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Nephroprotective Effect of *Hibiscus Sabdariffa* Leaf Flavonoid Extracts via KIM-1 and TGF-1β Signaling Pathways in Streptozotocin-Induced Rats

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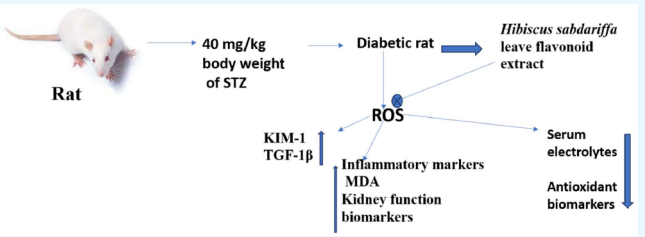
ABSTRACT: Diabetes-induced kidney damage represents a substantial health hazard, emphasizing the imperative to explore potential therapeutic interventions. This study investigates the nephroprotective activity of flavonoid-rich extracts from *Hibiscus sabdariffa* leaves in streptozotocin-induced diabetic rats. The flavonoid-rich extracts of *H. sabdariffa* leaves was obtained using a standard procedure. The animals were induced with streptozotocin and thereafter treated with both low (LDHSFL) and high doses (HDHSFL) of flavonoid-rich extracts from *H. sabdariffa* leaves and metformin (MET), and other groups are diabetic control (DC) and normal control (NC). The study assesses diverse renal parameters, encompassing kidney redox stress biomarkers, serum electrolyte levels, kidney inflammatory biomarkers, serum concentrations of creatinine, urea, and uric acid, kidney phosphatase activities, renal histopathology, and relative gene expressions of kidney injury molecule-1 (KIM-1) and transforming growth factor beta-1 (TGF-1β), comparing these measurements with normal and diabetic control groups (NC and DC). The findings indicate that the use of extracts from *H. sabdariffa* leaves markedly ($p < 0.05$) enhanced renal well-being by mitigating nephropathy, as demonstrated through the adjustment of various biochemical and gene expression biomarkers, indicating a pronounced antioxidative and anti-inflammatory effect, improved kidney morphology, and mitigation of renal dysfunction. These findings suggest that *H. sabdariffa* leaf flavonoid extracts exhibit nephroprotective properties, presenting a potential natural therapeutic approach for the treatment of diabetic nephropathy.



1. INTRODUCTION

Chronic kidney disease, often exacerbated by diabetes, poses a substantial health threat worldwide, necessitating the exploration of novel therapeutic interventions. Diabetes-associated renal damage is a critical concern, underscoring the urgency to identify effective treatments.[1,2] Historically, medicinal plants have played a crucial role in traditional and complementary medicine, providing remedies for treating, managing, and preventing various diseases throughout human existence.[3] Notably, the growing preference for herbal medicines over conventional treatments can be credited to the effectiveness of their active ingredients as natural curing agents, coupled with their availability, accessibility, affordability, and recognized low or nontoxic effects.[4] Medicinal plants and their bioactive compounds have received a lot of attention in the last ten years because of their demonstrated effectiveness in treating chronic and life-threatening conditions like cancer, neurodegenerative diseases, cardiovascular diseases, diabetes, stroke, and arthritis, as well as helping the elderly with their medical needs.[5−7] Phytochemicals in these plants act as antioxidants, neutralizing free radicals implicated in the onset of various diseases, and researchers have harnessed insights from natural product studies to develop new drug molecules through organic synthesis or isolating pure, active compounds from medicinal plants, resulting in the production of several drugs.[8,9]

The genes *KIM-1* (kidney injury molecule-1) and *TGF-1β* (transforming growth factor-1beta) play vital roles in kidney function and are implicated in diabetic-induced nephropathy.[10] *KIM-1*, normally absent in healthy kidneys, is induced in response to kidney injury, aiding in the repair of damaged tubular cells. Elevated *KIM-1* levels, associated with acute kidney injury, can serve as a biomarker for renal damage, particularly in the context of diabetic nephropathy.[11] In

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diabetes, *KIM-1* expression increases, indicating potential kidney injury, which makes it a valuable indicator for monitoring the damage extent.[12] *TGF-β1*, a multifunctional cytokine, is integral to various cellular processes in normal kidney function. In diabetic nephropathy, *TGF-β1* overexpression is common, contributing to the development of renal fibrosis and impaired function. Strategies targeting *TGF-β1* are explored for mitigating diabetic kidney complications.[13,14] A good understanding of these genes, their roles, and dysregulations is crucial for the development of effective diagnostics and therapeutics for individuals with diabetes and kidney issues.

Hibiscus sabdariffa, commonly known as Roselle or Hibiscus, originates from West Africa but is now cultivated globally and belongs to the Malvaceae family. The plant is valued for its vibrant red calyces, widely used in herbal teas and culinary creations.[15] In ethnobotany, it holds cultural significance, being integrated into traditional practices for centuries. The plant's diverse applications extend to traditional medicine, where its leaves and seeds are utilized for reported diuretic, antioxidant, and anti-inflammatory properties. Rich in bioactive compounds, such as polyphenols, anthocyanins, and vitamin C, *H. sabdariffa* boasts antioxidant properties, potentially mitigating oxidative stress and inflammation.[16,17] Scientific studies suggest cardiovascular benefits including blood pressure reduction and improved lipid profiles. Additionally, ongoing research explores its antidiabetic effects, diuretic properties, and potential antimicrobial and anticancer applications. In essence, *H. sabdariffa* intertwines its etiology, ethnobotanical significance, and multifaceted therapeutic effects, capturing global interest and sustaining cultural practices.[18,19] Despite the various bioactivities present in *H. sabdariffa*, there appears to be limited information on the impact of *H. sabdariffa* and its flavonoids or phenolic contents on the expression of *KIM-1* and *TGF-1β*, as well as other related genes associated with nephropathy in diabetic rats. The aim of the current study is to investigate how flavonoid-rich extracts of *H. sabdariffa* leaf affect the expressions of *KIM-1* and *TGF-1β* in streptozotocin-induced cardiomyopathy rats.

2. MATERIALS AND METHODS

2.1. Plant Materials Source and Authentication. Oja-Oba Market, Ado-Ekiti, Ekiti State, Nigeria, was the source of *Hibiscus sabdariffa* leaves. The identification and authentication of the leaves were carried out at the Forestry Research Institute of Nigeria, (FRIN), Ibadan, Nigeria (voucher number FHI: 113742).

2.2. Chemicals, Reagents, and Enzyme Kits. Chemicals including absolute ethanol, sulfuric acid, methanol, fructose, dilute ammonium hydroxide, concentrated ammonium hydroxide, streptozotocin (STZ), 10% formalin, phosphate buffer, and sodium citrate buffer were purchased from Sigma-Aldrich, Germany, and all chemicals were used without further purification. Enzyme kits were obtained from the Randox Laboratory (Crumlin, United Kingdom).

2.3. Extraction of Flavonoid-Rich Extract. Leaves of *H. sabdariffa* were dried at room temperature for a period of 2 weeks. Electronic blender was used to ground the air-dried leaves into a powder. The powdered sample was macerated for 72 h in 80% methanol, and the solution was filtered with muslin cloth. A rotary evaporator was used to concentrate the filtrate that was produced. Then 20 g of the residue was dissolved in 200 mL of 10% H_2SO_4 and heated to 100 °C for 30 min in a water bath to initiate hydrolysis. The mixture was left on ice for 15 min to obtain a precipitate called flavonoid aglycones.

After the flavonoid aglycone was dissolved in 50 mL of warm 95% ethanol, the mixture was filtered off. A rotary evaporator was then used to concentrate this. The filtrate was then precipitated using ammonium hydroxide, which had been concentrated. To extract the flavonoid, the entire solution was allowed to settle, and the precipitate was collected and washed with diluted ammonium hydroxide.

2.4. Experimental Animals. Two and a half-month-old male Wistar rats with a body weight of 150 ± 20 g were obtained from Show-Gold Animal House Idofin, Oye-Ekiti, Ekiti State, Nigeria. The rats were housed in groups of five (5) in a conventional laboratory. The lab temperature was adjusted to 22 ± 20 °C, and rats were adapted to a 12 h:12 h light/dark (LD) cycle. They were quarantined for 3 weeks.

2.5. Induction of Diabetes. Experimental diabetic rats were induced by feeding normal rat pellets chow and 20% fructose water for a week, while the control group of rats was given only water.[20] 12 h before the induction of streptozotocin (STZ), rats were served with only water. Then 40 mg/kg body weight of STZ was administered to the experimental animals. Control rats were administered an equivalent volume of citrate buffer pH 7.4. Only animals with fasting blood glucose ≥250 mg/dL at 72 h of STZ injection were utilized.[21]

2.6. Experimental Design and Animal Treatment. The animals were divided into five groups comprising eight rats in each group as follows:

- Group I: normal control.
- Group II: diabetic control, diabetic rats without treatment
- Group III: diabetic rats administered low dose of *H. sabdariffa* flavonoid-rich leaf extract (LDHSLF) (150 mg/kg body weight).
- Group IV: diabetic rats administered high dose of *H. sabdariffa* flavonoid-rich leaf extract (HDHSLF) (300 mg/kg body weight).
- Group V: diabetic rats placed on 200 mg/kg of metformin (MET). This treatment of the animals lasted for 21 days.

2.7. Tissue Collection and Processing. On the twenty-second day of this study, the rats were sacrificed using halothane anesthesia. Before this, the animals were fasted for 12 h. Cardiac puncture was utilized to withdraw blood samples immediately from each rat. Then, blood samples were collected into a plain bottle and centrifuged for 5 min at 5000 rpm. The serum samples were then separated and kept in a refrigerator for further analysis. Each rat's kidney was separated. The separated kidneys were washed using normal saline and cleaned by filter paper. The weighed and cleaned kidneys were then homogenized in 0.1 M potassium phosphate buffer (pH 6.5). The obtained suspension was subjected to centrifugation at 4000 rpm for 15 min to be prepared for further investigation.[22]

2.8. Kidney Oxidative Stress Biomarker Assays. The concentration of kidney malondialdehyde (MDA) was determined using the method of Oloyede et al.[23] Superoxide dismutase activity of kidney tissue was examined using Misra and Fridovich method.[24] Glutathione transferase (GST) activity was examined using the method of Habig et al.[25] with slight modifications. The activity of catalase in the kidney

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Table 1. Primer Sequences

genes	forward primer	reverse primer
KIM-1	5′-TGGCAGATTCTGTAGCTGGTT-3′	5′- AGAGAACATGAGCCTCTATTCCA-3′
TGF-1β	5′- CTTCTCCACCAACTACTGCTTC-3′	5′- GGGTCCCAGGCAGAAGTT-3′
GAPDH	5′-CATCTTCTTTTGCGTCGCCA-3′	5′-TTAAAAGCAGCCCTGGTGACC-3′

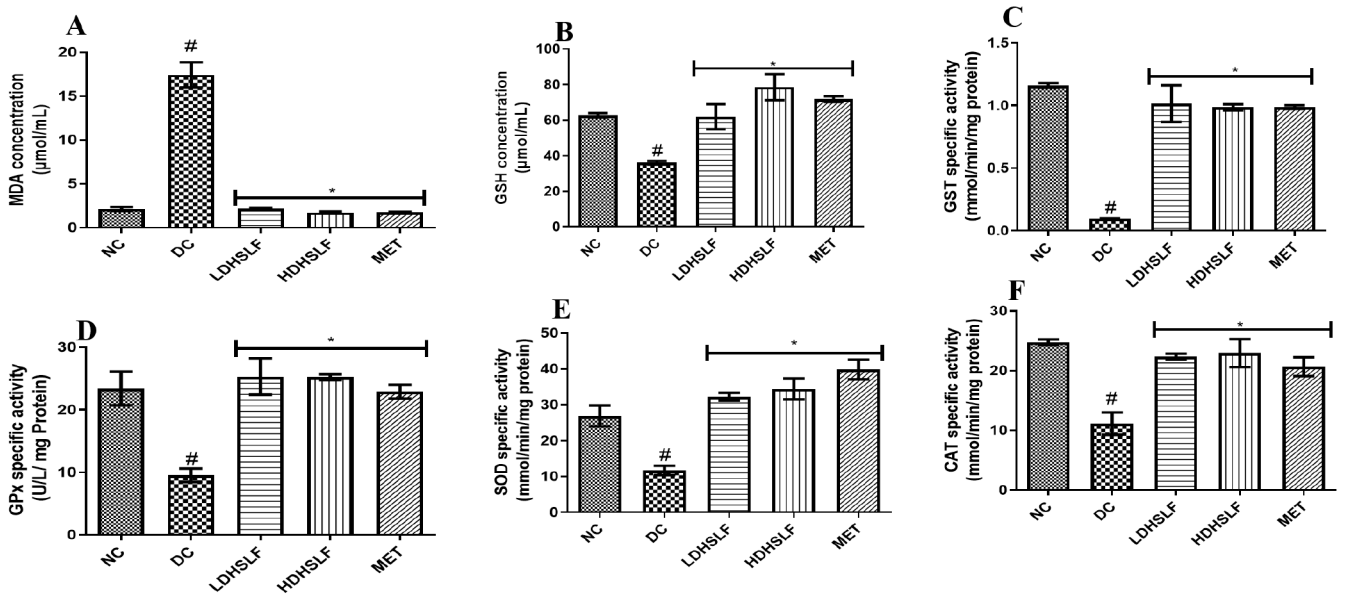

Figure 1. Kidney redox stress biomarkers (A–F) of HSLF in diabetic rats. Each value is a mean of eight determinations ± SD. #$p < 0.05$ vs NC, *$p < 0.05$ vs DC. NC: normal control, DC: diabetic control, HSLF: flavonoid-rich extract of *Hibiscus sabdariffa* leaf, LDHSLF: diabetic rats were administered a low dose (150 mg/kg body weight) of a flavonoid-rich extract of *Hibiscus sabdariffa* leaf, HDHSLF: diabetic rats were administered a high dose (300 mg/kg body weight) of a flavonoid-rich extract of *Hibiscus sabdariffa* leaf, MET: diabetic rats were administered 200 mg/kg of metformin, MDA: malondialdehyde, GSH: reduced glutathione GST: glutathione-S-transferase, CAT: Catalase, GPx: glutathione peroxidase, and SOD: superoxide dismutase.

homogenates was determined using the method used by Beers and Sizer.[26] The activity of kidney glutathione peroxidase was determined using the method of Haque et al.[27] The level of reduced glutathione (GSH) in the kidney homogenate was measured applying the Ellman's procedure.[28]

2.9. Renal Serum Electrolyte Levels Biomarker Assays. These were all measured according to the specified method using commercial kits. The serum electrolyte levels assayed for were bicarbonate (HCO_3^-) by the method of Forrester et al.;[29] potassium (K^+) and sodium (Na^+) were measured by the method of Henry;[30] and chloride (Cl^-) was measured using Tietz's method.[31] Serum calcium (Ca^{2+}) and magnesium (Mg^{2+}) levels were analyzed using Doğru Pekiner et al.[32]

2.10. Kidney Inflammatory Biomarkers Assays. ELISA kit was used to determine interleukin-1β (IL-1β), interleukin-6 (IL-6), and tumor necrotic factor-α levels applying the method described by Bergqvist et al.[33]

2.11. Renal Histopathological Examination. This was carried out utilizing hematoxylin and eosin (H&E) staining as mentioned by Blume et al.[34] Before embeding in paraffin wax, freshly excised kidney tissues were settled in 10% formalin solution for 12 h. A microtome was used to make thin slices of paraffin containing the tissues (4 μm). The specimen was picked up on a microscope slide, air-dried, and heated. To dissolve residual paraffin, it was trated with an acid-alcohol followed by water rinsing to get a red color of the acid-alcohol. Hematoxylin staining technique was used to stain slides, and the excess (bluing solution) was eliminated by water rinsing.

2.12. Serum Kidney Function Biomarker Assays. Serum concentrations of uric acid, urea nitrogen, and creatinine were determined using commercial kits on an automated biochemical analyzer. The serum urea level was determined utilizing Weatherburn's method;[35] serum creatinine, as well as urea levels, were estimated using the method described by Ran et al.[36]

2.13. Kidney Phosphatase Activities Biomarker Assays. The activities of alkaline phosphatase (ALP) and acid phosphatase (ACP) were carried out using a commercial Randox kits.

2.14. Relative Gene Expression of KIM-1 and TGF-1β. Total RNA was separated from kidney tissue with a Quick-RNA Kit (Zymo Research), and DNase I (NEB, Cat: M0303S) was used to remove the DNA contaminant. This was then converted to cDNA via reverse transcriptase and then subjected to a polymerase chain reaction (PCR) for the amplification process.[37] The GAPDH was used as the housekeeping gene, and "image J" software was used for the quantification of band intensity. Table 1 shows the primers used.

2.15. Statistical Analysis. All results obtained from the experiments were shown as mean ± SD ($n = 8$). Statistical significances were inspected by ANOVA followed by Tukey's multiple comparison (post hoc test) utilizing a GraphPad Prism, Version 5.0 software. $p < 0.05$ was fixed as statistically significant.

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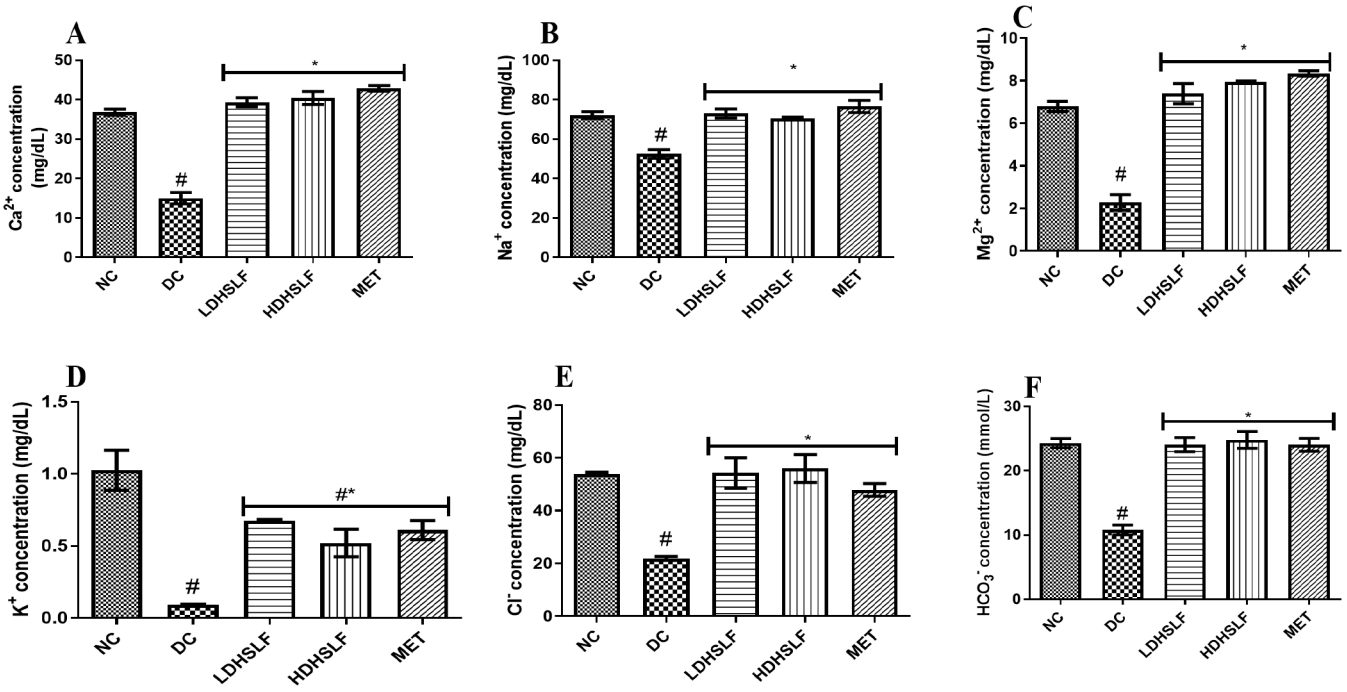

Figure 2. Electrolyte levels (A−F) of HSLF in STZ-induced diabetic rats.

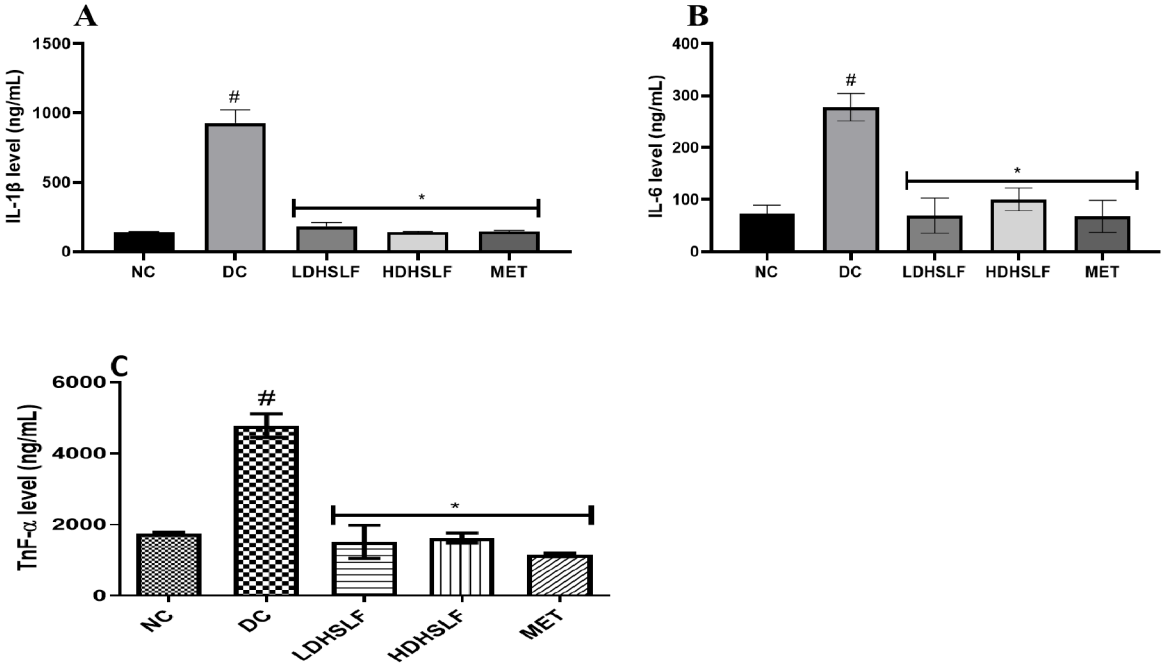

Figure 3. Kidney inflammatory biomarkers (A−C) of HSLF in STZ-induced diabetic rats.

3. RESULTS

3.1. Effect of *H. Sabdariffa* Flavonoid-Rich Leaf Extract (HSLF) on Activities/Levels of Redox Stress Biomarkers in the Kidney of STZ-Induced Diabetic Rats. Figure 1 shows the effect of HSLF on the activities or levels of kidney redox stress biomarkers in STZ-induced diabetic rats. There was a considerable ($p < 0.05$) increment in lipid peroxidation (measured by MDA level) and a sharp ($p < 0.05$) decline in antioxidant enzymes activities (glutathione-S-transferase, GST; catalase, CAT; glutathione peroxidase, GPx; and superoxide dismutase, SOD). Additionally, the level of reduced glutathione (GSH) decreased dramatically ($p < 0.05$) in the diabetic untreated group (DC) comparing with the normal one (NC). However, the administration of low and high doses of HSLF significantly mitigated these mis-normal in addition to metformin administered to near normalcy.

3.2. Effect of HSLF on Serum Electrolyte Levels in the Kidney of STZ-Induced Diabetic Rats. As shown in Figure 2, STZ administration resulted in a significant ($p < 0.05$) decrease in the concentrations of serum electrolyte levels (Ca^{2+}, Na^{+}, Mg^{2+}, K^{+}, Cl^{-}, and HCO_3^{-}) in the diabetic rats (DC) comparing with those in the normal group (NC). These

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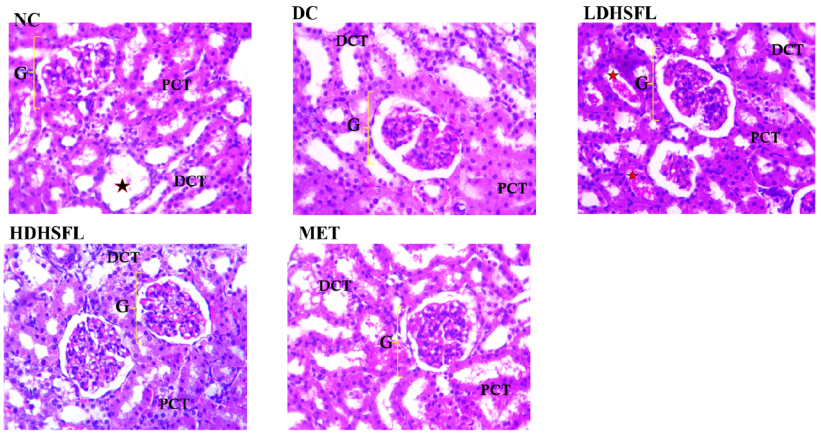

Figure 4. Kidney photomicrograph examination of HSLF in STZ-induced diabetic rats. Stained with H&E (mag. ×800).

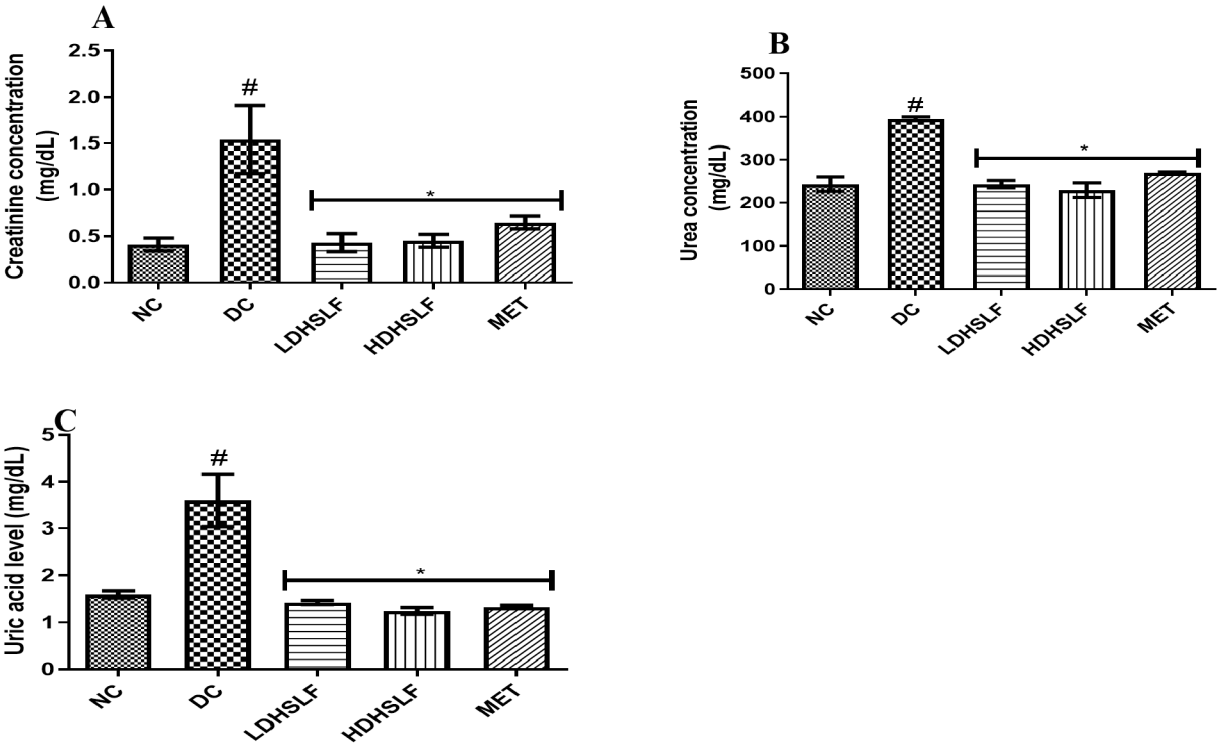

Figure 5. Creatinine (A), urea (B), and uric acid (C) concentrations of HSLF in STZ-induced diabetic rats.

dangerous decreases in renal serum electrolyte levels were enhanced significantly ($p < 0.05$) by the post-treatment of HSLF (150 mg/kg bwt and 300 mg/kg bwt) in addition to metformin (200 mg/kg bwt) as standard drug.

3.3. Effect of HSLF on Levels of Inflammatory Biomarkers in the Kidney of STZ-Induced Diabetic Rats. Figure 3 revealed the effect of HSLF on the levels of inflammatory biomarkers in the kidneys of STZ-induced diabetic rats. There was a considerable ($p < 0.05$) increment in the levels of interleukin-1β (IL-1β), interleukin-6 (IL-6), and tumor necrotic factor-α (TnF-α) in the diabetic rats (DC) compared with the normal rats (NC). Nevertheless, near-normal conditions were observed as a result of HSLF administration in low and high doses as well as metformin administration in a dose-dependent manner.

3.4. Effect of HSLF on Histopathological Changes in the Kidney of STZ-Induced Diabetic Rats. Figure 4 shows the effect of HSLF on histopathological modifications in the kidneys of diabetic rats (DC). Injurious histological modifications, such as enlargement of the glomerulus urinary space, severe loss of PCT epithelial cells, severe hemolysis, and fatal degeneration of glomerular cells, were noticed in the photomicrograph of the diabetic rats (DC) compared with the normal rats (NC). Nevertheless, HSLF administration in low and high doses led to remarkably ameliorating these observed abnormalities and standard upon close scrutiny of the photomicrograph of the diabetic-treated rats.

3.5. Effect of HSLF on the Levels of Serum Kidney Function Biomarkers in the Kidney of STZ-Induced Diabetic Rats. STZ administration to the diabetic rats caused a remarkable ($p < 0.05$) increment in the levels of serum kidney function biomarkers such as creatinine, uric acid, and urea in diabetic rats as displayed in Figure 5 compared with the normal one. Conversely, the treatment with low and high doses

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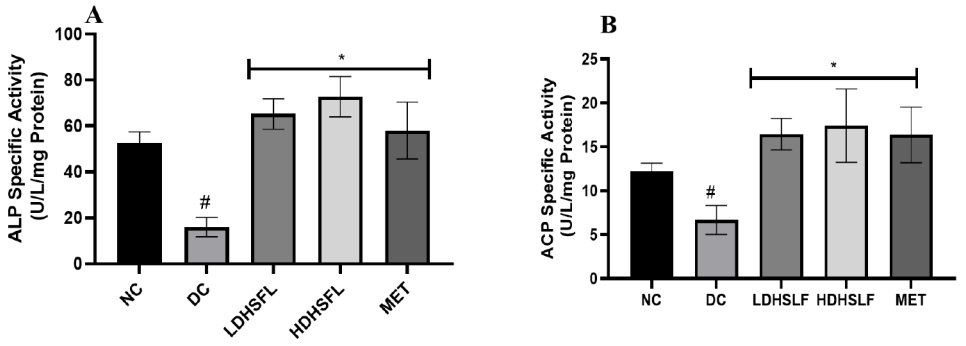

Figure 6. Kidney phosphatase activities (A,B) of HSLF in STZ-induced diabetic rats.

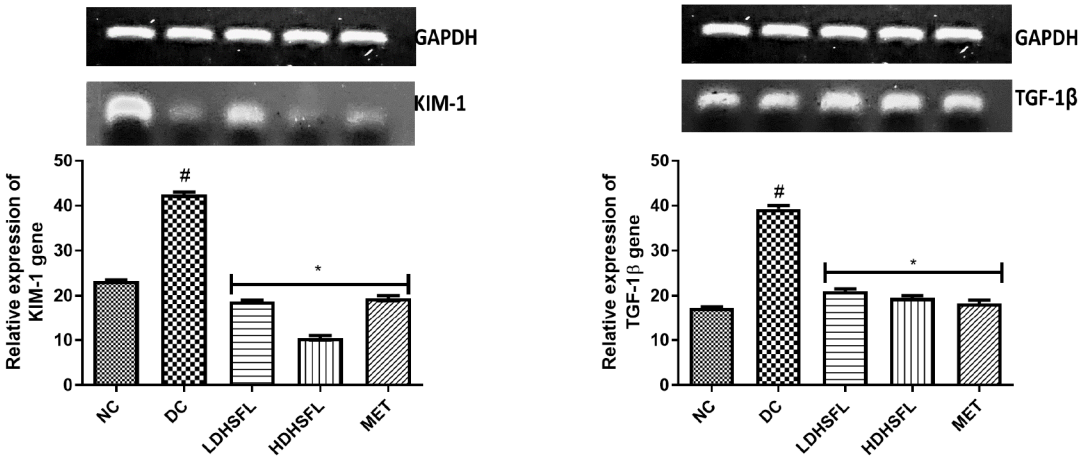

Figure 7. Relative gene expressions of KIM-1 and TGF-1β of HSLF in STZ-induced diabetic rats.

of HSLF as well as metformin caused a significant ($p < 0.05$) abrogation and eventually regulation of this deleterious trend of serum kidney function biomarkers levels in the kidney.

3.6. Effect of HSLF on Phosphatase Activity Assays in the Kidney of STZ-Induced Diabetic Rats. Figure 6 shows the effect of HSLF on phosphatase activities in the kidneys of diabetic rats. The activities of alkaline phosphatase (ALP) and acid phosphatase (ACP) decreased sharply ($p < 0.05$) in the diabetic group (DC) compared with the normal (NC). However, the administration of low and high doses of HSLF overturned markedly ($p < 0.05$) these injurious anomalies to near-normal conditions more than that of standard metformin.

3.7. Effect of HSLF on Levels of Relative Gene Expressions of *KIM-1* and *TGF-1β* Genes in the Kidney of STZ-Induced Diabetic Rats. Figure 7 shows the relative gene expression patterns of KIM-1 (kidney injury molecule-1) and TGF-1β (transforming growth factor-1beta) in the kidney of STZ-induced diabetic rats and the consequent recuperative effect of HSLF. Using glycealdehyde-3 phosphate dehydrogenase (GAPDH) as the house-keeping gene, there was a considerable ($p < 0.05$) upregulation of *KIM-1* and *TGF-1β* genes in the diabetic control (DC) compared with the normal one (NC). HSLF as well as metformin administration was, however, able to remarkably ($p < 0.05$) downregulate and eventually restore the relative gene expression of the genes (*KIM-1* and *TGF-1β*) in a dose-dependent manner.

4. DISCUSSION

The study investigated the nephroprotective impact of HSLF on various parameters related to renal health in streptozotocin (STZ)-induced diabetic rats. STZ is commonly utilized in experimental settings to induce diabetes, with a particular focus on exploring diabetic nephropathy.[38] STZ selectively damages pancreatic beta cells, causing insulin deficiency and the resultant hyperglycemia. This chemical induction leads to various manifestations indicative of diabetic nephropathy. Hyperglycemia ensues from disrupted insulin production, a hallmark of diabetes that fuels the progression of nephropathy.[39] Glomerular dysfunction, reflected in altered filtration rates and permeabilities, emerges as an early sign. Oxidative stress, indicated by increased reactive oxygen species and diminished antioxidant defenses, contributes to renal damage. Inflammation, evidenced by elevated cytokines, is intertwined with diabetic nephropathy. Impaired renal function, mirrored in elevated biomarkers like creatinine, urea, and uric acid, underscores the kidney's compromised filtration and excretion functions.[40,41] Histopathological changes, including hypertrophy and fibrosis, further characterize diabetic nephropathy. Alterations in antioxidant enzyme activities, such as SOD, CAT, and GPx, underscore the oxidative stress component.[42,43] Evaluating these biochemical parameters aids researchers in understanding the mechanisms at play in STZ-induced diabetic nephropathy, offering insights into potential therapeutic mediations and enhancing comprehension of the disease's pathophysiology.

Results from Figure 1 revealed a significant ($p < 0.05$) increase in lipid peroxidation (MDA level) and a decrease in antioxidant enzyme activities (GST, CAT, GPx, SOD) as well as reduced glutathione (GSH) in diabetic untreated rats. This feat has been corroborated by the previous study.[43,44]

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Antioxidant enzymes are pivotal in maintaining redox balance and safeguarding the normal kidneys against oxidative stress. SOD combats superoxide radicals by converting them into hydrogen peroxide, while CAT and GPx neutralize hydrogen peroxide, preventing the accumulation of reactive oxygen species (ROS).[45,46] This collective defense mechanism preserves the kidney's structural and functional integrity. In pathological states, such as kidney diseases, an imbalance between ROS production and antioxidant defenses leads to oxidative stress. Elevated ROS levels inflict damage on cellular components, fostering renal pathology.[40] Biomarkers like malondialdehyde (MDA) signify lipid peroxidation and abnormal renal function, serving as indicators of oxidative stress and impaired renal function.[47] However, these abnormalities observed in antioxidant activities/levels were significantly ($p < 0.05$) mitigated by both low and high doses of *Hibiscus sabdariffa* flavonoid-rich extract, approaching near-normal levels comparable to the standard drug, metformin. This suggests a potential antioxidative effect of the extract in combating oxidative stress associated with diabetes.

Figure 2 demonstrated a significant ($p < 0.05$) decrease in serum electrolyte levels (Ca^{2+}, Na^{+}, Mg^{2+}, K^{+}, Cl^{-}, and HCO_3^{-}) in diabetic-untreated rats compared to the normal group. Since electrolyte balance and levels have been extensively utilized as a renal functioning and pathophysiology indication, they are highly regarded as vital renal function indicators.[48,49] Serum electrolyte levels are essential for maintaining kidney function and serve as key indicators of various pathological conditions. In a healthy kidney, these electrolytes are meticulously regulated to uphold physiological balance, ensuring fluid equilibrium, cellular integrity, nerve impulse transmission, bone health, muscle function, and blood pH regulation.[50] Conversely, disruptions in serum electrolyte levels signify underlying kidney pathology, commonly observed in conditions such as acute kidney injury (AKI) or chronic kidney disease (CKD). Impaired filtration and excretion functions may lead to electrolyte imbalances, such as hyperkalemia, posing risks of cardiac arrhythmias.[51,52] Keenly observing and watching the level of these electrolytes is crucial for diagnosing and managing kidney disorders, allowing healthcare professionals to implement timely interventions, restore balance, and prevent complications associated with abnormal levels.[53] The administration of flavonoid-rich extract from *H. sabdariffa*, as well as metformin, significantly ($p < 0.05$) ameliorated these decreases, indicating a protective effect on renal electrolyte balance.

Figure 3 exhibited significantly ($p < 0.05$) elevated levels of inflammatory biomarkers (IL-1β, IL-6, and TNF-α) in diabetic untreated rats in line with previous literature.[54,55] In normal kidney physiology, the role of inflammatory biomarkers, including interleukin-1β (IL-1β), interleukin-6 (IL-6), and tumor necrosis factor-α (TNF-α), is typically limited and their expression is tightly regulated. These biomarkers are part of the immune response and contribute to maintaining homeostasis. IL-1β, IL-6, and TNF-α play vital roles in immune cell regulation, inflammation modulation, and tissue repair in response to injury or infection.[56−58] In pathological conditions such as kidney diseases, the expression of these inflammatory biomarkers can become dysregulated, leading to an overactive immune response and chronic inflammation. Elevated levels of IL-1β, IL-6, and TNF-α in the kidneys may contribute to tissue damage, fibrosis, and the progression of various kidney disorders, including glomerulonephritis, tubulointerstitial nephritis, and diabetic nephropathy.[58−60] It is very important to note that monitoring the physiological levels of these inflammatory biomarkers helps in understanding the immune response in kidney pathology and can thus guide therapeutic interventions to mitigate inflammation and prevent further damage.[61] Treatment with HSLF and metformin was able to significantly ($p < 0.05$) reverse these harmful increases in pro-inflammatory biomarker levels, suggesting an anti-inflammatory effect of the HSLF.

In Figure 4, histopathological examination revealed injurious modifications in the kidneys of diabetic untreated rats, including enlargement of the glomerulus urinary space and severe loss of PCT epithelial cells as well as severe hemolysis and fatal degeneration of glomerular cells. These histopathological alterations confirmed previous researches[62,63] and signify the progression of diabetic nephropathy, a common complication of diabetes characterized by kidney damage. The detrimental impact on kidney histology underscores the importance of STZ-induced diabetic rat models in studying the pathophysiology of diabetic nephropathy.[42] The enlargement of the glomerulus urinary space indicates structural alterations in the primary kidney filtration unit, possibly linked to increased permeability and compromised filtration.[64] The severe loss of PCT epithelial cells signifies damage to the crucial tubular structure responsible for reabsorption. Severe hemolysis suggests disruption of blood vessels, compromising renal blood flow and nutrient supply.[65,66] The fatal degeneration of glomerular cells underscores the progressive kidney damage caused by persistent hyperglycemia. These histopathological findings emphasize the urgent need for targeted interventions to address the structural and functional consequences of diabetic nephropathy and its associated complications.[63] Administration of HSLF markedly restored kidney morphology, indicating a nephroprotective effect against structural damage.

Figure 5 showed a significant ($p < 0.05$) increase in serum kidney function biomarkers (creatinine, uric acid, and urea) in diabetic untreated rats in tandem with prior reports.[67,68] Serum kidney function biomarkers, including creatinine, uric acid, and urea, are integral for evaluating kidney physiology and serve as indicators of various pathological conditions.[69] In a healthy kidney, these biomarkers are maintained within normal ranges, reflecting efficient filtration and excretion functions. Creatinine, a byproduct of muscle metabolism, is consistently excreted, while uric acid and urea, which are waste products of purine metabolism and protein breakdown, respectively, are also effectively eliminated.[70,71] Disruptions in kidney function, observed under conditions such as acute kidney injury or chronic kidney disease, result in abnormalities in these biomarkers. Elevated levels of serum creatinine signal impaired glomerular filtration, and increased serum uric acid levels may indicate reduced excretion or overproduction, while elevated urea levels can signify compromised renal excretory capacity.[72,73] However, the treatment with the flavonoid-rich extract of *H. sabdariffa* significantly ($p < 0.05$) normalized these elevated levels, indicating a potential role in mitigating diabetic-induced nephropathy.

Figure 6 demonstrated a significant ($p < 0.05$) reduction in alkaline phosphatase (ALP) and acid phosphatase (ACP) activities in diabetic untreated rats in corroboration of previous researches.[74,75] ALP and ACP are enzymes integral to normal kidney physiology, with ALP contributing keenly to phosphate metabolism and bone health as well as renal tubular health,

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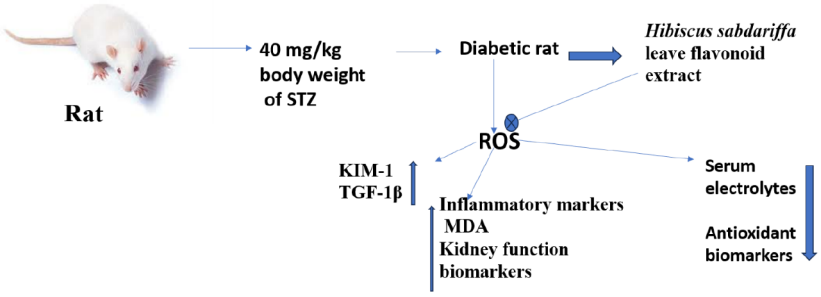

Figure 8. The proposed mechanism of action of HSLF weight.

while ACP participates in cellular processes and acidic environments. In pathological circumstances, alterations in these enzyme activities serve as crucial indicators of kidney health.[76] Dsyregulated renal ALP levels may signal kidney tubule or bone disorders, providing insight into impaired phosphate regulation. Detrimental changes in ACP activity can reflect cellular damage or inflammation in the kidney.[77] Following ALP and ACP activities is very essential for a comprehensive assessment of renal function and dysfunction as well as the management of kidney disorders.[78] The administration of HSLF, in both low and high doses, significantly ($p < 0.05$) restored and normalized phosphatase activities, suggesting its protective effect on kidney function.

The relative gene expression analysis, presented in Figure 7, indicates a significant ($p < 0.05$) upregulation of *KIM-1* and *TGF-1β* genes in diabetic untreated rats in tandem with a recent study.[10] This may be linked to the production of reactive oxygen species (ROS) under diabetic conditions. *KIM-1* and *TGF-1β* genes in healthy nephrophysiology have distinct roles, all of which are very crucial to normal kidney functioning. *KIM-1* is usually inactive in healthy kidneys but becomes activated in response to injury, contributing to the repair and regeneration of damaged tubular cells.[79] Conversely, *TGF-1β* is a versatile cytokine involved in maintaining tissue balance and regulating the extracellular matrix under normal conditions. In diabetic nephropathy, these genes gain significance due to their dysregulation. Elevated *KIM-1* expression in diabetes serves as a potential biomarker for kidney injury, while increased *TGF-β1* expression contributes to renal fibrosis, a characteristic of diabetic nephropathy.[80,81] It is very important to monitor these genes because of their cruciality in understanding and addressing kidney damage in individuals with diabetes, aiding in diagnostic and therapeutic interventions for diabetic nephropathy.[82,83] Interestingly, treatment with HSLF significantly ($p < 0.05$) downregulated these genes, indicating a potential molecular mechanism underlying the nephroprotective effects of the extract. This may be probably linked to the antioxidant nature of this extract. The obtained results were in line with the previous studies. Also, the proposed mechanism of action is illustrated in Figure 8.

5. CONCLUSION

The results revealed that diabetic rats exhibited significant alterations in redox stress biomarkers, electrolyte levels, inflammatory biomarkers, histopathological features, serum kidney function biomarkers, phosphatase activities, and gene expressions. Hence, the results suggest that HSLF possesses nephroprotective properties and supports the local use of this plant in the management of diabetic nephropathy.

■ ASSOCIATED CONTENT

Data Availability Statement

Only on special request from the corresponding author.

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Author Contributions

Conceptualization was done by B.O.A., T.H.F., and B.E.O.; methodology by C.D.F., D.M.N., B.M.A., and O.S.A.; software by B.E.O., A.O.E., and H.A.A.; validation by B.O.A., A.O.E., H.A.A., and C.D.F.; formal analysis by B.O.A., T.H.F., B.E.O., and C.D.F.; investigation by B.O.A., C.D.F., D.M.N., B.M.A., and O.S.A.; resources by C.D.F., D.M.N., B.M.A., and O.S.A.; data curation by C.D.F., D.M.N., B.M.A., and O.S.A.; writing—original draft preparation by C.D.F., C.D.F., D.M.N., B.M.A., and O.S.A.; writing—review and editing by B.O.A., C.D.F., D.M.N., O.S.A., T.H.F., A.O.E., H.A.A., S.G. and B.E.O.; supervision by B.O.A., T.H.F., and B.E.O.; project administration by C.D.F., C.D.F., D.M.N., B.M.A., and O.S.A.; and funding acquisition by A.O.E. and H.A.A. All authors have read and agreed to the published version of the manuscript

Notes

All experimental protocols were approved by FUOYE Faculty of Science Ethics Committee (FUOYEFSC 201122-REC2022/008).

The authors declare no competing financial interest.

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