Hypoglycemic and Diuretic Effects of Hibiscus sabdariffa Calyx and Flower in Wistar Rats: An Integrative Review of Preclinical Evidence

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Abstract

Preclinical research over the last decade has repeatedly shown that Hibiscus sabdariffa offers meaningful biological effects that touch two important aspects of metabolic health: blood sugar control and kidney function. Studies using Wistar rats reveal that both the calyx and flower extracts help lower glucose levels, improve the way the body handles insulin, and calm oxidative and inflammatory stress two drivers of metabolic dysfunction. At the same time, the plant acts as a natural diuretic, gently increasing urine output and supporting renal filtration without the harsh electrolyte loss seen in synthetic drugs. These benefits appear to stem from a rich mix of anthocyanins, hibiscus acid, organic acids, and flavonoids that work together rather than individually. However, the existing evidence is uneven; researchers often use different extraction approaches, doses, and animal models, making it difficult to compare results across studies. Moving forward, clearer standardization, stronger pharmacokinetic data, and carefully planned clinical research will be necessary to understand how these promising preclinical findings may translate into human therapies.

Keywords: Hibiscus sabdariffa, Hypoglycemic activity, Diuretic activity, Wistar rats, Anthocyanins, Hibiscus acid, Polyphenols, Insulin sensitivity, Oxidative stress, Renal biomarkers, Natural products, Phytotherapy

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1. Introduction

Hibiscus sabdariffa, widely recognized for its bright red calyces and pleasant sour taste, has gained increasing scientific attention due to its rich phytochemical profile and diverse biological activities. Traditionally consumed as a refreshing beverage in many tropical and subtropical regions, the plant has become a focus of contemporary research owing to its potential therapeutic effects. Over the past decade, studies have highlighted its antioxidant, anti-inflammatory, hypoglycemic, and diuretic properties, making it a promising botanical candidate for managing metabolic and renal-related disorders (Amin & Hamid, 2018).

1.1 Overview of Hibiscus sabdariffa

Hibiscus sabdariffa Linn., commonly known as Roselle, belongs to the Malvaceae family and is cultivated across Africa, Asia, and the Caribbean. The plant is characterized by its fleshy red calyces, which are rich in bioactive compounds such as anthocyanins, flavonoids, organic acids, and polysaccharides. These constituents contribute not only to its distinct color and flavor but also to its broad therapeutic potential. Roselle extracts are widely used in beverages, food products, and traditional medicine systems due to their recognized pharmacological benefits (Mahadevan et al., 2009). Recent phytochemical analyses continue to validate the presence of potent antioxidants, particularly delphinidin-3-sambubioside and cyanidin-3-sambubioside, which are responsible for many of its health-enhancing effects (Christian & Jackson, 2009).

1.2 Traditional Medicinal Uses

Historically, H. sabdariffa has been utilized in various traditional healing practices for its medicinal value. In African folk medicine, the calyx infusion is commonly used to control blood pressure, manage fever, and support kidney function. In Asian traditional systems, the plant has been employed as a gentle diuretic, digestive aid, and liver tonic. The dried calyces are also consumed as herbal teas to soothe inflammation, relieve mild constipation, and improve metabolic well-being. These long-standing uses reflect the plant's safety profile and its potential for addressing both acute and chronic health conditions (Dhar et al., 2014). The

widespread use of Roselle across cultures suggests a strong foundation for its continued investigation in modern pharmacology (Alarcon-Aguilar et al., 2007).

1.3 Rationale for Investigating Hypoglycemic and Diuretic Effects

The growing global prevalence of metabolic disorders, particularly diabetes mellitus and hypertension-associated renal complications, highlights the need for safe and affordable complementary therapies. Hibiscus sabdariffa has emerged as a promising candidate due to its demonstrated effects on glucose metabolism and renal function. Preclinical studies indicate that its polyphenolic compounds may enhance insulin sensitivity, modulate carbohydrate-digesting enzymes, and reduce oxidative stress mechanisms, collectively contributing to improved glycemic control (Olatunji & Afolayan, 2017). Additionally, its natural diuretic activity, attributed to organic acids and flavonoids, supports increased urine output and helps manage fluid retention, blood pressure, and urinary system health (Ali et al., 2018). Given these dual pharmacological benefits, investigating the hypoglycemic and diuretic properties of H. sabdariffa is scientifically justified and clinically relevant, especially for populations at risk of diabetic kidney complications (Haji Faraji & Haji Tarkhani, 1999).

2. Botanical Profile of Hibiscus sabdariffa

Hibiscus sabdariffa is an annual shrub cultivated widely in tropical and subtropical regions for its medicinally valuable calyces and flowers. Over the years, the plant has gained significant attention due to its rich phytochemistry and diverse pharmacological applications. Its morphological characteristics, taxonomic position, and part-specific chemical profiles contribute to its broad therapeutic relevance. Recent botanical and pharmacognostic studies have refined our understanding of the species and its medicinally important components (Rashid et al., 2021).

TABLE 2.1: Taxonomic Classification of Hibiscus sabdariffa

Taxonomic Rank	Description	Reference
Kingdom	Plantae	Pratama et al., 2022
Division	Magnoliophyta	Pratama et al., 2022
Class	Magnoliopsida	Rashid et al., 2021
Order	Malvales	Rashid et al., 2021
Family	Malvaceae	Sowunmi et al., 2023
Genus	*Hibiscus*	Rashid et al., 2021
Species	*Hibiscus sabdariffa* L.	Pratama et al., 2022
Common Names	Roselle, Karkade, Gongura	Sowunmi et al., 2023

TABLE 2.2: Morphological Features of Hibiscus sabdariffa

Plant Part	Morphological Characteristics	Reference
Stem	Erect, reddish, branched; height 1.5–2 m	Rashid et al., 2021
Leaves	Deeply lobed (3–5); alternate arrangement	Pratama et al., 2022
Flowers	Pale yellow petals with maroon center	Sowunmi et al., 2023
Calyces	Fleshy, bright red, acidic	Pratama et al., 2022
Seeds	Brown, kidney-shaped	Rashid et al., 2021
Root	Taproot with lateral fibers	Sowunmi et al., 2023

TABLE 2.3: Major Phytochemical Constituents of H. sabdariffa

Phytochemical Group	Key Compounds	Biological Role	Reference
Anthocyanins	Delphinidin-3-sambubioside, Cyanidin-3-sambubioside	Antioxidant, cardioprotective	Nguyen et al., 2020
Flavonoids	Quercetin, Hibiscetin, Kaempferol	Anti-inflammatory, hepatoprotective	Alqahtani et al., 2021
Phenolic Acids	Protocatechuic acid, Caffeic acid	Anti-diabetic, antioxidant	Rahman et al., 2022

Organic Acids	Hibiscus acid, Hydroxycitric acid	Diuretic, lipid control	Adegbuyi et al., 2024
Polysaccharides	Pectins, soluble fibers	Gut health support	Adegbuyi et al., 2024
Volatiles	Terpenes, aldehydes	Antimicrobial effects	Alqahtani et al., 2021

TABLE 2.4: Part-Specific Differences (Calyx vs. Flower)

Parameter	Calyx	Flower	Reference
Anthocyanins	Very high	Moderate	Alqahtani et al., 2021
Flavonoids	Moderate	High (quercetin-rich)	Rahman et al., 2022
Organic Acids	High	Medium	Adegbuyi et al., 2024
Volatile Oils	Low	Higher	Alqahtani et al., 2021
Total Phenolics	High	Moderate	Nguyen et al., 2020
Major Use	Hypoglycemic, diuretic	Anti-inflammatory	Rahman et al., 2022

3. Pathophysiology of Hyperglycemia and Fluid Retention

Hyperglycemia represents a central metabolic abnormality that underlies the progression of numerous endocrine and renal disorders, particularly in preclinical diabetes models. Persistent elevation of blood glucose disrupts cellular homeostasis, enhances oxidative stress, and activates inflammatory signaling pathways that collectively impair organ function. Experimental models of diabetes frequently replicate these metabolic disturbances, enabling researchers to understand how chronic high glucose levels alter physiologic processes such as insulin signaling, glucose utilization, and renal handling of electrolytes. These mechanisms ultimately contribute to pathological fluid retention and metabolic instability observed in diabetic states (Lin et al., 2021; Gaur et al., 2024).

3.1 Mechanisms of Hyperglycemia in Experimental Models

Experimental hyperglycemia is commonly induced to mimic type 1– or type 2–like diabetic states, depending on the research objective. Chemical induction using streptozotocin (STZ) or alloxan remains widely used for generating insulin-deficient hyperglycemia. STZ selectively damages pancreatic β-cells through DNA alkylation and excessive free-radical generation, leading to reduced insulin synthesis and persistent increases in circulating glucose (Ghasemi & Jeddi, 2023). In contrast, dietary interventions particularly high-fat or high-sucrose diets—promote insulin resistance, dyslipidemia, and impaired glucose tolerance, replicating metabolic patterns seen in type 2 diabetes (Martín-Carro et al., 2023).

Regardless of the induction method, chronic hyperglycemia alters multiple biochemical pathways. These include activation of the polyol pathway, accumulation of advanced glycation end products (AGEs), and stimulation of protein kinase C (PKC), all of which increase oxidative stress and inflammatory cytokine production within target tissues. Experimental studies consistently demonstrate that these mechanisms contribute to endothelial dysfunction, mitochondrial damage, and impaired glucose utilization, thereby perpetuating metabolic dysregulation (Sharma et al., 2022). Such models are therefore essential for evaluating therapeutics aimed at mitigating oxidative injury or improving insulin sensitivity.

3.2 Renal Regulation of Fluid Balance

The kidneys maintain body fluid homeostasis through a finely regulated system involving glomerular filtration, tubular reabsorption, and hormonal modulation. Hormones such as antidiuretic hormone (ADH), aldosterone, and natriuretic peptides govern the reabsorption of water and electrolytes, allowing the body to adjust fluid volume according to physiological demands. Specialized proteins including aquaporins, SGLT2 transporters, and various sodium channels facilitate these regulatory processes within different segments of the nephron (Suh et al., 2022).

In hyperglycemic states, renal physiology undergoes significant alterations. Excess glucose filtered at the

glomerulus increases sodium-glucose reabsorption via SGLT2, reducing sodium delivery to the macula densa and consequently triggering glomerular hyperfiltration. Over time, this promotes fluid retention, hypertension, and early nephron injury. Furthermore, oxidative stress and inflammatory mediators generated during chronic hyperglycemia impair tubular function, leading to disturbances in electrolyte excretion and progressive renal dysfunction (Okpechi et al., 2021). The interplay between hyperglycemia and altered fluid handling is therefore a key target in diabetes research.

3.3 Justification for Using Wistar Rats in Preclinical Studies

Wistar rats remain the preferred choice in preclinical metabolic and renal studies due to their well-documented physiology, stable reproductive characteristics, and predictable response to diabetogenic agents. Their larger body size compared to mice allows repeated blood sampling, detailed biochemical monitoring, and consistent evaluation of renal biomarkers such as creatinine, urea, urine output, and electrolyte excretion (Patel et al., 2024).

Moreover, Wistar rats exhibit reproducible susceptibility to STZ-induced β-cell toxicity, making them ideal for generating insulin-deficient models. Their calm temperament and ease of handling reduce procedural variability, which is crucial for maintaining reliable metabolic readings. Importantly, extensive baseline data—spanning hematological, biochemical, and behavioral parameters—facilitate accurate interpretation of treatment-related changes in hyperglycemia or fluid balance (Kole & Chowdhury, 2020). For these reasons, Wistar rats continue to serve as a robust, ethically manageable, and scientifically validated model for preclinical evaluation of hypoglycemic and diuretic interventions.

4. Preclinical Evidence of Hypoglycemic Effects

Preclinical investigations on Hibiscus sabdariffa (calyx and flower) have expanded significantly over the past decade, driven by its traditional use for glycemic control and emerging experimental support. Rodent models, particularly Wistar rats, provide a controlled platform to examine its influence on glucose homeostasis, insulin function, oxidative stress, and inflammatory pathways linked to hyperglycemia. Recent studies highlight that both aqueous and hydroalcoholic extracts exhibit promising antidiabetic potential through multimodal mechanisms (Adeyemi et al., 2021; Hassan & Mohammed, 2023).

4.1 Experimental Designs Used in Animal Studies

Experimental studies on Hibiscus sabdariffa commonly employ STZ-induced, alloxan-induced, or diet-induced hyperglycemic models to simulate Type 1 and Type 2 diabetes in Wistar rats. These models reproduce key metabolic alterations such as impaired insulin secretion, elevated fasting glucose, and oxidative stress. In most protocols, animals are divided into control, diabetic, extract-treated, and standard-drug groups. Treatment typically spans 14–28 days, allowing adequate time to observe metabolic and biochemical changes. Such structured designs ensure reproducibility and allow comparison with standard antidiabetic therapies (Kazeem et al., 2020; Singh et al., 2022).

4.2 Extract Types and Dosage Variations (Calyx & Flower)

Both calyx and flower extracts of H. sabdariffa have been evaluated, with significant differences attributed to variations in anthocyanin, flavonoid, and organic acid content. Aqueous extracts are commonly used due to their traditional relevance, while ethanol and methanol extracts offer higher phytochemical yields. Dosages typically range from 100–500 mg/kg, depending on extract concentration and study objectives. Comparative studies show that calyx extracts, being richer in delphinidin-3-sambubioside and cyanidin derivatives, often exert stronger glycemic modulation, whereas flower extracts contribute additional antioxidant effects (Alarcon-Aguilar et al., 2021; Bole et al., 2023).

4.3 Effects on Blood Glucose Levels

Consistent reductions in fasting blood glucose and post-prandial glucose levels have been reported in diabetic Wistar rats treated with H. sabdariffa extracts. Hypoglycemic activity is generally observed within the first week of treatment, with greater reductions seen in higher-dose groups. Mechanistic observations suggest that the extracts enhance peripheral glucose uptake and reduce hepatic gluconeogenesis, leading to improved glycemic control. These findings are supported by studies demonstrating 25–45% reductions in blood

glucose following calyx extract administration (Adeyemi et al., 2021; Nwosu et al., 2024).

4.4 Modulation of Insulin Secretion and Sensitivity

Several investigations highlight the role of H. sabdariffa in preserving β-cell function, improving insulin secretion, and enhancing insulin sensitivity. Anthocyanins and protocatechuic acid major constituents of the plant have been shown to protect pancreatic tissues from oxidative injury, thereby sustaining insulin synthesis. Additionally, improvements in HOMA-IR and HOMA-β indices suggest enhanced insulin responsiveness in peripheral tissues. This dual effect on secretion and sensitivity is comparable to pharmacological agents that target insulin resistance (Rahman et al., 2022; El-Sayed et al., 2023).

4.5 Antioxidant and Anti-inflammatory Pathways

Oxidative stress and inflammation are central contributors to hyperglycemia-induced tissue damage. H. sabdariffa extracts significantly enhance antioxidant enzyme activities such as SOD, CAT, and GPx while reducing MDA levels, indicating decreased lipid peroxidation. Anti-inflammatory action is mediated through suppression of TNF-α, IL-6, and NF-κB, which are typically elevated in diabetic states. Such modulation supports improved metabolic regulation and protects against complications associated with chronic hyperglycemia (Oboh et al., 2020; Chukwuma & Ibrahim, 2022; Nwosu et al., 2024).

4.6 Comparison With Standard Antidiabetic Drugs

When compared with standard antidiabetic medications such as metformin, glibenclamide, or pioglitazone, H. sabdariffa extracts demonstrate substantial and sometimes comparable improvements in glycemic parameters. Although the plant extracts may show slightly slower onset, their multitarget actions covering insulin activity, antioxidant defense, and anti-inflammatory signalling provide a broader therapeutic profile. Combined-therapy studies have even reported synergistic effects, suggesting potential for complementary use with existing pharmacological agents (Adebayo et al., 2021; Hassan & Mohammed, 2023).

Table 4.7: Preclinical Evidence of Hypoglycemic Effects of Hibiscus sabdariffa

Section	Key Findings	References
Experimental Designs Used in Animal Studies	Studies mostly use Wistar rats with STZ-induced, alloxan-induced, or high-fat diet–induced hyperglycemia. Typical protocols include diabetic control, extract-treated, and standard-drug groups over 14–28 days.	Kazeem et al., 2020; Singh et al., 2022
Extract Types and Dosage Variations (Calyx & Flower)	Both calyx and flower extracts are used in aqueous or hydroalcoholic form. Effective doses commonly range from 100–500 mg/kg. Calyx extracts often show stronger effects due to higher anthocyanin levels.	Alarcón-Aguilar et al., 2021; Bole et al., 2023
Effects on Blood Glucose Levels	Significant reductions in fasting and post-prandial glucose are observed. Calyx extract treatment typically reduces glucose levels by 25–45% after repeated dosing.	Adeyemi et al., 2021; Nwosu et al., 2024
Modulation of Insulin Secretion & Sensitivity	Extracts help preserve β-cell function, enhance insulin secretion, and improve insulin sensitivity indices. Protocatechuic acid and	Rahman et al., 2022; El-Sayed et al., 2023

	anthocyanins play major roles.	
Antioxidant & Anti-inflammatory Pathways	Extracts increase SOD, CAT, GPx and reduce MDA. Anti-inflammatory effects include lowering TNF-α, IL-6, and reducing NF-κB activation.	Oboh et al., 2020; Chukwuma & Ibrahim 2022; Nwosu et al., 2024
Comparison With Standard Antidiabetic Drugs	Effects of *H. sabdariffa* extracts are comparable to metformin and glibenclamide in many parameters; some studies show synergistic effects when combined with standard therapy.	Adebayo et al., 2021; Hassan & Mohammed, 2023

5. Preclinical Evidence of Diuretic Effects

Preclinical investigations have consistently demonstrated that Hibiscus sabdariffa particularly its calyx and flower components exerts measurable diuretic effects in rodent models. These effects are largely attributed to its phytochemical constituents, including anthocyanins, hibiscus acid, organic acids, and flavonoids, which modulate renal tubular handling of water and electrolytes. Experimental studies in Wistar rats show that administration of aqueous or hydroethanolic extracts leads to increased urine output, enhanced natriuresis and kaliuresis, and improvements in renal clearance parameters. These findings support its traditional use as a natural diuretic and highlight its potential relevance as an adjunctive therapy for conditions marked by fluid retention or mild hypertension (Olatunji et al., 2021; Ibrahim & Chukwuma, 2023).

5.1 Urine Volume and Electrolyte Excretion

A consistent observation across preclinical studies is the significant increase in urine volume following administration of Hibiscus sabdariffa extracts. This diuretic response is accompanied by enhanced sodium (Na^+) and chloride (Cl^-) excretion, with a moderate rise in potassium (K^+) excretion depending on the extract concentration. Calyx extracts, in particular, demonstrate stronger diuretic activity due to their higher organic acid content, which acts similarly to mild carbonic anhydrase inhibitors. Increased electrolyte clearance suggests improved renal tubular flow and reduced reabsorption in the proximal and distal tubules. Studies comparing low and high doses consistently report dose-dependent increases in total urine output within 24 hours of treatment (Adeyemi et al., 2022; Asante et al., 2024).

5.2 Effects on Renal Function Biomarkers

Beyond changes in urine volume, H. sabdariffa extracts influence renal biomarkers such as serum creatinine, urea, and creatinine clearance. In diabetic or toxin-induced renal stress models, calyx extracts demonstrate renoprotective effects by reducing serum urea and creatinine levels while increasing glomerular filtration markers. These findings indicate not only diuretic activity but also preservation of renal function under physiological and pathological conditions. Antioxidant components of the plant reduce oxidative injury to nephron structures, thereby supporting healthier tubular and glomerular performance (Rahman et al., 2021; Eze & Nwankwo, 2023).

5.3 Bioactive Compounds Involved in Diuresis (Anthocyanins, Hibiscus Acid)

The diuretic effects of H. sabdariffa can be traced to specific bioactive components. Hibiscus acid, one of the major organic acids in the calyx, promotes diuresis by inhibiting sodium–potassium ATPase in renal tubular cells, reducing solute reabsorption and promoting greater urinary flow. Anthocyanins, including delphinidin and cyanidin derivatives, exert vasodilatory and antioxidant actions that improve renal perfusion, further enhancing urine production. Flavonoids and polyphenols also modulate inflammatory pathways within renal tissues, supporting more efficient filtration and volume regulation (Mahadevan et al., 2022; Ogunlesi et al., 2023).

5.4 Synergistic Effects Between Calyx and Flower Extracts

Although calyx extracts are more widely studied, flower extracts also contribute to the overall diuretic potential of H. sabdariffa. Flower fractions contain higher proportions of flavonoids and volatile compounds that complement the organic acids and anthocyanins found in the calyx. Studies testing

combined calyx–flower formulations show amplified diuretic effects compared to either component alone, suggesting synergism in enhancing urine output, electrolyte excretion, and renal antioxidant status. This synergy is particularly pronounced when extracts are prepared using hydroethanolic solvents, which yield a broader spectrum of active constituents (Bello et al., 2020; Ibrahim & Chukwuma, 2023).

5.5 Comparison With Standard Diuretics

When compared with conventional diuretics such as furosemide, H. sabdariffa displays milder but physiologically meaningful diuretic activity. Unlike loop diuretics, which act rapidly and cause substantial electrolyte loss, H. sabdariffa extracts produce a more balanced effect moderate natriuresis with minimal potassium depletion. This positions the plant as a potential safer alternative for mild fluid retention or as an adjunctive therapy for long-term management of hypertension or metabolic disorders. Some studies report that high-dose calyx extract achieves approximately 40–60% of the natriuretic effect of furosemide, but with added antioxidant and anti-inflammatory benefits (Olatunji et al., 2021; Asante et al., 2024).

TABLE 5.6: Preclinical Evidence of Diuretic Effects of Hibiscus sabdariffa

Section	Key Findings	References
Preclinical Evidence of Diuretic Effects	*H. sabdariffa* calyx and flower extracts increase urine output, promote natriuresis and kaliuresis, and improve renal clearance. Effects attributed to anthocyanins, hibiscus acid, organic acids, and flavonoids. Useful for fluid retention and mild hypertension.	Olatunji et al., 2021; Ibrahim & Chukwuma, 2023
Urine Volume and Electrolyte Excretion	Increased urine volume and electrolyte excretion (Na^+, Cl^-, and mild K^+). Calyx extracts show stronger effect due to higher organic acid content. Dose-dependent diuresis seen in Wistar rats.	Adeyemi et al., 2022; Asante et al., 2024
Effects on Renal Function Biomarkers	Reduced serum creatinine and urea, improved creatinine clearance. Shows renoprotection in diabetic and nephrotoxic models due to antioxidant effects.	Rahman et al., 2021; Eze & Nwankwo, 2023
Bioactive Compounds Involved in Diuresis	Hibiscus acid inhibits Na^+/K^+-ATPase; anthocyanins improve renal perfusion; flavonoids exert antioxidant and anti-inflammatory actions supporting diuretic activity.	Mahadevan et al., 2022; Ogunlesi et al., 2023
Synergistic Effects Between Calyx and Flower Extracts	Combined extracts amplify diuretic effect—higher urine output, more electrolyte excretion, stronger antioxidant action. Hydroethanolic extracts show strongest synergy.	Bello et al., 2020; Ibrahim & Chukwuma, 2023
Comparison With Standard Diuretics	Produces 40–60% of the natriuretic activity of furosemide but with minimal potassium loss; acts more gently and offers antioxidant benefits vs. synthetic diuretics.	Olatunji et al., 2021; Asante et al., 2024

6. Integrated Analysis of Hypoglycemic and Diuretic Actions

Preclinical evidence indicates that Hibiscus sabdariffa exerts both hypoglycemic and diuretic effects through a collection of overlapping biochemical pathways. These mechanisms operate in parallel, enabling the plant to modulate glucose homeostasis, renal electrolyte handling, oxidative balance, and vascular tone. Together, these integrated actions support its therapeutic potential in conditions characterized by metabolic dysregulation, hypertension, and fluid retention (Ibrahim et al., 2023; Asante et al., 2024).

TABLE 6.1: Integrated Analysis of Hypoglycemic and Diuretic Actions of Hibiscus sabdariffa

Section	Key Findings	Mechanism/ Explanation	Calyx vs. Flower Differences	References
Shared Mechanisms	Both effects are linked to antioxidant, anti-inflammatory, and vasorelaxant actions.	Polyphenols (anthocyanins, hibiscus acid, quercetin) reduce ROS, modulate NF-κB, and enhance NO release → improves glucose control & renal perfusion.	Calyx: stronger antioxidant + organic acid contribution. Flower: stronger flavonoid-mediated protection.	Mahadevan et al., 2022; Ogunlesi et al., 2023; Rahman et al., 2021
Differential Actions (Calyx vs. Flower)	Calyx shows stronger diuretic effects; flower shows stronger metabolic effects.	Calyx rich in hibiscus acid & anthocyanins → diuretic potency. Flower rich in flavonoids → glycemic & antioxidant effects.	Combined extracts show synergism (enhanced natriuresis & antioxidant action).	Bello et al., 2020; Ibrahim & Chukwuma, 2023
Dose–Response Relationships	Dose-dependent improvements in glucose reduction and urine output.	100–200 mg/kg = mild effect; 300–600 mg/kg = significant hypoglycemic + diuretic activity. Hydroethanolic > aqueous extract.	Calyx responds more steeply to dose increases.	Adeyemi et al., 2022; Asante et al., 2024
Safety & Toxicity	Generally safe at experimental doses. High doses can cause mild renal/hepatic stress.	Preserves kidney/liver histology; reduces oxidative damage in diabetic models. Toxicity at >2000 mg/kg.	Calyx slightly more acidic → potential stress at very high dose.	Eze & Nwankwo, 2023; Olatunji et al., 2021

7. Methodological Limitations in Current Preclinical Evidence

Although preclinical studies provide valuable insights into the hypoglycemic and diuretic actions of Hibiscus sabdariffa, several methodological constraints limit the strength, reproducibility, and translational value of

current findings. These limitations arise from inconsistencies in extraction procedures, variations in experimental design, and insufficient standardization across laboratories. Understanding these constraints is essential for improving future research quality and for strengthening the evidentiary basis required to advance H. sabdariffa toward clinical application.

7.1 Variability in Extraction and Standardization

One of the most persistent limitations in preclinical research is the wide variability in extraction methods used to prepare H. sabdariffa samples. Studies frequently differ in the choice of plant part (calyx, flower, leaf), solvent system (aqueous, ethanol, methanol, hydroethanolic), extraction temperature, and phytochemical characterization. This variability leads to pronounced differences in concentrations of key bioactive constituents such as anthocyanins, hibiscus acid, flavonoids, and organic acids. Recent investigations have emphasized that extraction conditions significantly influence antioxidant capacity and total phenolic content, making comparisons between studies challenging (Sowbhagya et al., 2021). Seasonal variation, soil composition, and post-harvest drying also affect phytochemical stability, suggesting that even naturally sourced samples may differ markedly in composition (Umeokoli et al., 2022). The absence of uniform phytochemical profiling especially anthocyanin quantification limits the ability to correlate biological activity with specific constituents, thereby reducing the reproducibility of study outcomes (Leal et al., 2023).

7.2 Lack of Harmonization in Dosage and Treatment Duration

Significant differences also exist in the dosage and duration of H. sabdariffa administration across preclinical studies. Some investigations utilize single-dose, short-term protocols to assess acute diuretic responses, while others employ prolonged treatment periods to evaluate hypoglycemic effects or renoprotection. These inconsistencies hinder the ability to establish reliable dose–response relationships and complicate cross-study interpretation. Several studies have used doses ranging from 50 mg/kg to over 500 mg/kg without standardized justification related to extract potency or phytochemical load (Okoro et al., 2020). Differences in treatment duration—sometimes from as short as 24 hours to as long as 8–12 weeks further add to the variability in outcomes (Chukwuma et al., 2021). Without harmonized dosing frameworks or validated pharmacokinetic data, it remains difficult to determine optimal therapeutic doses or predict potential toxicity thresholds (Ibrahim et al., 2023).

7.3 Small Sample Sizes and Model Variability

Many preclinical studies involving H. sabdariffa rely on small sample sizes, often with fewer than six animals per group. Such limited numbers reduce statistical power, increase the likelihood of false-positive or false-negative results, and weaken the reliability of reported biological effects. Moreover, differences in animal models such as variation in species, strain, age, sex, and induction method for metabolic or renal disorders lead to inconsistent physiological responses. For example, chemically induced diabetic models may respond differently to treatment than high-fat diet models due to variations in metabolic pathways (Oboh et al., 2022). Environmental factors such as housing, diet composition, and handling can further influence outcomes, making inter-study comparisons difficult. Recent methodological evaluations emphasize that natural-product research is particularly vulnerable to these limitations, underscoring the need for more rigorous reporting and experimental controls (Martins et al., 2023).

7.4 Translational Barriers to Human Application

Despite promising preclinical findings, translating the biological effects of H. sabdariffa into clinical settings remains challenging. The complexity of plant-based extracts, which contain multiple interacting compounds, complicates efforts to identify definitive mechanisms of action or isolate the primary bioactive constituents. Furthermore, differences in human metabolism, renal physiology, and comorbid conditions may lead to responses that differ from those observed in controlled animal studies. Recent clinical assessments highlight variability in responses to H. sabdariffa supplements due to differences in formulations, such as teas, powders, capsules, or standardized extracts (Nduka et al., 2022). Limited long-term safety studies also restrict our understanding of potential adverse effects, particularly with chronic consumption or when combined with antihypertensive or diuretic medications. As emphasized in contemporary pharmacology literature, overcoming these translational gaps requires well-designed human

trials supported by standardized, reproducible preclinical data (Serrano-Medina et al., 2024).

8. Future Directions for Research

The growing body of preclinical evidence highlights the therapeutic potential of Hibiscus sabdariffa, yet several scientific gaps remain before its safe and effective translation into clinical use can be realized. Future work must therefore prioritize standardized pharmacokinetic analysis, consistent extract profiling, and targeted mechanistic investigations. Moreover, integrating H. sabdariffa into multi-herbal formulations and designing ethically sound clinical trials will be crucial to advancing its role in modern phytotherapy.

8.1 Need for Structured Pharmacokinetic Studies

Despite extensive preclinical findings, the pharmacokinetic behaviour of the bioactive constituents of H. sabdariffa including anthocyanins, quercetin derivatives, and hibiscus acid remains insufficiently explored. Rigorous absorption, distribution, metabolism, and excretion (ADME) studies are required to determine how these compounds behave in vivo, particularly with respect to oral bioavailability and metabolic stability. Recent phytopharmacology research emphasizes that polyphenolic molecules undergo rapid conjugation, influencing not only their therapeutic efficacy but also their safety profile (Martins et al., 2023). Furthermore, rodent studies suggest that the metabolic fate of standardized extracts varies substantially across dose ranges, highlighting the need for dose-dependent kinetic evaluation using LC–MS/MS-based analytical platforms (Ibrahim & Chukwuma, 2023). Comprehensive pharmacokinetic modelling will thus serve as a foundational step for bridging preclinical findings with human therapeutic potential (Serrano-Medina et al., 2024).

8.2 Standardization of Extracts and Active Compounds

One of the most pressing priorities is the establishment of uniform extraction, characterization, and standardization protocols. Variability in extraction solvents, plant maturity, geographical origin, and processing parameters leads to inconsistent bioactive profiles, thereby limiting reproducibility across laboratories. Recent comparative studies demonstrate that anthocyanin concentrations may vary more than two-fold depending on extraction methodology, thereby altering antioxidant and metabolic outcomes (Sowbhagya et al., 2021). Additionally, seasonal and environmental influences have been shown to significantly modify the phytochemical richness of the calyces, calling for tighter control of raw-material sourcing (Umeokoli et al., 2022). Standardized markers such as delphinidin-3-sambubioside and cyanidin-3-sambubioside should be adopted globally to ensure consistency in both experimental and commercial formulations (Leal et al., 2023). Establishing such benchmark standards will enhance reproducibility and facilitate regulatory acceptance.

8.3 Exploration of Molecular Targets

While antioxidant, diuretic, and metabolic benefits of H. sabdariffa have been documented, the molecular mechanisms underlying these effects remain only partially defined. Advanced omics-based approaches including transcriptomics, proteomics, and metabolomics could reveal new signalling pathways influenced by polyphenols and organic acids present in the plant. Current evidence hints at involvement of AMPK activation, modulation of renal transporters, and inhibition of key enzymes in lipid and carbohydrate metabolism (Oboh et al., 2022). However, these pathways have not been universally validated across different experimental models. Future studies should employ CRISPR-based gene modulation or receptor-binding assays to more precisely define pharmacodynamic targets (Chukwuma et al., 2021). Furthermore, systems-biology frameworks may help connect multiple biochemical pathways into an integrated therapeutic model.

8.4 Potential for Formulation of Combined Herbal Therapeutics

Given its diverse pharmacological properties, H. sabdariffa may serve as a central component in multi-herb formulations aimed at synergistic metabolic or renal benefits. Polyherbal combinations have shown promise in enhancing bioavailability, balancing phytochemical profiles, and lowering toxicity, but scientific validation remains limited. Studies in metabolic-disorder models indicate that combining H. sabdariffa with other polyphenol-rich botanicals could amplify antioxidant and lipid-modulating effects (Oboh et al., 2022). Nanoparticle-assisted delivery systems and encapsulation technologies may further optimize the stability and targeted release of key

compounds. Future formulation science must therefore prioritize pharmacodynamic synergy, dose-ratio optimization, and controlled-release systems to improve therapeutic efficacy.

8.5 Roadmap Toward Clinical Trials

The translation of preclinical evidence into meaningful human applications necessitates well-designed clinical research. Existing clinical studies on H. sabdariffa supplements show inconsistent outcomes, largely due to variability in formulations, dosage ranges, and phytochemical content (Nduka et al., 2022). To move toward regulatory approval, future trials must adopt standardized extracts with validated phytochemical markers and transparent manufacturing protocols. Phase I clinical studies should first establish safety thresholds, followed by phase II trials evaluating dose-response relationships in metabolic, renal, or cardiovascular conditions. Additionally, integration of pharmacokinetic data, long-term safety monitoring, and biomarker-based outcome measures will strengthen the scientific rigor of these clinical investigations (Serrano-Medina et al., 2024). Developing international guidelines for herbal-based clinical trials will also be essential to harmonize methodologies across research centers.

9. Conclusion

The accumulated preclinical evidence strongly supports the therapeutic relevance of Hibiscus sabdariffa as a multifunctional botanical with meaningful hypoglycemic and diuretic activity. Across diverse experimental models including STZ-induced diabetes, diet-induced metabolic dysfunction, and renal stress paradigms extracts prepared from the calyx and flower consistently demonstrate improvements in glucose regulation, renal clearance, and oxidative balance. These health-promoting effects are closely linked to the plant's distinctive phytochemical profile, enriched with anthocyanins, hibiscus acid, flavonoids, and organic acids, each contributing synergistically to its metabolic protection.

The hypoglycemic actions of H. sabdariffa extend beyond simple blood glucose reduction; they involve preservation of β-cell integrity, enhancement of insulin sensitivity, and attenuation of oxidative and inflammatory stressors that drive metabolic deterioration. Parallel diuretic responses characterized by increased urine volume, improved electrolyte excretion, and normalization of renal biomarkers further highlight the plant's utility in supporting cardiovascular and renal health. These dual actions are particularly relevant in the context of modern chronic diseases, where metabolic syndrome, hypertension, and early-stage renal dysfunction often coexist and require interventions that are both effective and physiologically gentle.

Despite these encouraging findings, interpretation must be approached with caution. Existing studies reveal substantial methodological variability, from inconsistent extraction procedures and non-standardized phytochemical content to disparities in dosages, treatment durations, and experimental models. Such variations complicate cross-study comparisons and limit the formulation of universally applicable dosing recommendations. The predominance of small sample sizes and limited toxicological profiling also prevents robust assessment of long-term safety. In addition, the translation of findings from rodent models to human physiology remains a significant challenge, with human clinical studies still too few and often compromised by inconsistent supplement quality.

Looking ahead, the advancement of H. sabdariffa as a scientifically validated therapeutic agent will require a more unified and rigorous research framework. Priority should be given to pharmacokinetic studies, standardized extract characterization, identification of precise molecular targets, and investigations into calyx–flower synergism. Integrating advanced omics platforms such as metabolomics and transcriptomics may further clarify mechanism-level interactions and guide optimized formulation development. Ultimately, bridging these preclinical insights with well-designed human clinical trials will be crucial for establishing dosage guidelines, confirming therapeutic efficacy, and ensuring safety.

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