



## Effect of Aqueous Extract of *Hibiscus Sabdariffa* Calyces Against Alloxan-Induced Kidney Damage in Adult Female Wistar Rats



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### ABSTRACT

Diabetes mellitus is a chronic disorder that is both capable of disrupting metabolism and causing severe long-term complications, diabetic nephropathy being an example. The potential of the *Hibiscus sabdariffa* calyces aqueous extract against alloxan-induced kidney damage in adult Wistar rats (female) was evaluated in this study. Ten of the rats (80 –150 g) were group into five. Group I, the normal control received distilled water only. Groups II–V were induced with diabetes via a single administration of alloxan monohydrate (200 mg/kg) intraperitoneally to induce type 1 diabetes mellitus. While group II remained an untreated, groups III and IV were treated with 200 and 500 mg/kg aqueous extract of *Hibiscus sabdariffa* calyces respectively, and Group V, metformin (150 mg/kg) for seven days. The kidney tissues were harvested for histological examination and stained with Hematoxylin and Eosin following euthanasia. The result of the diabetic control (Group II) revealed renal impairment, glomerular enlargement, narrowed bowman's space, and tubular distortion. Group III (200 mg/kg aqueous extract) showed persistent damage and glomerular necrosis. Conversely, Group IV (500 mg/kg aqueous extract) demonstrated remarkable recovery, with the renal architecture with the glomeruli and bowman's capsules appearing normal, similar to the control and metformin groups indicating that *Hibiscus sabdariffa* calyces possess protective properties in the kidney. The structural renal damage caused by diabetes was effectively mitigated at 500 mg/kg of the aqueous extract, showing possible efficacy comparable to metformin. This suggests that *H. sabdariffa* is a promising therapeutic agent, able to manage complications in diabetic kidney.

### Keywords:

Alloxan,  
Diabetes mellitus,  
*Hibiscus sabdariffa*,  
Nephropathy,  
Wistar rats

### INTRODUCTION

Diabetes is a major metabolic syndrome, involving inappropriately enhanced glucose levels or defective insulin secretion. When we speak about diabetes, hypoglycemia, lipid metabolism, carbohydrates, and proteins are the key factors that compose the whole picture of this specific disease (Davis *et al.*, 2006). The disease predisposes individuals to a spectrum of lasting adverse complications such as renal diseases, retinopathy, and heart disease (American diabetes association, 2023). The high figure of 150 million people worldwide is already suffering with diabetes and this is predicted to double that amount in 2025 making it 300 million in 2025 (Aziz *et al.*, 2007).

A total of eight hundred and thirty million humans around the world are affected by diabetes, more than half of these people reside in countries earning low and middle income (World Health Organization (WHO), 2016). Individuals with diabetes can experience heart failure, stroke, kidney failure limb amputation and blindness (Mohd *et al.*, 2025). Diabetes mellitus include type 1 (T1DM), type 2 (T2DM), maturity-onset, gestational, neonatal diabetes and secondary cause could be due to endocrinopathies, or steroid use. Common forms are the type 1 (T1DM) and the type 2 (T2DM). T1DM occurs mostly in children or adolescence meanwhile T2DM is thought to affect adults who have hypoglycemia as a result of poor dietary choices.

Type 1 diabetes occurs when there is pancreatic failure to keep pace with production of insulin due to the beta cells having entered a state of failure. People affected with type 1 diabetes totally depend on insulin in synthetic form while type 2 diabetes (insulin independent) affected individuals can be treated by diet modifications, frequent exercise and medication (Matteucci *et al.*, 2000). Diabetic kidney disease, also referred to as diabetic nephropathy, is an acute complication of both T1DM and T2DM. It is the primary cause of chronic renal failure (Rabkin, 2003). Insulin and hypoglycemic drugs usage for treating diabetes may have repercussions in the form of side effects (Aziz *et al.*, 2007). Medicinal plants have been used all through centuries and even in recent times they are commonly used. Several species have been studied concerning their antidiabetic potentials.

*Hibiscus sabdariffa*, also called Roselle is a shrubby annual or perennial plant and its family is Malvaceae (Frank *et al.*, 2012; Rasool *et al.*, 2024). The plant being edible is known for different names by different regions: Roselle or Red sorrel (English), Bissap (Senegal), Sobolo (Ghana), Folera (Cameroon), Zobo or Zaborodo (Nigeria), Jamaica (Spanish), Karkadeh (Arabic), Gongura/Lal-ambari/Patwa (Hindi), Pulachakiri (Kannada) and Polechi (Malayalam) (Anokwuru *et al.*, 2011). *Hibiscus sabdariffa* enjoys popularity since almost every portion whether seeds, stems, leaves and flowers have medicinal, nutritional, and industrial purposes (Riaz & Chopra, 2018). The most pharmacologically significant part is assumed to be the calyces (Hapsari *et al.*, 2021). Ancient Chinese records tell about its application as a tea to cure hypertension, fever, bladder and kidney stones, and inflammation (Tahir *et al.*, 2017; Lee *et al.*, 2018). The calyces and leaves of plants infused in India, Mexico, and Africa are used as diuretics, choleric, antipyretics, hypotensives, and they enhanced intestinal motility and blood viscosity (Da-Costa-Rocha *et al.*, 2014). The Indians use to relieve indigestion or painful urination, while in Thailand it is taken to relieve urinary and kidney stones (Riaz & Chopra, 2018). Likewise, in Egypt, infusions were made to treat cardiovascular diseases, neuropathies and fever (Da-Costa-Rocha *et al.*, 2014). Phytochemistry and pharmacology have demonstrated that *Hibiscus sabdariffa* has neuroprotective, antidiabetic, hepatoprotective, antihypertensive, vasodilatory, as well as hypolipidemic properties (Da-Costa-Rocha *et al.*, 2014). Besides having therapeutic value, roselle has usage in food preparation as a food source, as a natural dye for food and textile sectors. It has been reported that *Hibiscus sabdariffa* contain ascorbic acid in high amount, as well as iron, niacin, riboflavin, calcium and anthocyanins with low sugar concentration (Builders *et al.*, 2010; Aganbi *et al.*, 2017; Teye *et al.*, 2019). Its seeds are also a valuable source of minerals such as calcium,

phosphorus, magnesium and sodium (Salami & Afolayan, 2021).

## MATERIALS AND METHODS

### Collection of plant, authentication and extraction

Calyces of the plant, *Hibiscus sabdariffa* were bought at Masaka local market, in Nasarawa State and authenticated at the Department of Pharmacognosy, Faculty of Pharmaceutical Sciences, Bingham University, Karu, Nasarawa State, Nigeria (Voucher number-DPHBHU 0083). The plant extraction was carried out in the Chemistry department of Bingham University. *Hibiscus sabdariffa* dry calyces were hand-picked and cleaned to eliminate dirt. The calyces were dried following washing under running water. Following immersion in distilled water, the calyces were left to macerate for three days at room temperature. The mixture was agitated intermittently to enhance the efficiency of the aqueous extraction. The mixture was passed through a fine mesh to isolate the liquid extract. The liquid after filtration was concentrated using rotary evaporator under vacuum and at temperature approximating 70°C.

### Ethical clearance

All protocols and procedures of treatment were in accordance to the guidelines of Animal Care and Use Committee (National Research Council, 2011) and as approved by the Faculty of Basic Medical Sciences Ethics Review Committee Bingham University, Karu, Nasarawa State, Nigeria. The approval number is BHUCAUC/CMS/2025/0010.

### Experimental Animals

Ten adult female wistar rats (80-150 g) were procured and housed at the Bingham University animal house. To ensure physiological stabilization, the rats were given two weeks to habituate to their surroundings. Standardized conditions were strictly maintained, with temperature at about 25°C and a synchronous 12-hour light/dark rhythm. Nutritional requirements were met through daily feeding with standard rodent chow. Following a 12-hour overnight fast, diabetes mellitus (type 1) was induced via single administration of alloxan monohydrate (200 mg/kg) intraperitoneally, which had been freshly dissolved in 0.9% normal saline. Diabetes mellitus was confirmed 72 hours post-induction using an Accu-Chek Active glucometer; when blood glucose concentrations exceeded 200 mg/dL (Ighodaro *et al.*, 2017; Banda *et al.*, 2018). This was performed after fasting the rats' for 12 hours and obtaining blood from the tail. The blood was dropped on the glucometer and readings taken (Idu *et al.*, 2024). After a 7-day treatment regimen of aqueous *Hibiscus sabdariffa* extract and metformin, the animals were euthanized via chloroform inhalation for tissue

collection on day 8. Table 1 describes the experimental design.

**Table 1: The Design of the Experiment**

Groups	Dosage	Duration (Days)
I	Control (distilled water) 2 ml/kg	1 – 7
II	AM (200 mg/kg)	1 – 7
III	AM (200 mg/kg) + AEHS (200 mg/kg)	(1 – 3) + (4 – 7)
IV	AM (200 mg/kg) + AEHS (500 mg/kg)	(1 – 3) + (4 – 7)
V	AM (200 mg/kg) + MTFM (150 mg/kg)	(1 – 3) + (4 – 7)

AM (Alloxan monohydrate), AEHS (Aqueous extract of *Hibiscus sabdariffa*), MTFM (Metformin)

### Histological analysis and photomicrographs

The kidneys were removed following euthanasia and fixed in 10% formalin. Histological analysis and stain were carried out using Bancroft & Gamble (2007) method. The slides were view using a light microscope and photomicrographs obtained at x100 and x400.

### RESULTS AND DISCUSSION

Figure 1 (A1-E2) shows the photomicrograph of the rats kidneys after the completion of the experiment. The normal control group 1 (A1 and A2) follows normal

histoarchitecture of the kidney. The bowman capsule and space, glomerulus and convoluted tubules appear normal. Group 2, the diabetic control (B1 and B2) which was induced with alloxan monohydrate (200 mg/kg/bw) with no further treatment presented with enlarged glomerulus, narrow bowman space and proximal tubule and distal tubule alteration. Group 3 diabetic treatment group (C1 and C2), treated with 200 mg/kg/bw *Hibiscus sabdariffa* calyces aqueous extract showed histoarchitecture alteration and necrosis of the glomerulus. Group 4 diabetic treatment group (D1 and D2) treated with 500 mg/kg/bw *Hibiscus sabdariffa* calyces aqueous extract shows recuperation with bowman capsule, glomerulus and tissue architecture presenting as normal. Group 5 diabetic treatment (E1 and E2) administered metformin shows normal histoarchitecture of the kidney.

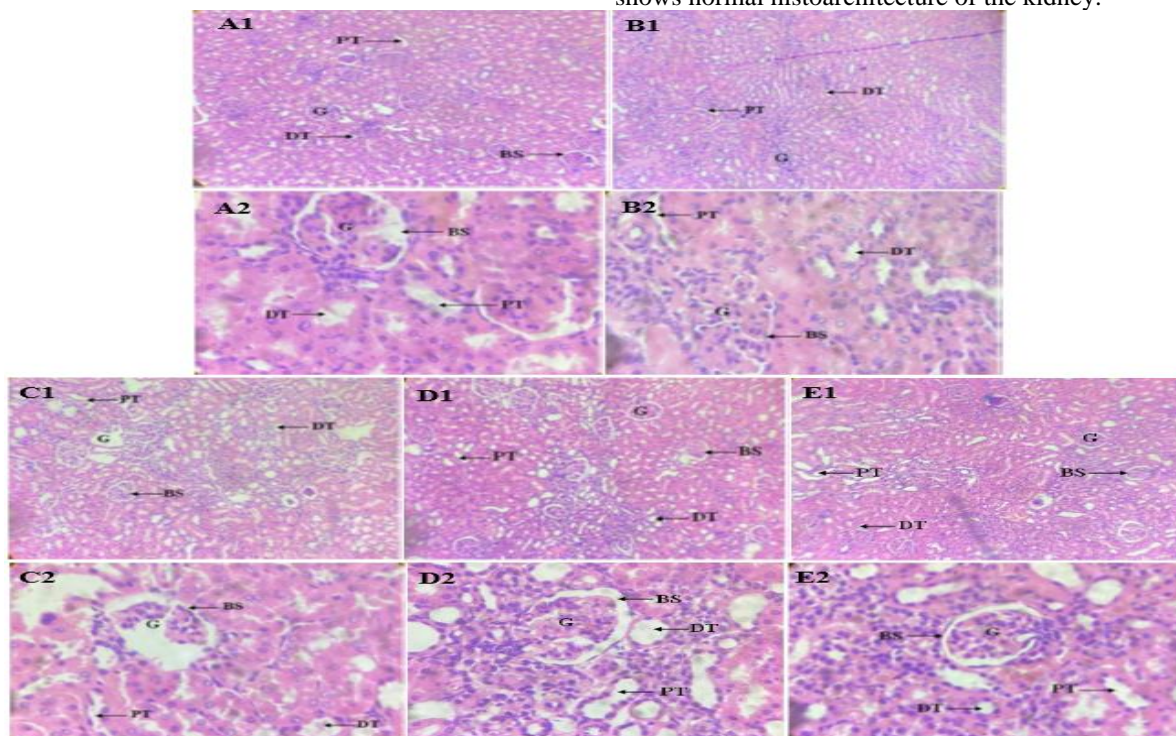


Figure 1: Photomicrograph of longitudinal section of kidney of adult female wistar rat group 1-5. Control group 1 given distilled water (A1, X100; A2, X400). Shows normal glomerulus, bowman capsule, proximal tubule and distal tubule. Diabetic control group 2 administered alloxan monohydrate only (B1, X100; B2, X400). Presence of enlarged glomerulus and narrow bowman space with alteration in tissue architecture. Group 3 rat given alloxan monohydrate and 200 mg/kg/bw of aqueous extract of *Hibiscus sabdariffa* calyces (C1, X100; C2, X400). Distorted glomerulus and alteration in tissue architecture. Group 4 given alloxan monohydrate and 500 mg/kg/bw of aqueous extract of *Hibiscus sabdariffa* calyces (D1, X100; D2, X400). Bowman capsule, glomerulus, bowman space, proximal tubule and distal tubule appear normal suggesting recuperation. Group 5 given alloxan monohydrate and metformin (E1, X100; E2, X400). Presence of normal tissue architecture suggesting recuperation. G= Glomerulus, PT= Proximal tubule, BS= Bowman capsule, DT = Distal tubule. H&E, Magnification: x100.

Diabetes mellitus is a chronic disorder that is both capable of disrupting metabolism with persistent hyperglycemia as a result of both impaired secretion and defective action of insulin, or both (American Diabetes Association (ADA), 2024; Yameny, 2024). Complications arising from it such as nephropathy, retinopathy, neuropathy, and cardiovascular disease results from long standing hyperglycemia, and is largely due to oxidative stress and inflammation (Ceriello, A., 2006; Magliano & Boyko, 2021). Globally, diabetic nephropathy particularly, is a leading cause of chronic renal disease (Rout & Jialal, 2026). Animal models used for experiment such as alloxan-induced diabetes mellitus in adult wistar rats provide valuable understanding into the origin of diabetes, serving as platforms for testing potential therapeutic agents.

The effect of the calyces of *Hibiscus sabdariffa* (Roselle) aqueous extract on the kidney of alloxan-induced diabetic adult female wistar rats was evaluated in this study. The plant is consumed commonly as a beverage in Nigeria and many parts of Africa, and is abundant in bioactive substances including anthocyanins (cyanidin-3-sambubioside, delphinidin-3-sambubioside), phenolic acids and flavonoids reported to exert properties against oxidative stress, hypertension and diabetes (Da-Costa-Rocha *et al.*, 2014; Riaz & Chopra, 2018). These compounds with the potential to improve glycemic control, clear free radicals, and diminish lipid peroxidation, they possess nephron protection. The findings are discussed in relation to the histological integrity of the kidney of the rats.

The histological analysis provides compelling evidence of both the toxic effect of alloxan-induced diabetes mellitus and the defensive role of aqueous extract of the

calyces of *Hibiscus sabdariffa* on the kidney. Normal tissue architecture was maintained in the normal control group I, as the bowman capsule, bowman space, glomerulus and convoluted tubules appeared normal. The diabetic control group II exhibited significant renal damage, characterized by glomerular enlargement, narrowing of the bowman's space, and architectural distortion of the proximal and distal tubules. These findings are consistent with studies that have established pathology of diabetic nephropathy, where glomerular hypertrophy, mesangial expansion, and tubular injury were observed (Reutens & Atkins, 2011; Karalliedde *et al.*, 2016). The group treated with 200 mg/kg/bw of the aqueous extract, group III showed continued damage with glomerular necrosis, suggesting its insufficiency in counteracting the diabetic mellitus damage. The group treated with the 500 mg/kg/bw of the aqueous extract, group IV demonstrate renal histoarchitecture preservation. Normalcy of the kidneys appearance, with distinct glomeruli, clear bowman's space, and well-organized tubules, comparable to the normal control (Group I) and the metformin-treated group (Group V). Thus, the high-dose aqueous extract of *Hibiscus sabdariffa* possesses significant kidney protective properties, effectively preventing the structural damage typically caused by hyperglycemia. This effect is suggested to be attributable to *Hibiscus sabdariffa* rich array of bioactive compounds, such as anthocyanins (e.g., delphinidin-3-sambubioside), phenolic acids and flavonoids, known for their potent activities against oxidative stress and inflammation activities (Da-Costa-Rocha *et al.*, 2014; Riaz & Chopra, 2018).

## CONCLUSION

This research demonstrates that the kidney can be damaged by diabetes mellitus induced by alloxan monohydrate. The aqueous extract of the calyces of *Hibiscus sabdariffa*, especially at high dose possess remarkable protective effects against this damage and effectively preserved the renal architecture. These findings suggests *Hibiscus sabdariffa* as a promising complementary agent for managing kidney complications arising due to diabetic mellitus and recommends investigation using isolated active constituents of the plant and elucidating precisely their molecular pathways.

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## Conflict of Interest

The authors report no competing interests.

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