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Exploring the NRF2/HO-1 and NF-κB Pathways: Spirulina Nanoparticles as a Novel Approach to Combat Diabetic Nephropathy

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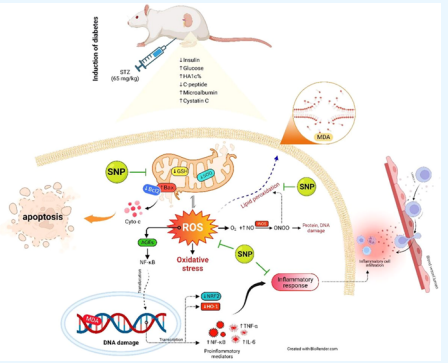
ABSTRACT: *Arthrospira platensis* has been the subject of plentiful studies due to its purported health advantages; nevertheless, additional investigation is required to determine whether several chronic diseases may be treated or avoided with its nanoform. Therefore, we set out to examine *A. platensis* nanoparticles (SNPs) to protect against kidney impairment caused by Streptozotocin (STZ) in diabetic rats, precisely focusing on its effect and the cellular intracellular pathways involved. Male Wistar rats were assigned into four groups: Group 1 was set as control, comprising the normal rats; group 2 was administered SNPs (0.5 mg/kg BW, once/day) orally for 84 consecutive days; group 3, STZ-diabetic rats were injected with STZ (65 mg/kg BW); and group 4, in which the diabetic rats were treated with SNPs. After inducing diabetes in rats for 84 days, the animals were euthanized. The results disclosed that SNP treatment substantially ($P < 0.05$) improved the glucose and glycated hemoglobin levels (HbA1c %), insulin, C-peptide, and cystatin C deterioration in diabetic rats. Furthermore, SNP administration significantly lowered ($P < 0.05$) nitric oxide (NO) and malondialdehyde (MDA) levels in renal tissue and enhanced kidney function metrics, as well as improved the antioxidant capacity of the renal tissue. In addition, oral SNPs overcame the diabetic complications concerning diabetic nephropathy, indicated by downregulation and upregulation of apoptotic and antiapoptotic genes, respectively, along with prominent modulation of the antiangiogenic marker countenance level, improving kidney function. SNP modulated the nuclear factor erythroid 2-related factor 2 and heme oxygenase-1 (NRF2/HO-1) pathways and inhibited the nuclear factor-κB (NF-κB) expression, strengthening the SNP pathways in alleviating diabetic nephropathy. The histopathology results corroborated the obtained biochemical and molecular observations, suggesting the therapeutic potential of SNPs in diabetic nephropathy via mechanisms other than its significant antioxidant and hypoglycemic effects, including modulation of antiangiogenic and inflammatory mediators and the NRF2/HO-1 pathways.



1. INTRODUCTION

Diabetes mellitus is a socioeconomic burden on health systems worldwide. Type I diabetes is an autoimmune illness illustrated by hyperglycemia due to absolute insulin insufficiency.[1] This hyperglycemia provokes macrovascular and microvascular diseases, including renal injury and neuropathy.[2] Diabetic kidney disease (DKD) can be clinically diagnosed via albuminuria or a low estimated glomerular filtration rate. Diabetic renal impairment can be detected early by measuring serum cystatin C rather than microalbuminuria or serum creatinine.[3,4] Hyperglycemia can trigger the polyol pathway, oxidative injury, and mitochondrial dysfunction in the endothelial cells and promote immune cell recruitment.[5] These events eventually progressed to tubular and podocyte dysfunction. Glomerular endothelial cells (GEnCs) are specialized vascular cells that form the ramparts of the glomerular tufts and are crucial for renal homeostasis. Endothelial cell dysfunction can aggravate apoptosis and increase endothelial permeability, ultimately leading to the loss of fenestration of GEnCs, causing albuminuria.[6] GEnCs are covered by a glycocalyx, a linkage of endothelial polysaccharide layers. It was previously reported that the albuminuria degree correlates with glycocalyx loss. Nevertheless, its role in DKD remains contentious.[7]

The incidence of diabetes-associated kidney disease has been on the rise, particularly affecting the elderly at a higher rate. Moreover, there is a notable link between the progression of

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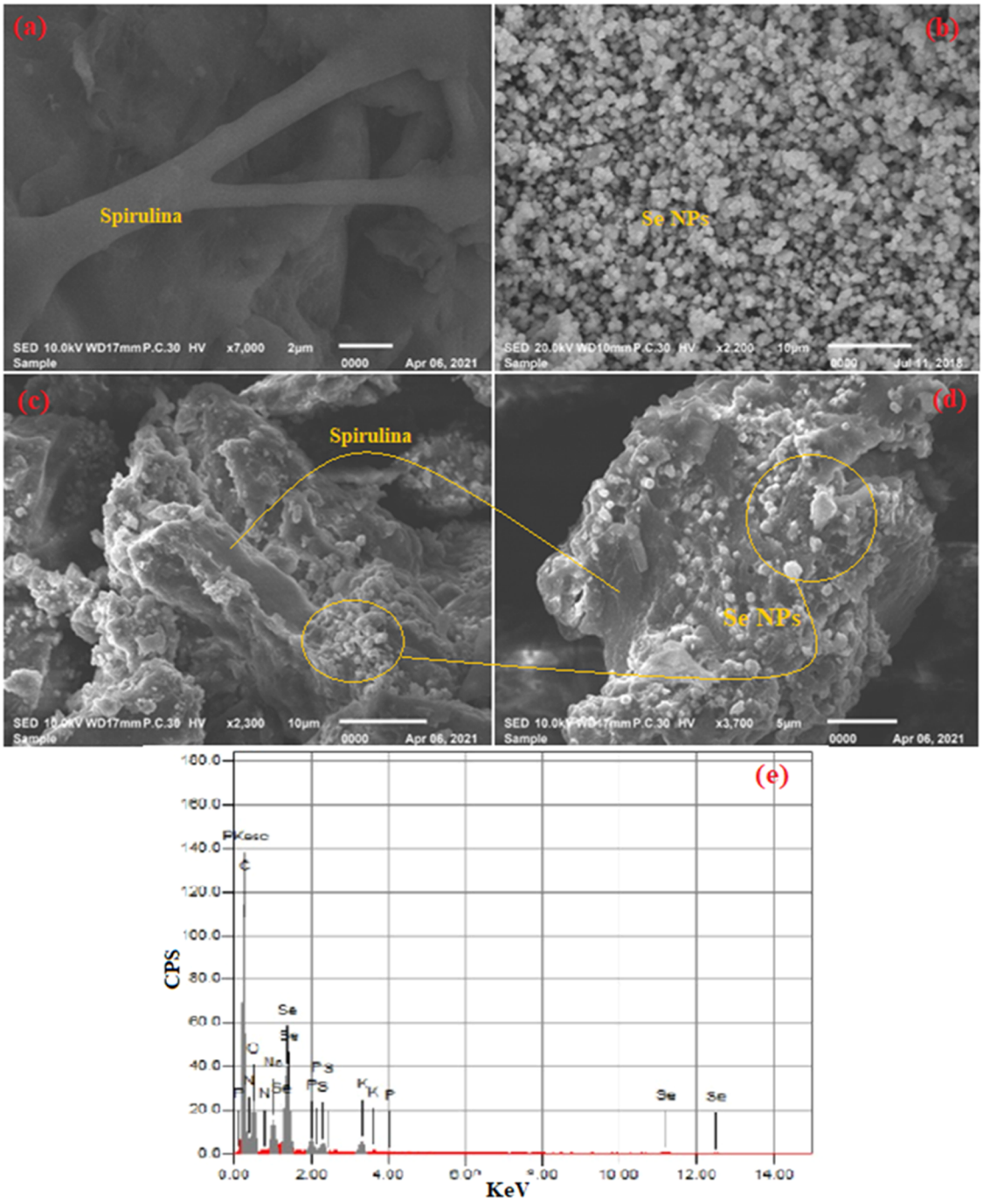

Figure 1. Field-emission scanning electron microscopy (FESEM) micrograph of fabricated nanomaterials: (a) Spirulina, (b) Se NPs, and (c, d) Sp@Se nanocomposite with different magnifications. (e) DX analysis for Sp@Se.

DKD stages and increased mortality rates.[8] Research indicates that annually, about 1% of diabetic patients progress to end-stage renal disease (ESRD), and roughly 6% of those with significant albuminuria advance to ESRD each year.[9] Recent investigations indicate that the occurrence of DN among individuals with type 2 diabetes mellitus (T2DM) falls within a range of 20 to 40% in all people with diabetes.[10] In individuals with type 2 diabetes, the occurrence rate of diabetic nephropathy (DN) complications stood at 35.24%.[11] The proportion of end-stage renal disease (ESRD) attributable to diabetes alone ranges from 12 to 55%. People with diabetes and clinical nephropathy experience 50% higher health expenditures compared to those with diabetes but without clinical nephropathy.[12] Between 2000 and 2016, diabetic nephropathy (DN) was a significant cause of diabetes-related deaths due to vascular complications, with the largest proportion of these deaths attributed to DN itself. Specifically, of the total 1,904,787 deaths from vascular complications reported during this period in 108 countries, 71.1% (1,355,085 cases) were due to diabetic nephropathy.[13]

Traditional medicine has extensively pursued different Physalis plants to manage chronic diseases. Among these, *A. platensis* has gained a substantial attraction for its use in diabetes mellitus.[14,15] Spirulina is a filamentous blue−green

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cyanobacterium that was ancestrally cultivated and utilized worldwide due to its incredible nutritional value and pharmacological effects.[16,17] Spirulina is reported to possess antioxidant, anti-inflammatory, antimetastatic, immunostimulatory, and cardioprotective properties, which are attributed to its functional bioactive β-carotene contents, tocopherols, phenolic acids, and phycobiliproteins, including phycocyanin.[18,19] Hence, it has been effective in the treatment of fatty liver,[20,21] allergic respiratory disorder,[22] neuropathies,[23] and neurobehavioral and cognitive deficits.[24,25]

Furthermore, drug delivery has come a long way recently, and further development is expected.[26] Nanoparticles are unique among active compounds because of their small size and active surface, besides the advantage of a longer half-life that enhances efficiency and activity.[27] In light of this background, this study sought to investigate whether *A. platensis* nanoparticles (SNPs) could have a moderating influence on diabetic nephropathy. Moreover, this experiment tackled the underlying molecular trials associated with SNP's antioxidant, anti-inflammatory, antiangiogenic, and antiapoptotic assets.

2. MATERIALS AND METHODS

2.1. Ethical Application. The Animal House Ethical Committee, Faculty of Veterinary Medicine, officially approved the experiment at Kafrelsheikh University, Egypt (KFS-IACUC/133/2023). The rats were treated by following the ethical guidelines established by the National Institutes of Health (NIH).

2.2. Diabetes Induction. Streptozotocin (STZ), at a dose of 65 mg/kg body weight,[28−31] was intraperitoneally injected after 16 h of fasting. Before STZ injection, STZ was dissolved in a 0.1 M citrate buffer at pH 4.[32] After the STZ injection, the rats received a 5% glucose solution for 24 h to avoid early hypoglycemia. The control rats had identical volumes of citrate buffer injected intraperitoneally. One week later, a glucometer (IME-DC, Germany) was used to verify the blood glucose level in a blood sample collected from the tail vein. When the blood glucose level was over 250 mg/dL, it was deemed diabetic.[33−35]

2.3a. *A. platensis* Nanoparticle (SNP) Formulation. The Algae Biotechnology Unit, National Research Center, Giza, Egypt, supplied 1 g of *A. platensis*, which was suspended in 100 mL of double-distilled water for 1 h with stirring and ultrasonication; afterward, 50 mL of a suspension of synthetic nanoparticles (Se NPs) (0.5 g) was added. The mixture was ultrasonicated for 1 h, left to stir at room temperature overnight, examined for nanocomposite production by ultraviolet−visible (UV−vis) spectroscopy, and then centrifuged. The product was washed and dried, and then characterized using different techniques (particle size is 158 nm). Please see Figure 1 and S1 for the SNP characterization.

2.3b. ζ-Potential and Particle Size. ζ-Potential devices were used to measure synthesized nanoparticles' stability and surface charge; ζ-potential was conducted at room temperature and in double-distilled water. The ζ-potential of the synthesized materials is shown in Figure S1. The sample has a value of −22.6 mV, which refers to moderate stability in the solution. The stability of nanoparticles is due to the strong charge between the solvent and the charge of the nanoparticle's surface. A substantial value of more than ±60 mV referred to excellent stability, and that of ±36 mV referred to moderately durable material. The diameter of the fabricated materials was calculated by dynamic light scattering (DLS), where the sample has a diameter of 158 nm; this emphasized that the model was settled in the range of nanomaterials.

2.4. Scanning Electron Microscopy (SEM) Analysis. The study of the size and morphology of the fabricated nanomaterials was detected via the SEM device with different magnifications to illustrate the morphology of the surface and its details. Figure 1a gives a high-resolution SEM micrograph of spirulina, which appeared as little spirals featuring filaments and individual cells. Se NPs appeared as irregular pure nanosphere shapes (Figure 1b). The spirulina nanocomposite (Sp@Se) appeared as a high combination between the Se NPs and spirulina, which showed the appearance of a high level of overlap of Se NPs between the layers of spirulina (Figure 1c,d). The energy-dispersive X-ray spectroscopy (EDX) analysis of fabricated nanomaterials has been measured, confirming the pure formation of nanomaterials (Figure 1e). The chemical composition (wt %) of each component of the fabricated Sp@Se was documented at 52.03% (c), 27.29% (N), 17.56% (O), 1.13% (Na), 0.21% (P), 0.15% (S), 0.23% (K), and 1.39% (Se).

2.5. Experimental Design. Thirty-six male Wistar rats (175−185g, 55 ± 2 days) were housed in plastic cages with plenty of ventilation, provided unrestricted access to food and water, and subjected to a 12/12 h cycle of darkness and light each day at 27 ± 2 °C in this experiment. The standard rat chow diet provided by Al-Gomhoureya, Cairo, Egypt, consists of 306.2 kcal per 100 g, with 48.8% carbohydrates, 21% protein, and 3% fat.[36] The rats were adapted for 2 weeks. Then, the rats were randomized into four groups (9 rats/group). Group 1 was treated with saline orally as the control group; group 2, the normal rats received SNP dissolved in saline (0.5 mg/kg BW, once daily/orally)[27] for 84 consecutive days; group 3, STZ-diabetic rats were intraperitoneally (I/P) injected with STZ (65 mg/kg BW);[37] and group 4, in which the diabetic rats were treated with SNPs (0.5 mg/kg BW). A similar normal saline volume was administered to the control and diabetic rats. In addition, the same volume of the vehicle used to dissolve STZ (0.1 M citrate buffer, pH 4) was I/P injected into other groups of rats. Animals were sacrificed after 84 days of diabetes induction.[38] Please see the experimental time course (Figure S2).

2.6. Sampling and Biochemical Analyses. Rats were housed in metabolic cages for 1 day (before the end of the experiment), during which time urine was collected under a layer of toluene. One way in which the glomerular filtration rate (GFR) can be estimated is by measuring creatinine clearance (CCr), which is calculated using the following formula: CCr = 24 h urine creatinine × urine flow (mL/min)/serum creatinine[39] and then amended per weight of rat (kg).[40] Urine flow per minute was calculated by dividing the 24 h urine volume by 1440, representing the number of daily minutes. On the final day of the experiment, the animals were subjected to fasting for a period that could span approximately 12 to 18 h before undergoing anesthesia. A mixture of urethane (1.0 g/kg) and α-chloralose (0.01 g/kg) via intraperitoneal injection was used for rat anesthesia.[41] Rats were humanely euthanized by cervical decapitation. Blood samples were obtained in aliquots. A part was taken on EDTA for HbA1c determination, and another was left for coagulation and serum separation for other proposed biochemical analyses. Then, kidneys were obtained; one part was homogenized by hand in ice-cold phosphate-buffered saline (PBS). At the end of the

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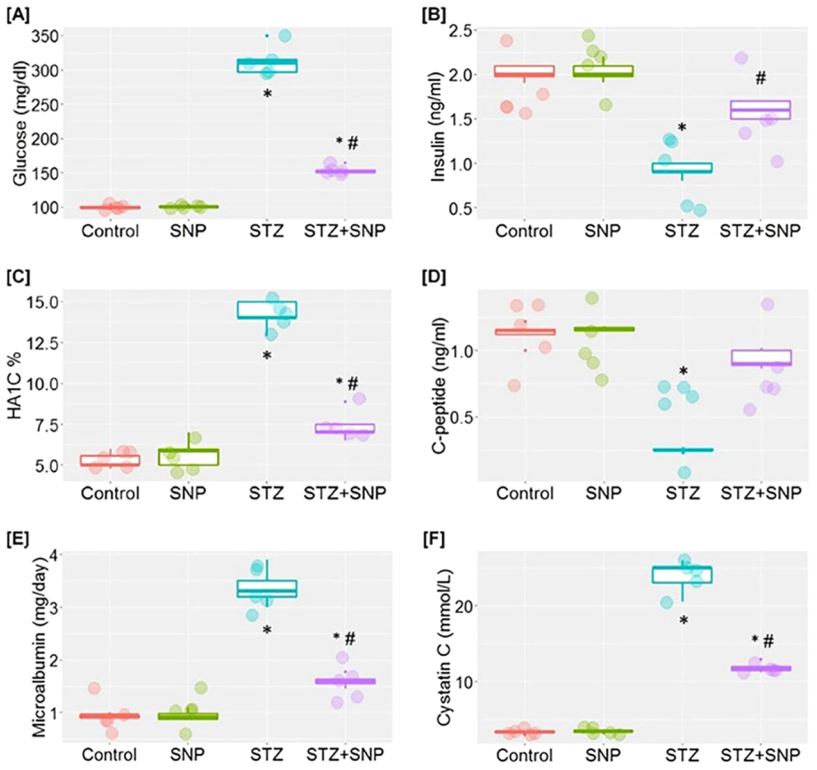

Figure 2. (A) Effect of *A. platensis* nanoparticles (SNPs) on serum glucose, (B) insulin, (C) glycated hemoglobin [HbA1c%], (D) C-peptide, (E) microalbumin, and (F) cystatin C in control and diabetic rats. The values are shown as medians ± whisker bar. Statistical significance was determined using a one-way ANOVA and Tukey's post hoc. $^{*}P < 0.05$ vs control group and $^{\#}P < 0.05$ vs STZ group.

homogenization process, the supernatant was centrifuged at 3000*g* for 10 min at 4 °C and saved at −20 °C. The second part was divided into two sections: one was placed in formalin fixed at 10% for histopathological analysis, and one was stored in RNA later and kept at −80 °C for further mRNA expression assays. The kidney weights and organ/body weight ratio were recorded at this step.

2.7. Biochemical Assays. Biomed Diagnostics (Egypt) reagent kits were used to measure serum creatinine and urea,[42,43] as well as urinary microalbumin levels,[44] according to the manufacturer's guidelines. Superoxide dismutase (SOD) activity and NO, glutathione (GSH), and MDA (a marker of lipid peroxidation) concentrations were determined in the kidney homogenate following the manufacturer's instructions.[45−47] Also, the Rat HbA1c assay kit for HbA1c analysis (Bio diagnostic, Giza, Egypt) was used.[48] Blood glucose levels were measured spectrophotometrically (1200 UNICO Instruments, Inc., Dayton, NJ.) using a commercial kit (Spinreact, Barcelona, Spain). Advanced glycation end-products (AGEs) and insulin were assessed using ELISA kits (NOVA Co. China and Crystal Chem. Co.), following the producer guidelines. Quantitative solid-phase ELISA (Multiskan EX, Thermo Scientific, Shanghai, China) was used to measure serum C-peptide concentrations using commercially available kits for the quantitative analysis of cystatin C in serum (Glory Science Co., Hangzhou, China). Quantitative evaluation of hydroxyproline content in the kidneys was performed by analyzing renal tissue to determine kidney collagen content.[49] In brief, after 24 h incubation at 37 °C, 100 mg of kidney tissue was exposed to 6 mL of 5% KOH. Following a 3 h incubation at 25 °C with chloramine T reagent, it was hydrolyzed with 10% NaOH. After adding Ehrlich's reagent, the reaction mixture was incubated in a water bath at 65 °C for 20 min. The result was the formation of a purple complex with an absorbance of 550 nm. Since hydroxyproline accounts for 13.5% collagen, the hydroxyproline content in the kidney was multiplied by 7.46 to get the total collagen content.[50,51]

2.8. Gene Expressions Using Real-Time PCR. Total RNA was extracted from 100 mg of kidney tissue using TRIzol (Invitrogen, Life Technologies, Carlsbad, CA). The concentration of RNA was determined using a Nanodrop (UV−vis spectrophotometer Q5000/Quawell), ensuring that RNA samples had an A260/A280 ratio of 1.8 or higher. Further cDNA synthesis was performed using a Fermentas, MA, kit. For cDNA amplification, SYBR Green Master Mix (Roche, Basel, Switzerland) and the primers listed in Table S1 were used. The amplification reaction had a final volume of 20 μL, containing 10 μL of SYBR Green Master Mix, 0.5 μL of forward primer, 0.5 μL of reverse primer, and 9 μL of template cDNAs. The glyceraldehyde-3-phosphate dehydrogenase gene (GAPDH) was used as a reference during the amplification process. Data collection was conducted during the amplification, and the authenticity of the polymerase chain reaction (PCR) products was confirmed using melting curve analysis. A Rotor-Gene Q instrument (Qiagen, Valencia, CA) was employed for automatic data collection and analysis of the threshold cycle value (Ct). The $2^{-\Delta\Delta Ct}$ method was utilized to analyze the amplification data..[52]

2.9. Histopathological Assessment. Following sacrificing, kidney samples were collected and fixed in a 10% neutral buffer formaldehyde solution for 24 h. After fixation, an increasing gradient of ethyl alcohol (from 70% to absolute alcohol) solutions was used to dehydrate the kidney specimens before they were cleaned in xylene and impregnated in paraffin

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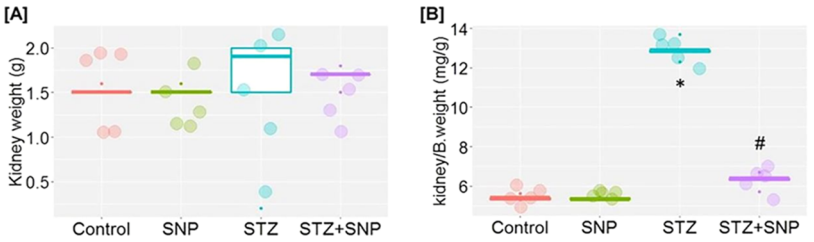

Figure 3. (A) Effect of *A. platensis* nanoparticles (SNPs) on kidney weight and (B) kidney/body weight ratio in control and diabetic rats. Values are presented as medians ± whisker bar. Statistical significance was determined using one-way ANOVA and Tukey's post hoc. $^{*}P < 0.05$ vs control group and $^{\#}P < 0.05$ vs STZ group.

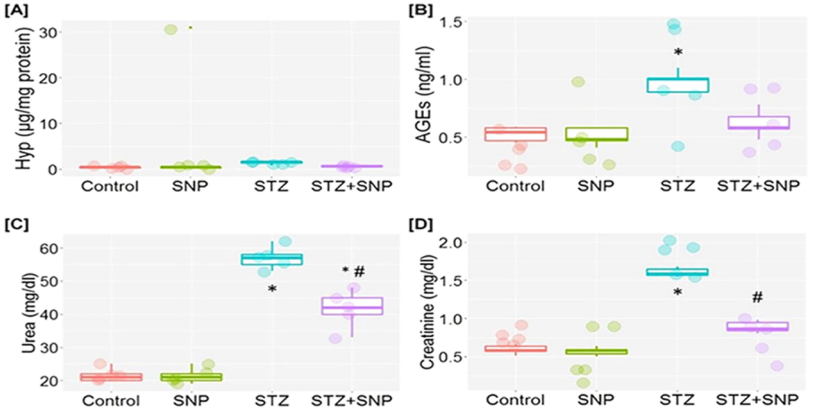

Figure 4. Effect of *A. platensis* nanoparticles (SNP) on renal hydroxyproline level (A), advanced glycation end-products (AGEs) (B), urea (C), and creatinine (D) in control and diabetic rats. Values are displayed as medians ± whisker bar. Statistical significance was determined using one-way ANOVA and Tukey's post hoc. $^{*}P < 0.05$ vs control group and $^{\#}P < 0.05$ vs STZ group.

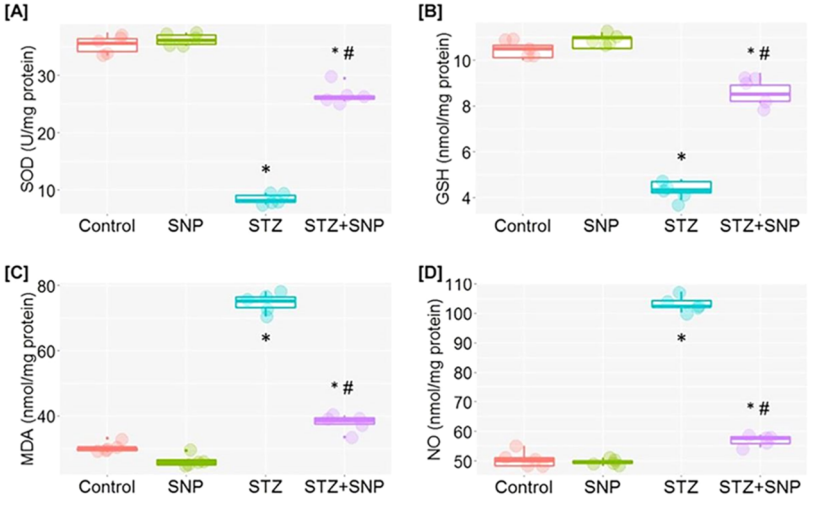

Figure 5. Effect of SNPs on renal superoxide dismutase (SOD) (A), glutathione (GSH) (B), malondialdehyde (MDA) (C), and nitric oxide (NO) (D) in control and diabetic rats. Values are displayed as medians ± whisker bar. Statistical significance was determined using a one-way ANOVA and Tukey's post hoc. $^{*}P < 0.05$ vs control group and $^{\#}P < 0.05$ vs STZ group.

wax. Thicknesses of 4−5 μm were achieved in tissue sectioning, utilizing a rotating microtome (Leica Microsystems, Wetzlar, Germany). Then, all sections were stained in H&E. Light microscopic analysis and imaging of the tissue were performed (Olympus CX 40, Olympus Corporation, Tokyo, Japan).

2.10. Statistical Investigation. The data are displayed as means ± SEM using GraphPad 8 Software, CA. The data were assessed using a one-way analysis of variance. When the results of the *F*-test for the analysis of variance were significant, the Tukey−Kramer postanalysis examined the means of the groups individually. The cutoff for statistical significance was set at $P < 0.05$. Data visualization, clustering heatmap, and variable importance projection (VIP) scores were generated using RStudio with R version 4.0.2.

3. RESULTS

3.1. Effect of *A. platensis* Nanoparticles (SNPs) on Glucose Homeostasis in Diabetic Rats. As illustrated in Figure 2, STZ induced significant increases in glucose levels, glycated hemoglobin (HbA1c%), microalbumin, and cystatin

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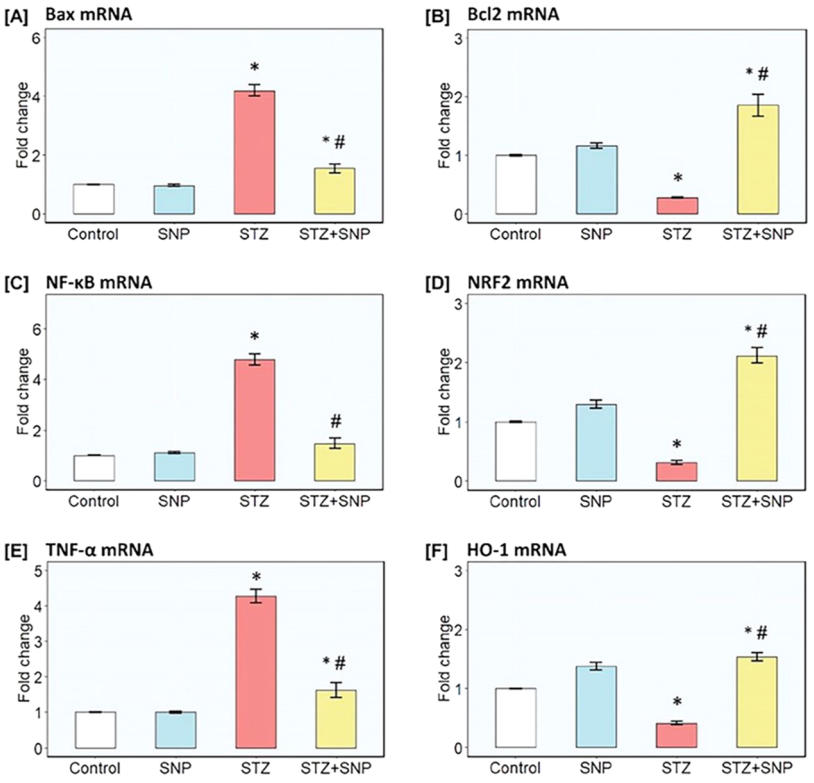

Figure 6. Effect of SNPs on renal gene expression of Bcl2-associated X-protein (Bax) (A), B-cell lymphoma 2 (Bcl2) (B), nuclear factor kappa-B (NF-κB) (C), nuclear factor erythroid 2-related factor 2 (NRF2) (D), tumor necrosis factor-α (TNF-α) (E), and heme oxygenase-1 (HO-1) (F) in control and diabetic rats. Values are displayed as medians ± whisker bar. Statistical significance was determined using one-way ANOVA and Tukey's post hoc. *P < 0.05 vs control group and $^{\#}P$ < 0.05 vs STZ group.

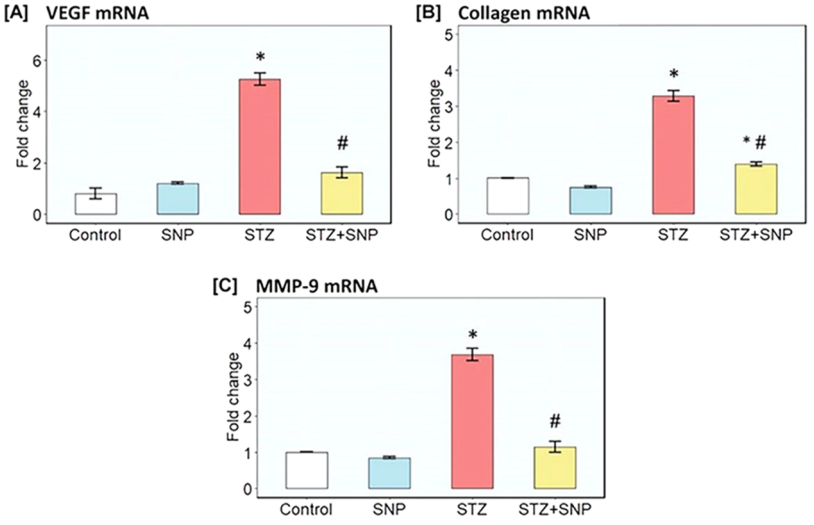

Figure 7. Effect of *A. platensis* nanoparticles (SNPs) on renal gene expression of vascular endothelial growth factor (VEGF) (A), collagen (B), and matrix metallopeptidase 9 (MMP-9) (C) in control and diabetic rats. Values are means ± SEM. Statistical significance was determined using one-way ANOVA and Tukey's post hoc. *P < 0.05 vs control group and $^{\#}P$ < 0.05 vs STZ group.

C. These derangements were connected with statistically substantial drops in serum insulin and C-peptide compared with the controls. However, the SNP supplementation did not substantially affect the above-mentioned parameters when administrated to the control rats. On the other hand, SNP significantly ameliorated those indices in diabetic rats, indicated by the marked reduction in the glucose level, HbA1c%, and cystatin C when coadministrated to the STZ-injured rats. Besides, SNP could enhance the insulin and C-peptide levels in those animals.

3.2. Effect of SNPs on Kidney Weight and Its Ratio to Body Weight in Diabetic Rats. In different studied groups, the kidney weight was not significantly changed, as exhibited in Figure 3. However, the kidney-to-body weight ratio was increased in diabetic rats when compared to normal ones. SNP significantly decreased this elevated ratio compared to the

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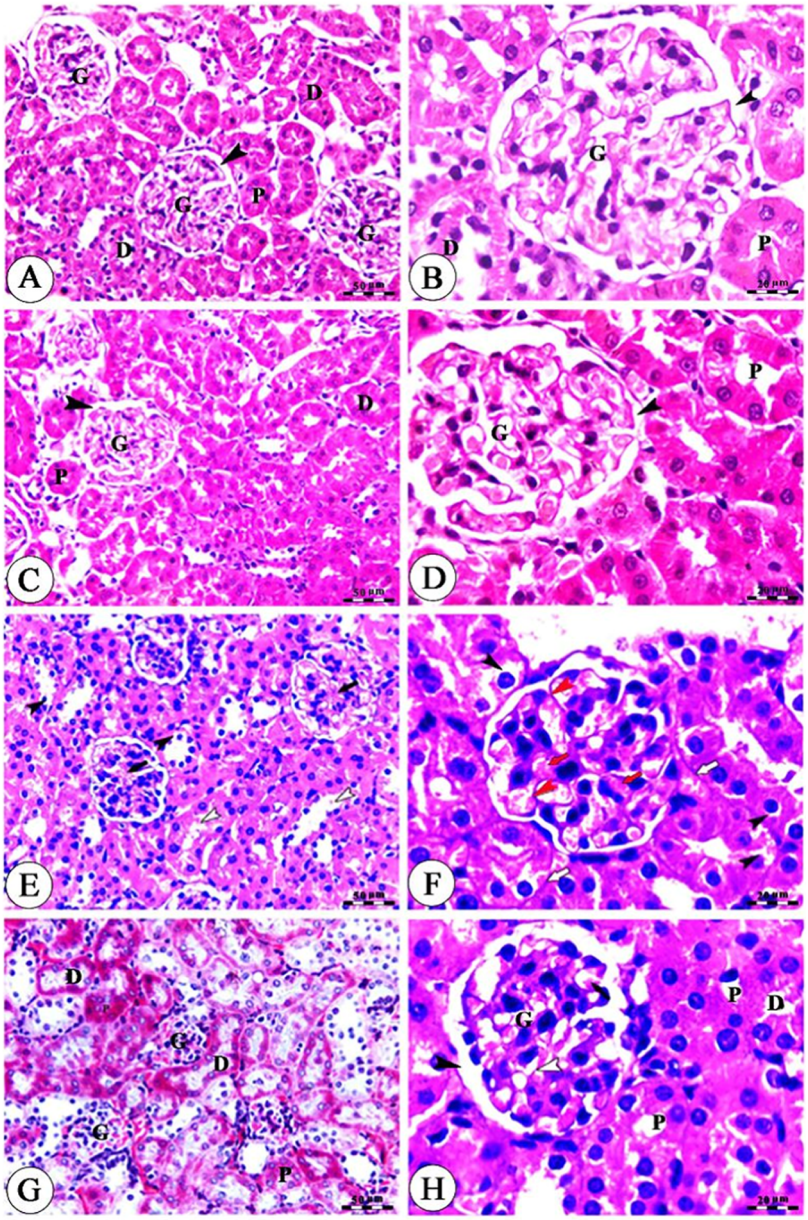

Figure 8. Effect of SNPs on the histopathological picture of diabetic nephropathy. Control (A, B) and SNP (C, D) groups exhibited normal architecture of renal parenchyma with intact renal corpuscles containing glomerulus (G) surrounded by narrow capsular space (black arrowheads) in addition to proximal (P) and distal (D) convoluted tubules. (E, F) STZ group shows mesangial expansion in the glomerulus tissue (black arrows), thickening of the basement membrane of glomerular capillaries (red arrowheads) and renal tubules (white arrows) in addition to vacuolar degeneration in some tubules (black arrowheads), congestion in glomerular capillaries (red arrows), and accumulation of a small proteinaceous substance in the lumen of some renal tubules (white arrowheads). (G, H) STZ + SNP shows renal glomeruli (G), mild enlarged capsular space (black arrowheads), and mild congestion of glomerular capillaries (black arrows) with a thin basement membrane (white arrowhead) in addition to intact proximal (P) and distal (D) convoluted tubules.

untreated diabetic group, and the amount reached the ratio in control rats.

3.3. Effect of SNPs on Kidney Injury Markers in Diabetic Rats. As shown in Figure 4, there were no appreciable variations in renal hydroxyproline levels across the various studied groups. The untreated diabetic rats, in contrast to the healthy control group, had remarkable increases in serum advanced glycation end product (AGEs), urea, and creatinine concentrations. Combined treatment of SNP and STZ showed reductions in the urea and creatinine levels compared to those of STZ sole treatment. However, there was no significant alteration in the AGEs after SNP supplementation in diabetic animals. Also, see Figure S3 for the creatinine clearance.

3.4. Effect of SNPs on Oxidative Stress Parameters in Diabetic Rats. Associated with the normal group, the STZ-injured rats exhibited a significant reduction in the renal contents of GSH and SOD activity and a marked increase in the MDA and NO concentrations (Figure 5). However, SNP supplementation in STZ-injured animals alleviated such alterations by enhancing the reduced levels of GSH and SOD and decreasing the high renal contents of MDA and NO.

3.5. Effect of SNPs on Apoptotic and Inflammatory Parameters in Diabetic Rats. As presented in Figure 6,

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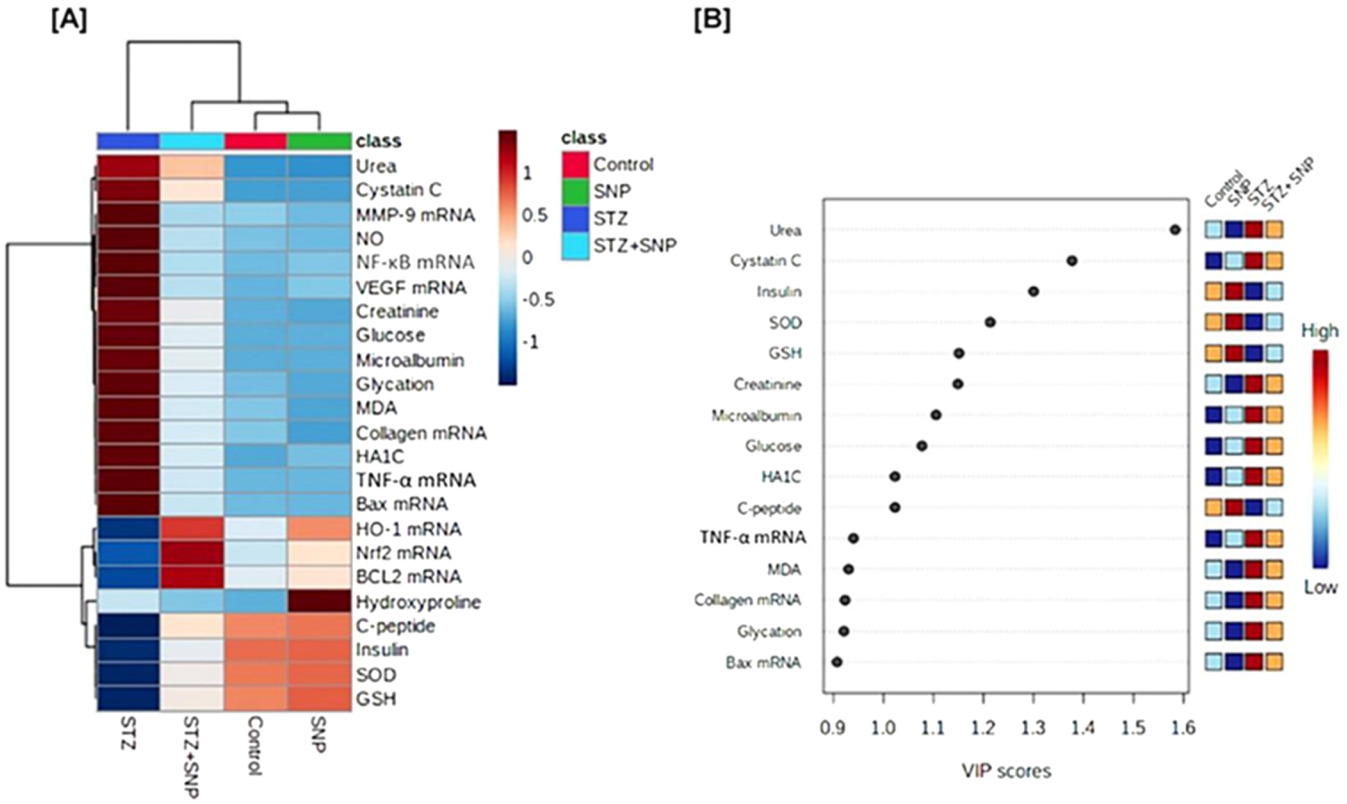

Figure 9. Hierarchical clustering heatmap and the variable important project (VIP) score facilitate data visualization. (A) Heatmap of clustering of the various treatments; concentration levels are shown on the map as colored cells; row averages and column treatments are shown separately. (B) Variable important project (VIP) score; the weighting of numerous criteria. The scale from red at the top to blue at the bottom indicates the relative importance of each contribution (blue color). Dark red (the highest value) is at the top of the gradient scale, while blue (the lowest value) is at the bottom (lowest value).

there was a significant upregulation of the renal content of Bax, NF-κB, and TNF-α mRNA expression levels in the uncured diabetic group. Moreover, there was substantial down-regulation in the renal content of Bcl2, NRF2, and HO-1 mRNA expressions in the same group. Alternatively, SNP-treated diabetic rats showed dramatic decreases in the kidney levels of Bax, NF-κB, and TNF-α, along with observable improvements in expression levels of Bcl2, NRF2, and HO-1 linked to the STZ group.

3.6. Impact of SNPs on Antiangiogenic Parameters in Diabetic Rats. Figure 7 shows noticeable upregulations in the renal gene expression of vascular endothelial growth factor (VEGF), collagen, and MMP-9 in the diabetic group compared with the control group. Conversely, SNP-treated diabetic rats reveal a significant retirement in the renal VEGF, collagen, and MMP-9 gene expressions compared to untreated diabetic rats.

3.7. Effect of SNPs on the Histopathological Picture of Diabetic Nephropathy. As shown in Figure 8, our histopathological result revealed a typical architecture of renal parenchyma with intact renal corpuscles containing glomeruli in control (Figure 8A,B) and SNP-treated rats (Figure 8C,D). In contrast, the diabetic kidneys showed vacuolar tubular degeneration, glomeruli congestion, and the basement membrane thickening of renal tubules, as shown in Figure 8E,F. However, the SNP-treated diabetic rats exhibited improvements in the renal tissue, seen by mild congestion of glomerular capillaries with thin basement membrane and restoration of the integrity of the proximal and distal renal tubules (Figure 8G,H). For semiquantitative scoring of histopathological lesion of the kidney, see Table S2.

3.8. Clustering Heatmap and Variable Importance Projection (VIP) Scores. The concentration values of all observed biochemical parameters are summarized in the clustering heatmap shown in Figure 9A, which gives an intuitive overview of all data sets. Higher concentrations of HbA1c%, microalbumin, cystatin C, AGEs, urea, creatinine, MMP-9, VEGF, TNF-α, NF-κB, NO, and MDA and lower concentrations of HO-1, Bcl2, NRF2, GSH, SOD, insulin, and C-peptide were observed in STZ-treated animals, as indicated by redder cells in the heatmap's gradient scale. On the other hand, the STZ + SNP group's cells displayed moderate levels of color intensity throughout the board, indicating possible improvement in response to STZ treatment. The next step was to use the VIP score to identify the variable that most affected the outcomes of various therapies. Figure 9B reveals that the most influential factors in STZ-induced diabetic nephropathy were urea, cystatin C, insulin, SOD, GSH, creatinine, microalbumin, and HbA1c%.

4. DISCUSSION

STZ is frequently utilized in diabetes mellitus research because it precisely targets β-cells and decreases blood insulin levels, resulting in hyperglycemia and simulating diabetes mellitus.[53] 20−50% of all people with diabetes develop a diabetic injury, which is depicted by persistent albuminuria and a deterioration in kidney function.[54] In the current research, the renoprotective effects of SNPs against diabetic nephropathy were

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demonstrated. The present data revealed that blood glucose (HbA1c%) and cystatin C significantly increased in response to STZ treatment. Serum insulin and C-peptide levels dropped considerably in rats compared with the control rats. However, SNP dramatically improved their levels in diabetic rats. Although SNPs significantly reduced glucose, HbA1c%, and cystatin C compared with STZ-diabetic rats, SNP considerably elevated insulin and C-peptide compared with the STZ group sole treatment. These data are consistent with the literature,[27,55] which documented that the hypoglycemic antidiabetic characteristics of *A. platensis* reduced glucose and HbA1c% with improved insulin levels, resulting in enhanced glucose tolerance and weight gain in diabetics. *A. platensis* is known to have antioxidant[56] and anti-inflammatory activities.[57] It has been thought to protect against STZ diabetes by lowering oxidative stress and maintaining the integrity of pancreatic β-cells. Besides, spirulina has been reported to shield the pancreatic β-cells, maintaining blood sugar levels.[58]

The present findings suggest that the SNP is functional for warding off the consequences of diabetes caused by poor glucose metabolism. There are several potential mechanisms by which the SNP functions as a hypoglycemic agent. These effects include elevating peripheral blood glucose levels, boosting pancreatic islet cell insulin secretion, and promoting glucose absorption.[59,60] Furthermore, our data proposed that cystatin C might be an additional biomarker for diabetic nephropathy. Diabetic rats in the current study exhibited higher cystatin C levels than nondiabetic animals. This result was in harmony with that obtained by Kandil,[61] reflecting the importance of cystatin C as an adiabatic marker.

Moreover, our results revealed low insulin and C-peptide levels at pathogenic levels in diabetic nephropathy. Herein, cotreatment with SNPs led to a modest restoration of the normalcy of such parameters. Diabetic renal impairment caused by STZ would result in microalbuminuria.[62] SNP cotreated with diabetes significantly induced decreases in microalbuminuria in diabetic rats. These data were in harmony with a previuos study,[63] which revealed that the elevated level of microalbumin in the cisplatin-treated rat was decreased after *A. platensis* supplementation. In addition, we found that STZ-induced diabetes in rats led to increasing kidney weight, which was opposite to the SNP treatment; this finding was in line with Jensen et al. and Sharma and Ziyadeh,[64,65] who returned the increased kidney weight of the diabetic rat to regional shifts in the synthesis of growth factors and/or their receptors and overexpression of transforming growth factor.

Our data reported that kidney injury markers, serum AGEs, urea, and creatinine were significantly alleviated by SNP treatment. Hyperglycemia's increased AGE production causes diabetic nephropathy. AGEs interact with RAGE to initiate the NF-κB cascade and produce proinflammatory cytokines and fibrogenic mediators, establishing an intracellular oxidative state and persistent inflammation.[66] AGE alters protein function or induces receptor-mediated reactive oxygen species generation, compromising cell hemostasis.[67] Furthermore, diabetic rats developed kidney hypertrophy, hyperglycemia, and renal dysfunction with significantly increased urea, uric acid, and creatinine.[68] Increased blood creatinine levels indicate that a kidney's filtering capacity is impaired, which can lead to nephron destruction.[69] Significant improvements in renal biomarkers imply that spirulina supplementation in diabetic rats could mitigate STZ-induced kidney damage, as supported by the data concluded by Gargouri et al.[69] Diabetic nephropathy and its potential progression to severe renal fibrosis have been related to elevated levels of the extracellular matrix protein hydroxyproline and collagen in the kidneys. SNPs effectively inhibited the rise in the hydroxyproline concentration, leading to a reduction in fibrosis. This observation is consistent with Al-Mokaddem et al.,[70] who reported the antifibrotic activity of *A. platensis* against thioacetamide-induced liver fibrosis in rats. Furthermore, SNP-treated diabetic rats restored the harmful effect of STZ concerning the antioxidant enzymes; this finding was in harmony with a previous study,[71] whereby it was observed that MDA and NO levels were significantly elevated in the kidneys of diabetic rats due to oxidative injury and ROS, which leads to membrane destruction.[72] It is thought to be an essential causal element connecting diabetes with pathogenic consequences in several tissues.[73]

In addition, the present data additionally demonstrate that STZ lowered the renal GSH concentration and SOD activity in diabetic rats. Excess ROS creation may be blamed for the inactivation of the free radical scavenging enzyme SOD, which reduces its activity. Since superoxide cannot diffuse across a lipid barrier, it cannot generate H_2O_2 and must be disputed by the SOD. H_2O_2, the primary agent in tissue destruction, is known to be neutralized by computed axial tomography (CAT). Damage to the second line of antioxidant protection occurs, as indicated by the considerable reduction of GSH in the kidneys of diabetic rats.[74] Thus, decreased SOD, CAT, and GSH levels could weaken the antioxidant defense against free radicals.[75]

Vitamins C and E in *A. platensis* may account for its antioxidant potential. These vitamins have antioxidant assets, are central to scavenging free radicals, and offer better protection. However, vitamin E may stop the peroxidation of lipids in cell membranes by sequestering peroxyl radicals and maintaining the GSH levels.[76] Chlorophyll in *A. platensis* and its derivatives neutralize free radicals.[77] Either by attachment to RAGEs or by production of ROS, AGEs activate the NF-κB signaling cascade. Inflammatory cytokines and fibrogenic mediators are created mainly due to the work of NF-κB, a transcription factor. In its inactive state, NF-κB resides in the cytoplasm and is associated with inhibitory B (IkB). Upon activation by stimuli like AGEs, IkB dissociates from NF-κB, allowing NF-κB to translocate to the nucleus, where it promotes the upregulation of proinflammatory cytokines, including IL-6 and TNF-α.[78] In our study, SNPs pointedly decreased the level of NF-κB mRNA expression in diabetic rats; this may explain its anti-inflammatory properties and ability to prevent or postpone the onset of diabetic nephropathy. This attests to what has been previously documented as *A. platensis*, which has an anti-inflammatory effect on the brain against the damage brought by free proinflammatory cytokines via decreasing oxidative injury and inhibiting the NF-κB signaling cascade.[79]

It is well-known that oxidative stress and inflammation promote apoptosis during diabetic nephropathy,[80] involving the upregulation of Bax and downregulation of Bcl2 mechanisms; these support our results concerning the apoptotic cascade in diabetic animals. However, in this study, SNP administration could overcome STZ-induced cell death. These findings were supported by Sadek et al.,[81] who found that supplementation of spirulina increased the Bcl2 and reduced Bax expression, affirming the antiapoptotic impacts of *A. platensis*. The antioxidant and anti-inflammatory reactions

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mediated by the transcription factor NRF2 are integral to the cell's defensive system.[82] Excess ROS can cause inflammation by damaging cellular macromolecules and activating redox-sensitive molecules like NF-κB.[83] Oxidative injury, NF-κB activation, and elevated proinflammatory conciliators have all been associated with diabetes complications and diabetic nephropathy.[84] TLR4/MyD88/NF-κB signaling also regulates the synthesis of proinflammatory mediators in diabetic kidneys, further escalating the inflammatory cascade.[85] Apoptosis and cell death are further consequences of diabetic nephropathy in the induction of oxidative injury and inflammation. NRF2 expression increases show an antioxidant response and balance restoration. NRF2, an antioxidant, regulates metabolic balance and eliminates lipid buildup and oxidative stress.[86] SNP activated NRF2 due to its antioxidant action, as evidenced by increased mRNA levels of NRF2 and HO-1 in the kidneys of diabetic rats.[87]

The process of angiogenesis is a therapeutic target for diabetic nephropathy.[88] Since VEGF is a crucial angiogenesis regulator linked to diabetic nephropathy,[88] we tested how SNPs affected this factor. Previous research has shown that diabetic rats had increased VEGF expression in their kidneys.[89] One possible mechanism by which SNP protects against diabetic nephropathy is reducing the quantity of VEGF mRNA in diabetic rats. Although the effect of SNPs on VEGF in diabetic kidneys has not been studied, spirulina has been shown to reduce VEGF in the context of full Freund's adjuvant-induced arthritis in rats.[90]

Cumulative evidence suggests the upregulation of MMP-9 in diabetic kidneys.[91] Consistently, the current data showed increased MMP-9 expression levels in diabetic rats, which were reduced after SNP supplementation. Our findings align with those obtained by Braune et al.,[92] who observed the downregulation of MMP-9 after administration of *A. platensis*. Furthermore, renal fibrosis is caused by the degeneration of the kidney's structure due to abnormal collagen accumulation. Renal tissue degradation was linked to increased collagen mRNA expression, as formerly documented.[93] Our findings, backed by the literature, showed that SNP therapy dramatically reduced collagen expression in diabetic rats,[94] which first reported that *A. platensis* inhibited the expression of collagen mRNA.

Diabetic kidney injury has been linked to changes in histology, including glomerular necrosis, interstitial hemorrhage, fibrotic and degenerative abnormalities, inflammatory cell infiltration, and perivascular lymphocytic accumulations.[95] SNP protects the kidneys by enhancing renal function and reversing structural changes brought about by hyperglycemia in diabetic rats. The potential role of *A. platensis* in restoring the damaged renal architecture following furan intoxication has been demonstrated.[96] Its antioxidant qualities probably saved lives and helped fix the kidney's messed-up tissue architecture.

The multivariate assessments seen by heatmaps and VIP scores confirmed the findings above. The clustering heatmap acquitted noticeable differences between the mean values of all parameters in diabetic rats and those cotreated with SNPs. These results strongly propose the mitigating mechanisms of SNP against STZ-induced oxidative damage, inflammation, and apoptosis of renal tissue. The VIP scores revealed that urea, cystatin C, glucose, SOD, GSH, creatinine, microalbumin, and HbA1c% were the most contributing variables in response to STZ and/or SNP treatments. The proposed mechanisms behind SNP's mitigating activity against diabetic nephropathy are summarized in Figure 9. Our investigation into spirulina nanoparticles (SNP) unveils their significant renoprotective potential against STZ-induced diabetic nephropathy, corroborating and extending existing knowledge on A. platensis's antioxidative and anti-inflammatory properties. Our study supports SNP's therapeutic efficacy through improvements in biochemical markers, glucose metabolism, and a decrease in microalbuminuria and kidney hypertrophy. It suggests cystatin C as a promising biomarker for diabetic nephropathy. Acknowledging limitations, such as the study's single-dose approach and focus on male subjects, we underscore further research to explore SNP's mechanisms of action more deeply, including dose−response relationships and gender differences, to bridge preclinical insights with clinical therapeutic potential.

Spirulina nanoparticles (SNPs) offer protection against streptozotocin (STZ)-induced diabetic nephropathy through a multipronged mechanism that includes antioxidative, anti-inflammatory, and antiapoptotic actions. By enhancing the cellular antioxidant defense via upregulation of NRF2 and HO-1, reducing oxidative stress markers, such as MDA and NO, and elevating antioxidant enzymes (SOD, CAT, GSH), SNP mitigates oxidative damage. It also suppresses inflammation by inhibiting NF-κB signaling and reduces renal cell apoptosis by modulating the Bax and Bcl2 expression. Furthermore, SNP improves renal function by lowering urea, creatinine, and cystatin C levels and protects the renal structure by preventing fibrosis. These combined effects and SNP's ability to improve glucose metabolism by enhancing insulin secretion and reducing hyperglycemia underpin its therapeutic potential in attenuating the progression of STZ-induced diabetic nephropathy.

5. CONCLUSIONS AND PROSPECTS

In summary, STZ could evoke diabetic nephropathy in rats; oxidative damage, inflammation, fibrosis, and apoptosis were implicated in such renal injury. Interestingly, the administration of SNP exerted a mitigating effect against the STZ-induced diabetic model. These improvements might be attributed to antioxidant, anti-inflammatory, and antiapoptotic activities of SNPs. We anticipate that the SNP would be a promising agent with therapeutic potential used for attenuating diabetic nephropathy. Research on novel biomarkers, genetic and proteomic profiles, and their potential use is essential for understanding the molecular pathways involved in diabetic nephropathy.

6. LIMITATION AND FUTURE DIRECTION

We did not assess blood pressure in this investigation, and there were difficulties in obtaining comparable amounts of cortical and medullary components for proteomics analysis. In addition to the lack of protein expression data (for some podocyte-related proteins, such as podocin and synaptopodin), there is a lack of hemodynamic measurement. Further investigation is necessary using broad distributions for age, thus allowing for more precise disease staging. In addition, studying the alteration induced between blood pressure and the renin−angiotensin−aldosterone system and proteome analysis is one of the most influential and valuable tools for detecting novel proteins associated with various kidney diseases and studying renal ultrastructural changes.

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■ ASSOCIATED CONTENT

Data Availability Statement

The data is available throughout the manuscript and supporting files.

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.4c02285.

Experimental design; XRD, ζ-potential and SNP particle size; creatine clearance; primers for gene expression; scoring of histopathological lesion of kidney (PDF)

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Author Contributions

F.A., A.A., S.H.B., and M.A.K., supervised, designed, performed experiments, and revised the manuscript. F.A.F., M.A.A., and S.F.I. performed experiments, analyzed data, wrote and finalized the manuscript. L.F., F.I., and M.A. performed experiments. S.A.E., H.I.G., and M.S. performed experiments, wrote, interpreted data, and finalized the manuscript.

Notes

The authors declare no competing financial interest.

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