffa*) extract on pancreatic integrity in Wistar rats following alloxan-induced diabetes. As a metabolic condition, diabetes results in profound morphological and functional pancreatic degradation, primarily through the depletion of insulin-secreting beta cells. The use of natural plant extract such as *Hibiscus sabdariffa* has gained attention because of their antioxidant and regenerative properties.

Alloxan, a widely used chemical, induces type 1 diabetes mellitus in experimental laboratory animals because of its selective toxicity to pancreatic beta cells. Its mechanism involves the rapid generation of reactive oxygen species (ROS), which precipitates an overwhelming oxidative burden and cellular necrosis, leading to insulin deficiency and hyperglycemia [25]. This mechanism copies the beta-cell destruction seen in human type 1 diabetes and provides a reliable platform for studying antidiabetic agents. In this study, the elevated glucose levels observed after alloxan administration confirmed successful induction of diabetes.

The histological analyses of this study reveals that *Hibiscus sabdariffa* (Zobo) calyces' aqueous extract promotes structural recovery of pancreatic tissues damaged by oxidative stress from alloxan induction. The untreated diabetic group (group 2) presented clear signs of pancreatic injury including disrupted pancreatic acini and complete absence of islets confirming the cytotoxic effects of alloxan. In contrast, the *Hibiscus sabdariffa* (Zobo) calyces aqueous extract treated groups (3 and 4) shows progressive improvement; with the high-dose group (group 4), demonstrating almost complete restoration of pancreatic histoarchitecture. This regenerative potential highlights the possible role of bioactive constituents, particularly anthocyanins and flavonoids in cellular repair and beta-cell protection [21,22]. The similarity in histological outcomes between the high-dose *Hibiscus sabdariffa* (Zobo) calyces group and the metformin group further supports the potential of the plant's calyces' as an effective alternative or adjunct therapy.

CONCLUSION

This study concludes that aqueous extract of *Hibiscus sabdariffa* (Zobo) calyces possesses potent restorative properties. By protecting beta cells from oxidative stress-induced apoptosis, it facilitates pancreatic regeneration, offering a promising, affordable, and natural adjunct therapy for diabetes management.

Conflict of interest

The authors have no conflicts of interest to declare.

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Authors' contributions

SID conceived the idea of research and design, data interpretation as well as article drafting. TIC was involved in the article drafting and laboratory research. AA advised on research design as well as article drafting and revising. GD participated in the interpretation of result and article revising. MIA was involved in the result presentation, interpretation and article drafting and revising. EEO played advisory role in the methodology design and article drafting and revising.

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