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Ameliorative Effect of the Methanolic Extract of Spirogyra varians on Hyperglycemia and Nephrotoxicity in Male Rats Induced with Streptozotocin and Gentamicin

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ABSTRACT

Objective: *This study evaluates the therapeutic efficiency of the methanolic extract of Spirogyra varians obtainable in diabetes and renal failure with an experimentation model in a sampled mices.* ***Method:*** *The alga biomass was obtained, extracted and purified and its chemical elements was examined with the use of albino gas chromatography, mass spectrometry (GC–MS). The experimentation was carried out on the male albino laboratory with rats (200g) which was maintained under measured room and was randomly separated in to experimentation categories which include: (8 rats/group). Diabetes was tempted through streptozotocin (STZ), and nephrotoxicity was tempted with gentamicin (GN). After this process the rats obtained graded an oral dose of the methanolic algal throughout the period of treatment.* ***Results:*** *The findings showed the glucose level was elevated as well as the glucose levels and similarly the insulin levels also decreased to confirm the successful induction of diabetes. The renal function biomarkers which include urea and creatinine indicated an elevation. The isolated groups displayed essential enhancement following algal the administration of extract with does-inclined differences. The renal injury evaluation with the use of kidney injury molecule-1 (KIM-1) discovered an essential enhancement in the treatment groups, though, the effect was not the same across all the administered doses. Oxidative stress markers again indicated reformed levels of malondialdehyde (MDA) and superoxide dismutase (SOD) this follows the treatment and induction to support the inclusion of antioxidant mechanisms.* ***Novelty:*** *Generally, the findings present that the methanolic extract of Spirogyra varians uses significant antihyperglycemic and nephroprotective impacts in a diabetic nephropathy model to accompany the improvement in renal function oxidative stress status and biomarkers.*

INTRODUCTION

Diabetes mellitus is viewed as a composition of metabolic disorders which results in defects in the insulin action or insulin secretion or all, which leads to long hyperglycemia. These irregularities may occur to pancreatic beta cells which is responsible for insulin release to insulin at the level of the target cells [1]. Diabetes mellitus signifies one of the main cause of complications in diabetes, this comprises nephropathy, neuropathy and diabetic retinopathy, [2], [3]. Diabetes mellitus is grouped into different types which include the key insulin-inclined diabetes mellitus (IDDM), which occurs as a result of autoimmune annihilation of pancreatic beta cells, and non–insulin-inclined diabetes mellitus (NIDDM), which accounts for around 90% of diabetes problems. The latter one results as a result of insulin secretion and insulin resistance, which leads to impaired glucose consumption by cells. This problem is connected with metabolic glucose production especially during fasting time [4], [1].

Reports show that there is a universal increment in severe kidney disease. This highlights the vitality of protecting renal health and the use of some drugs such as nephrotoxic effects. Kidney damage brought by pharmaceuticals can happen via numerous tools, and some alterations. Drug-inclined through nephrotoxicity accounts for around 20% of severe kidney damage and serve as serious clinical challenge in disease managing. Numerous types of drugs are the causes of renal toxicity which includes antibiotics non-steroidal antivirals, anti-inflammatory drugs, chemotherapeutic drugs and, immunosuppressive agents, mostly described as "silent killers" of functional kidney as a result of their deceptive effects [5]–[7].

Diabetes mellitus forms a major universal health challenge, which necessitates early healing strategies. Natural products have been regularly used by different patients as an alternative source of treatment owing to their structural composition and biological activities. Algae specifically draw important interest as possible agent for the management of diabetes because of their quality in biological active elements antihyperglycemic properties [8].

Algae have established notable chemical variety and are seen as economically and environmentally friendly with conventional drug synthesis. Marine-inclined natural substance like prokaryotic and eukaryotic types and produce a broad range of secondary metabolites comprising of alkaloids, polyphenols, flavonoids, fatty acids, terpenoids, steroids, and polysaccharides which display numerous biological activities [9]. Similarly, dietary imbalance plays a central function in the growth of diabetes mellitus and its complexities [10].

Innovations in algal biotechnology have become broadened in medicine, pharmacy, and nutrition. Algae represent an essential source of biological active elements utilized for the treatment and prevention of different classes of the diseases. These elements possess antioxidant, anticancer, antimicrobial anti-inflammatory, antiviral, and immunomodulatory actions. They clearly assert their impacts through the interaction with cell membranes, receptors and enzymes, and eventually influencing the cellular pathways and metabolic processes [11].

Spirogyra varians is a green filamentous alga belong to the groupings of belonging Chlorophyta and order Zygnematales. It is considered by its ability to create diverse bioactive elements with several antioxidant, antibacterial, and antiviral and inflammatory elements [12]. One of the main recompences of the natural products used is their chemical composition, especially algae which is a secondary form of metabolites comprising of flavonoids, polyphenols, steroids, polysaccharides, and terpenoids which help in their pharmacological potential [13]. Prior studies have stated the isolation and characterization of bioactive mixtures from algae with advanced therapeutic effects. For instance, the phenolic compound A-Calothrixin isolated from *Cladophora crispata* showed no cytotoxic effects on human erythrocytes, while phenolic compounds derived from medicinal plants have demonstrated potential benefits in diabetes management [14].

Furthermore, ethanolic extracts of green algae such as *Chlorella vulgaris* and *Ulva lactuca* have been shown to significantly reduce blood glucose and cholesterol levels in diabetic animal models, highlighting their potential as natural antidiabetic agents [15]–[17].

Based on the above findings, the present study aimed to investigate the therapeutic potential of the methanolic extract of *Spirogyra varians* in the management of diabetes mellitus and renal toxicity, and to provide comprehensive insight into the role of natural algal products in improving hyperglycemia and nephrotoxicity induced by streptozotocin and gentamicin in male albino rats.

Materials and Methods

Green freshwater algae used in the present study were collected from aquatic the environment in Basrah Governorate. *Spirogyra varians* was collected from the Shatt Al-Arab district. The samples were transported to the laboratory in clean, tightly sealed plastic containers. The algal material was thoroughly washed with tap water to remove adhering impurities and debris, followed by washing with distilled water several times. An ultrasonic cleaner (Sanicater) was used to ensure further purification. The samples were then prepared for subsequent purification procedures.

Identification and purification of algal isolate

Algal isolate was examined and identified using a light microscope to confirm taxonomic characteristics. Identification was carried out based on morphological characteristics which are the standard references and keys and confirmed utilizing the Algae database [18], [19]. The purification process was conducted based on the approach described by Weideman et al. [20]. To get axenic algal culture free of microbial pollution. The culture was then freeze and dried utilizing a freeze dryer and stored in sterile within a glass container at 40 °C until further use.

Preparation of the Methanolic Extract of the Alga

The methanolic abstraction of *Spirogyra varians* was carried with the method as described by Salman et al with little changes [21]. Momentarily 40 g of dried, crushed algal biomass was removed with 800 mL of 70% methanol utilizing a Soxhlet extraction apparatus. The algal material was placed in filter paper thimbles to prevent dispersion during extraction. The extraction process was carried out at a temperature range of 60–65 °C. After completion, the methanolic extract was concentrated and allowed to evaporate at room temperature using glass Petri dishes to obtain a dry residue. The dried extract was stored in airtight containers at −18 °C until use.

Chemical Characterization of the Algal Extract Using Gas Chromatography–Mass Spectrometry (GC–MS)

Chemical constituents of the methanolic extract of *Spirogyra varians* were identified using gas chromatography–mass spectrometry (GC–MS). Analysis was performed using an Agilent Technologies GC system model 7890 coupled with a mass selective detector (MSD) model 5977A. The analysis was conducted at one of the

laboratories affiliated with Agilent Technologies in Basrah. Identified compounds were characterized based on their retention times and mass spectral data.

Experimental Animals

The experimental study was conducted using 90 male albino laboratory rats. Animals were obtained from the College of Medicine, University of Basrah, and housed under controlled laboratory conditions [22].

Preparation of Therapeutic Doses

Therapeutic doses of methanolic extract of the alga Spirogyra varians were prepared according to the methods previously mentioned [23], [24]. The extract was weighed to obtain concentrations of 20, 40, 80, and 120 mg/kg body weight. The extract was dissolved in a few drops of Tween 80, and the final volume was adjusted to 1 mL with distilled water to obtain the required doses for oral administration.

Induction of Diabetes Mellitus and Nephrotoxicity

Diabetes mellitus was induced in male rats by a single intraperitoneal injection of streptozotocin (STZ) (Sigma, St. Louis, MO, USA) at a dose of 50 mg/kg body weight, dissolved in freshly prepared buffer solution, following the method described by Thongrung et al. [25]. Rats with fasting blood glucose levels exceeding 300 mg/dL on the fourth day after injection were considered diabetic. Nephrotoxicity was induced by intraperitoneal administration of gentamicin (GN) at a dose of 100 mg/kg body weight once daily for 10 consecutive days, following the method described by Sharma et al. [26].

Experimental Design

The experimental animals were randomly and evenly divided into seven groups, with eight rats per group. Rats were aged 10–12 weeks and weighed between 170–230 g, with an average body weight of 200 g. The experimental groups were as follows: Group A (Negative Control): Rats received physiological saline (1 mL) during the induction period, followed by 1 mL of distilled water orally for 28 days.Group B (Positive Control I): Rats received the methanolic alga extract at a dose of 80 mg/kg body weight orally for 28 days.Group C (Induced Positive Control): Rats were induced with diabetes and nephrotoxicity using STZ and GN without treatment. Group D Treatment groups (1,2,3,4): Induced rats received the methanolic alga extract at a doses of (20,40,80,120) mg/kg respectively body weight orally for 28 days.

Blood Serum Collection

At the end of the experimental period, rats were fasted for 12 h and then anesthetized using chloroform inhalation. Blood samples were collected directly from the heart and transferred into sterile test tubes. Samples were centrifuged to separate serum, which was then stored in deep-freeze conditions at −80 °C until biochemical analysis.

Biochemical Assays

Serum biochemical parameters were assessed, including blood glucose, urea, and creatinine levels, using enzymatic methods according to Tietz [27]. Serum insulin levels

and additional biomarkers, including kidney injury molecule-1 (KIM-1), malondialdehyde (MDA), and superoxide dismutase (SOD), were measured using enzyme-linked immunosorbent assay (ELISA) kits following the sandwich ELISA technique as described by Vaidya et al. [28].

Statistical Analysis

Data obtained from the present study were statistically analyzed using one-way analysis of variance (ANOVA) with the Statistical Package for the Social Sciences (SPSS, version 22). Variations between group means were observed numerically significant at $p \leq 0.05$. Results were articulated as mean ± standard deviation (SD).

Results

Morphological Identification of Spirogyra varians

Spirogyra varians is a non-branched filamentous green alga mostly found in freshwater ecosystem where it has a formation of floating mats like on the surface of the water. An analysis of it showed cylindrical cells with lengths which ranges from 30–70 µm as well as the widths range from 30–38 µm. The alga was categorised by the presence of spiral ribbon-shaped chloroplasts which extends along the filament that contains numerous pyrenoids. These morphological features affirmed the taxonomic identification of the alga as *Spirogyra varians* (Figure 1).

Taxonomic classification:
Division: Chlorophyta
Class: Zygnematophyceae
Order: Zygnematales
Family : Zygnemataceae
Genus: Spirogyra
Species: Spirogyra varians (Hassall) Kützing (1849)

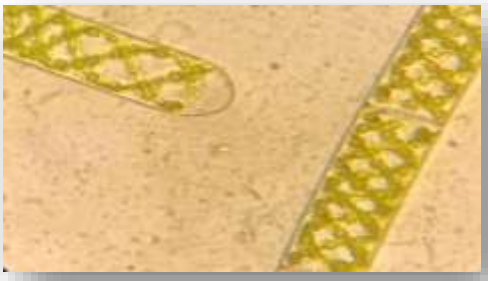

Figure 1. Spirogyra **varians**

Chemical composition of the Methanolic Extract of Spirogyra varians

Methanol, one of the polar solvents, was utilized in this study because of its ability to obtain a broad number of chemically and diverse bioactive elements. The methanolic extraction yielded approximately 2 g of dry extract per 40 g of dried algal biomass.

Gas chromatography–mass spectrometry (GC–MS) analysis of the methanolic extract of *Spirogyra varians* revealed the presence of 28 chemical compounds, as listed in Table (1). The identified compounds exhibited different molecular weights and retention times. The mass spectra of the isolated compounds are shown in Figure (2).

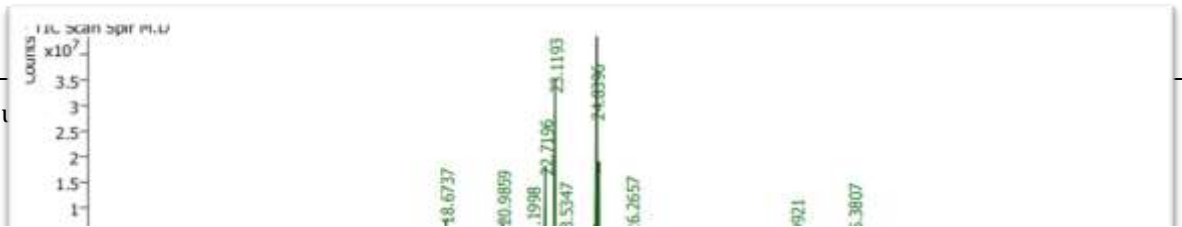

Figure 2. Mass spectrum of isolated and identified compounds from S. varians algae extract).

Table 1. Chemical compounds identified in Spirogyra varians algae extract.

Peak	زمن الاحتجاز	الوزن الجزيئي g/mol	المساحة %	الصيغة الجزيئية	Library/ID
5	19.324	172.26	5.335	C12H24O2	Dodecanoic acid
12	22.717	262.39	7.114	C17H26O2	Methyl 4,7,10,13-hexadecatetraenoate
14	23.123	270.45	18.291	C17H34O2	Hexadecanoic acid, methyl ester
15	23.536	256.42	3.541	C16H23O2	n-Hexadecanoic acid
20	24.841	292.46	29.669	C19H32O2	9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-
21	24.93	296.5	6.598	C20H40O	Phytol

Table 2. Glycemic and endocrine markers in diabetic rats with renal dysfunction

Insulin (mg/dL), Mean ± SD (Letter)	Blood glucose (mg/dL), Mean ± SD (Letter)	Groups
0.992 d±8.048	27.745 d± 118.572	Negative control
0.171 e±3.732	61.644 a± 324.866	Induced positive control
1.234 c±9.358	12.157 c± 178.438	Positive control (II)
1.19 b,c±10.428	18.522 cd±147.935	Treatment 1 (20 mg/kg)
1.458 a±12.290	41.989 c ±181.977	Treatment 2 (40 mg/kg)
1.246 b±11.077	30.179 b ±246.410	Treatment 3 (80 mg/kg)
1.265 a±12.605	31.140 d ±129.454	Treatment 4 (120 mg/kg)

Footnote: Different letters indicate significant differences among groups. One-way ANOVA with

letter-based post hoc mean separation; p ≤ 0.05 (n = 8/group).

The results presented in Table (2) showed a statistically significant increase (P ≤ 0.05) in blood glucose levels in the serum of the first induced positive control group compared with the negative control group. In contrast, the results clearly indicated the therapeutic role of the algal extract of *S. varians*, as a marked improvement in blood glucose levels was observed in all treated groups compared with the first induced positive control group. The most effective concentration in significantly reducing blood glucose levels was 120 mg/kg, at which the glucose level reached the normal level observed in the negative control group.

The results shown in Table (2) for *S. varians* also demonstrated a statistically significant decrease (P ≤ 0.05) in serum insulin hormone levels in male rats of the first induced positive control group compared with the negative control group. The results recorded a statistically significant increase and a marked improvement in serum insulin levels secreted by pancreatic beta cells in all treated groups compared with the first induced positive control group, reflecting the therapeutic efficiency of the algal extract.

Table 3. Renal function and injury biomarkers (merged).

KIM-1 (mg/dL), Mean ± SD (Letter)	Creatinine (mg/dL), Mean ± SD (Letter)	Urea (mg/dL), Mean ± SD (Letter)	Groups
0.085 c± 0.669	0.254 c± 1.065	4.229 d,e± 32.647	**Negative control**
0.088 a± 2.004	0.499 a± 2.736	10.374 a± 77.426	**Induced positive control**
0.219 b± 0.764	0.426 b± 1.734	3.363 e± 28.545	**Positive control (II)**
0.232 b± 0.908	0.710 a± 2.487	13.350 b,c±48.593	**Treatment 1 (20) mg/kg**
0.156 b,c± 0.779	0.437 b,c± 1.534	7.262 b± 55.124	**Treatment 2 (40) mg/kg**
0.344 b± 0.926	0.340 c± 1.106	5.464 c± 42.164	**Treatment 3 (80) mg/kg**
0.133 b± 0.872	0.636 a± 2.921	10.290 a± 76.482	**Treatment 4 (120) mg/kg**

Footnote: Different letters indicate significant differences among groups. One-way ANOVA with

letter-based post hoc mean separation; p ≤ 0.05 (n = 8/group).

The results of the present study for *S. varians*, as shown in Table (3), recorded a statistically significant increase (P ≤ 0.05) in serum urea levels in male experimental rats of the first induced positive control group compared with the negative control group. This indicates the effective role of streptozotocin and gentamicin in induction and in

causing dysfunction of pancreatic beta cells and renal functions. Serum urea levels decreased and showed improvement in the first three treatment groups, with the exception of the fourth treatment group (120 mg/kg), which did not show a statistically significant difference compared with the first induced positive control group.

The results for *S. varians*, as presented in Table (3), also demonstrated a statistically significant increase ($P \leq 0.05$) in serum creatinine levels in male rats of the first induced positive control group compared with the negative control group as a result of the induction process. The results further indicated that the algal extract had an effective role and efficiency in reducing serum creatinine levels in the second and third treatment groups compared with the first induced positive control group, whereas the first and fourth treatment groups did not show statistically significant differences compared with it.

The results of the present study for *S. varians*, as shown in Table (3), confirmed a statistically significant increase ($P \leq 0.05$) in serum KIM-1 levels in male rats of the first induced positive control group compared with the negative control group, possibly due to the effect of gentamicin on renal tubular epithelial cells. In contrast, all four treatment groups showed a statistically significant decrease as a result of the therapeutic efficiency of the algal extract in improving serum KIM-1 levels compared with the first induced positive control group.Treatment with the algal extract resulted in a significant reduction in KIM-1 levels in all treated groups compared with the induced positive control group.

Table 4. Oxidative stress and antioxidant defense (merged).

MDA (mg/dL), Mean ± SD (Letter)	SOD (mg/dL), Mean ± SD (Letter)	Groups
0.114 d ±0.952	1.909 a,b ±10.349	**Negative control**
0.352 a± 2.478	0.657 d± 5.720	**Induced positive control**
0.287 b,c± 1.333	1.870 b,c±9.659	**Positive control (II)**
0.238 b± 1.378	0.731 a,b± 10.411	**Treatment 1 (20) mg/kg**
0.122 c,d± 1.132	0.990 c± 8.600	**Treatment 2 (40) mg/kg**
0.288 b,c± 1.241	0.982 b,c± 9.218	**Treatment 3 (80) mg/kg**
0.197 c,d± 1.103	1.669 a± 11.40	**Treatment 4 (120) mg/kg**

Footnote: Different letters indicate significant differences among groups. One-way ANOVA with

letter-based post hoc mean separation; $p \leq 0.05$ (n = 8/group).

The results presented in Table (4) for *S. varians* recorded a statistically significant decrease ($P \leq 0.05$) in serum superoxide dismutase (SOD) levels in male rats of the first induced positive control group compared with the negative control group. Serum SOD levels showed marked improvement and a statistically significant increase due to the

therapeutic role of the algal extract in all four treatment groups compared with the induced positive control group, which may indicate the effective role of the algal extract in alleviating oxidative stress through controlling free radicals generated as a result of the induction process.

The results presented in Table (4) for *S. varians* demonstrated a statistically significant increase ($P \leq 0.05$) in serum malondialdehyde (MDA) levels in male rats of the first induced positive control group compared with the negative control group. In contrast, serum MDA levels improved due to the therapeutic role of the algal extract, as they showed a statistically significant decrease in all four treatment groups compared with the first induced positive control group, which may indicate an improvement in the level of oxidative stress resulting from the induction process.

Discussion

In the present study, 70% methanol was used as the extraction solvent. Methanol is a polar solvent that extracts most chemical compounds, as it forms various complex structures when interacting with them. Accordingly, most chemically active compounds were extracted in the methanolic algal extract, and this is consistent with the study of Hussein et al. [17].

The mass spectrum results of the present study revealed that the methanolic extract of the green alga *S. varians* contained twenty-eight chemical compounds, six of which exhibited the highest percentage in terms of peak area compared with the other chemical constituents of the extract. These compounds included 9,12,15-octadecatrienoic acid, methyl ester (Z,Z,Z), hexadecanoic acid methyl ester, methyl 4,7,10,13-hexadecatetraenoate, phytol, dodecanoic acid, and n-hexadecanoic acid, in addition to other compounds that differed in their percentage areas. The results of this study were in partial agreement with previous studies [15], [29].

The results showed a statistically significant increase in serum blood glucose levels in male laboratory rats of the first induced positive control group compared with the negative control group. This is considered clear evidence of the successful induction of the pathological condition in rats using streptozotocin and gentamicin through causing damage to pancreatic and renal functions, reflecting the combined effect of diabetes and nephrotoxicity in inducing severe disturbance in carbohydrate metabolic homeostasis. These findings are consistent with those reported in several studies conducted on laboratory animals [30]–[32]. Injection of STZ in male laboratory rats leads to the destruction, necrosis, and death of pancreatic beta cells, as STZ preferentially accumulates in these cells via the glucose transporter GLUT2 through multiple mechanisms, including DNA alkylation and depletion of cellular NAD^+ levels, thereby depriving cells of energy. This process created oxidative stress and raised production of nitric oxide (NO^-) [33].

Likewise, the results of the potential positive and controlled group indicated a numerically essential reduction in blood glucose levels in rats, which indicate that the algal substance have a clear therapeutic activity in the absence of pathological initiation.

This effect is attributed to the presence of biologically active compounds capable of improving cellular insulin sensitivity and regulating glucose metabolism. These compounds act by inhibiting intestinal carbohydrate-digesting enzymes; when alkaloid compounds bind to the selective sites of enzymes involved in digestion, they prevent the formation of the enzyme–substrate complex, thereby reducing the actual enzymatic pathway of the process. This interpretation is consistent with the findings of Rahman et al. [34].

This explanation is consistent with findings from studies confirming that methanolic algal extracts possess antidiabetic properties through inhibition of carbohydrate-digesting enzymes (α-amylase and α-glucosidase), stimulation of cellular glucose uptake, and regulation of gene expression associated with glucose metabolism [35].

The results of the present study demonstrated that induction of diabetes and nephrotoxicity by streptozotocin and gentamicin caused a significant and marked decrease in serum insulin levels in male rats of the first positive control induced group compared with the negative control group. These findings are consistent with the results reported by Boukorttand and Labbaci [36]. Gentamicin exerts a negative effect on the cells of the islets of Langerhans, leading to a pronounced reduction in insulin secretion through inhibition of the Ca^{2+} influx required for insulin granule exocytosis. Consequently, glucose-stimulated insulin secretion is impaired despite unchanged glucose metabolism within the islets. Moreover, gentamicin is associated with increased oxidative stress in experimental models, while β-cells are highly susceptible to oxidative damage; therefore, secretory dysfunction of β-cells may be exacerbated. Accordingly, the decline in insulin levels observed in diabetic groups treated with gentamicin may be explained by the combined effect of disrupted calcium signaling and oxidative stress associated with induction [37].

The improvement and elevation in insulin levels observed in the four algal-treated groups compared with the first positive induced control group can be attributed to the efficacy and potency of the methanolic algal extract. Regarding the results of *S. varians* extract, insulin levels improved in all four treated groups compared with the positive induced control group and exceeded the normal level when compared with the negative control group. In support of this, Alrasheedi et al. explained that the increase and improvement in insulin levels in algal extract-treated groups of streptozotocin-induced experimental animals may be due to their role in functional protection of β-cells through reduction of oxidative stress and regulation of inflammation and cytokines such as tumor necrosis factor-α (TNF-α) and interleukin-6 (IL-6) [38]. This occurs via scavenging free radicals, thereby maintaining mitochondrial efficiency and stability, given that β-cells are highly sensitive to oxidative damage. Algal extracts are rich in natural antioxidants, including phenolic compounds, carotenoids, and flavonoids, which reduce oxidative factors, contributing to functional stability of β-cells and supporting their ability to produce the energy required to maintain insulin secretory capacity [39], [40].

The results of the study also recorded a significant elevation in serum urea levels in male experimental rats of the first positive induced control group compared with the negative control group, due to induction by streptozotocin and gentamicin, which caused diabetes and nephrotoxicity in the experimental animals. These findings are in agreement with several studies reporting increased urea levels [41], [42]. This elevation in urea levels in diabetic conditions is attributed to prolonged hyperglycemia, which leads to macrovascular and microvascular complications resulting from advanced glycation end products and free radicals, causing oxidative stress in renal tissues and negatively affecting kidney function [43]. Persistent oxidative stress stimulates increased expression of several pro-inflammatory cytokines, such as tumor necrosis factor-α and interleukin-6, through activation of nuclear factor kappa B (NF-κB), leading to inflammation, apoptosis, and subsequent immune responses [44].

The reduction in urea levels may be due to the ability of the algal extract to enhance the activity of antioxidant enzymes, inhibit oxidative processes and oxidative stress, and improve glomerular filtration and renal function by scavenging free radicals and suppressing their harmful effects [45]. The improvement in renal function observed in the current study is consistent with the findings of Al-Halaseh et al. [46]. Among the bioactive compounds identified in *S. varians* extract is methyl 4,7,10,13-hexadecatetraenoate, a fatty acid compound with an effective antioxidant role [47].

The results for *S. varians* revealed a significant and marked increase in serum creatinine levels in male rats of the first positive induced control group compared with the negative control group. This increase resulted from the induction of diabetes and nephrotoxicity in experimental male rats using streptozotocin and gentamicin. The induction results of the present study are consistent with several previous studies [48]. The elevation in creatinine levels is attributed to damage to the renal glomeruli and tubules, leading to impaired renal function, as reflected by the significant increase in plasma urea and creatinine levels. In cases of hyperglycemia induced during the induction process, insulin signaling may be impaired and oxidative stress exacerbated through increased production of reactive oxygen species. Furthermore, reactive oxygen species and inflammatory cytokines can damage renal tissues by inducing apoptosis and fibrosis [49].

The results of the present study also demonstrated that the methanolic extract of the studied alga showed a significant effect in the second and third treatment groups of *S. varians*, whereas no significant difference was observed in the first and fourth treatment groups compared with the positive induced control group. This variation may be attributed to the bioactive compounds and their functional groups present in these extract. One of the identified compounds in S. varians is dodecanoic acid, known as lauric acid, a medium-chain saturated fatty acid characterized by rapid absorption and metabolism, which contributes to modulation of the body's inflammatory response [50].

The results for *S. varians* extract also showed a significant decrease in superoxide dismutase (SOD) levels in the serum of male rats in the first positive induced control

group treated with streptozotocin and gentamicin compared with the negative control group. This finding is consistent with the study reported by Aizzat et al. [51]. The reduction may be clarified by the fact that hyperglycemia itself is proficient of reducing mRNA countenance numerous antioxidant enzymes which include SOD. Raised the levels of the glucose and suppress the expression of gene under certain situations, this indicates that the decrease in SOD involves some impaired synthesis, which eventually contribute to the reduction in the defensive capacity [52].

On the other hand, serum SOD levels enhanced and increased broadly as a result of the therapeutic function of the algal extract of S. varians from all the treatment categories, the fourth treatment category showed the most visible enhancement in serum SOD stages, to show the therapeutic efficiency to induce control grouped. These results are in consonance with the findings obtained by Rayapu et al. [53]. Though SOD may rapidly increase the compensatory response but it remains inadequate if the lipid peroxidation, showed by MDA, persists. Here, the role of algal antioxidants will be obvious, as they serve to reduce the ROS and RNS stages, and as such reducing the load that may lead to enzymatic reduction. Thus, the final result is usually the normal part of the enzyme actions. treatment of algal extract can reduce the MDA levels as seen with C. glumerata, which is utilized as signs of lipid peroxidation and enhanced metabolic markers linked with insulin resistance. The accompanied pathways of oxidative stress-linked pathways like the NF-κB and regulation of the appearance of certain the defensive genes as well as enzymes, that comprises glutathione peroxidase GPx. This assertion is in line with the ROS impacts, but it may also influence the molecular control of antioxidant results [54].

The results established a momentous and noticeable increase in malondialdehyde (MDA) levels in the setrum of the male rats consisting of streptozotocin and gentamicin in the first positive administration in comparison with the negative control group [55]. support this result which was later interpreted as an indication of lipid peroxidation under oxidative stress situations. The procedure starts with the relative exposure of the pancreas β-cells to streptozotocin, and to be followed by the production of reactive oxygen and the nitrogen species (ROS/RNS). Subsequently, hyperglycemia additionally intensifies mitochondrial superoxide creation and activates the damage associated with the increment in ROS production, which leads to the damage of the membrane lipids and an eventual increment in MDA levels. Correspondingly, MDA in the STZ-induced diabetic model will present a reliable marker of the oxidative imbalance and membrane malfunction when evaluated along with antioxidant defense indicators [56].

Conversley, serum MDA stages linked with S. varians indicated an essential reduction and enhancement as a result of the therapeutic efficiency of the algal extract, because the MDA stages may decrease among the the four groups significantly in comparison with the first group. In a related study by Ontawong et al, the green alga *Cladophora glomerata* proved the capability to reduce renal lipid peroxidation in an HFD+STZ-induced diabetic model via the NF-κB inflammatory pathway [54]. This approach deals with the observed decrease and the slow stabilization of SOD actions as

confirmed experimentation by the reduction in lipid peroxidation and enhanced antioxidant defense systems in the STZ-inclined diabetic model.

The study findings also showed an essential increment in kidney damage molecule-1 (KIM-1) stages in the serum of male rats induced with streptozotocin and gentamicin in the initial positive group that is under control. The study by Udupa and Prakash exhibits similar result. KIM-1 is a seriously sensitive biomarker of proximal tubular injury with supplemented response to the toxic injury as well as injury to the renal tubular epithelial cells, often prior raises in creatinine and urea stages. In gentamicin-induced replicas, drugs accrual within the induces cytotoxic, oxidative, and inflammatory impacts that trigger the repair signaling results in a marked raise of KIM-1 levels. Gentamicin is freely sifted via the renal glomeruli but the selective features can accumulate the proximal tubular epithelial cells through receptor-mediated endocytic devices which invloves megalin and cubilin. This high degree of accumulation renders the proximal tubules the primary target of gentamicin-induced nephrotoxicity, explaining the pronounced tubular nature of renal injury

The results further indicated an improvement in KIM-1 levels, with a significant reduction recorded in the serum of rats treated with methanolic algal extract of *S. varians* across all experimental groups compared with the first positive induced control group. The therapeutic efficacy of the extract was most evident in the second treatment group, in which KIM-1 levels were reduced to values comparable to those of the negative control group. Given that elevated KIM-1 levels are associated with proximal tubular injury in gentamicin-induced models, the observed improvement in renal function, reduction of lipid peroxidation, and enhancement of antioxidant defenses attributed to the therapeutic action of polysaccharide-rich compounds provide a logical explanation for the attenuation of tubular injury severity and the consequent decrease in serum KIM-1 levels in treated male rats. Moreover, extracts of *Cladophora glomerata*, which are rich in polyphenols, have demonstrated antidiabetic and renoprotective effects through modulation of organic anion transporters Oat1 and Oat3 mediated by protein kinase pathways in diabetic mice [54].

CONCLUSION

Fundamental Finding : The results of this study demonstrate that the methanolic extract of Spirogyra varians possesses a notable efficacy in improving the metabolic and renal disturbances associated with diabetes mellitus in male rats, which is attributed to its content of biologically active compounds. **Implication :** These findings indicate the promising therapeutic potential of this alga as a natural supportive source in the management of renal complications associated with diabetes mellitus. **Limitation :** This effect was associated with an improvement in the endogenous antioxidant defense system, contributing to the reduction of cellular damage and the restoration of oxidative balance. **Future Research :** Further studies are required to explore the detailed

mechanisms and evaluate the clinical applicability of Spirogyra varians in the management of diabetic renal complications.

REFERENCES

[1] A. Petersmann *et al.*, "Definition, classification and diagnosis of diabetes mellitus," *Experimental and Clinical Endocrinology & Diabetes*, vol. 127, no. S01, pp. S1–S7, 2019.

[2] D. Mahmood, B. K. Singh, and M. Akhtar, "Diabetic neuropathy: therapies on the horizon," *Journal of Pharmacy and Pharmacology*, vol. 61, no. 9, pp. 1137–1145, 2009.

[3] S. Shelbaya *et al.*, "Study of the role of interleukin-6 and highly sensitive C-reactive protein in diabetic nephropathy in type 1 diabetic patients," *European Review for Medical and Pharmacological Sciences*, vol. 16, no. 2, pp. 176–182, 2012.

[4] D. Glovaci, W. Fan, and N. D. Wong, "Epidemiology of diabetes mellitus and cardiovascular disease," *Current Cardiology Reports*, vol. 21, pp. 1–8, 2019.

[5] J. Kaufman, M. Dhakal, B. Patel, and R. Hamburger, "Community-acquired acute renal failure," *American Journal of Kidney Diseases*, vol. 17, no. 2, pp. 191–198, 1991.

[6] J. B. Patel and A. Sapra, "Nephrotoxic medications," 2020.

[7] A. Džidić-Krivić *et al.*, "Unveiling drug induced nephrotoxicity using novel biomarkers and cutting-edge preventive strategies," *Chemico-Biological Interactions*, vol. 388, 110838, 2024.

[8] L. Pereira and A. Valado, "Algae-derived natural products in diabetes and its complications—current advances and future prospects," *Life*, vol. 13, no. 9, 1831, 2023.

[9] L. A. Minhas *et al.*, "Algae-derived bioactive compounds as potential pharmaceuticals for cancer therapy: A comprehensive review," *Algal Research*, vol. 78, 103396, 2024.

[10] A. Bocanegra, A. Macho-González, A. Garcimartín, J. Benedí, and F. J. Sánchez-Muniz, "Whole alga, algal extracts, and compounds as ingredients of functional foods," *International Journal of Molecular Sciences*, vol. 22, no. 8, 3816, 2021.

[11] E. S. Sruthy and E. C. K. Baiju, "Exploration of secondary metabolites from green algae as antimicrobial agents," *Botanica Serbica*, vol. 48, no. 2, pp. 127–140, 2024.

[12] T. Takano *et al.*, "Identification of 13 Spirogyra species by traits of sexual reproduction," *Scientific Reports*, vol. 9, 2019.

[13] K. Yeshi, D. Crayn, E. Ritmejerytė, and P. Wangchuk, "Plant secondary metabolites produced in response to abiotic stresses," *Molecules*, vol. 27, no. 1, 313, 2022.

[14] T. Behl *et al.*, "Alkaloidal phytoconstituents for diabetes management," *Molecules*, vol. 27, no. 18, 5851, 2022.

[15] S. S. Ghwenm, F. S. Kata, and A. M. Athbi, "Hypoglycemic and antioxidant effect of the ethanol extract of *Chlorella vulgaris* in alloxan-induced diabetic mice," *Biochemical & Cellular Archives*, vol. 20, 2020.

[16] F. Z. Labbaci and F. O. Boukortt, "Beneficial effects of Algerian green alga *Ulva lactuca* on insulin resistance," *Preventive Nutrition and Food Science*, vol. 25, no. 4, pp. 353–360, 2020.

[17] A. S. Hussein, M. M. Zidan, and H. M. Hwihy, "Therapeutic effects of marine algae extracts on diabetic albino rats," *Egyptian Journal of Aquatic Biology & Fisheries*, vol. 27, no. 5, 2023.

[18] G. W. Prescott, *Algae of the Western Great Lakes Area*, 6th ed. Dubuque: Wm. C. Brown Publishers, 1975.

[19] M. D. Guiry, "AlgaeBase," World-wide electronic publication, 2013.

[20] V. E. Weideman, P. R. Walne, and F. R. Tainor, "A new technique for obtaining axenic cultures of algae," *Canadian Journal of Botany*, vol. 42, pp. 958–959, 1984.

[21] N. D. Salman, A. S. Dwaish, and S. M. Kareem, "Effect of methanolic extract of *Spirogyra varians* on biofilm genes expression," 2024.

[22] K. K. Al-Fartosi, *Physiological Studies of the Effects of Benzene in Laboratory Mice and Humans*, PhD thesis, University of Basrah, 2004.

[23] A. Ontawong *et al.*, "Antioxidant and renoprotective effects of *Spirogyra neglecta* extract in diabetic rats," *BioMed Research International*, 2013.

[24] B. Mesbahzadeh *et al.*, "Beneficial effects of *Spirogyra neglecta* extract on antioxidant factors," *Biomolecular Concepts*, vol. 9, no. 1, pp. 184–189, 2018.

[25] R. Thongrung *et al.*, "Moringa oleifera leaf extract ameliorates early stages of diabetic nephropathy," *Journal of Applied Pharmaceutical Science*, vol. 13, no. 8, pp. 158–166, 2023.

[26] I. Sharma *et al.*, "Modulation of gentamicin-induced acute kidney injury," *JCI Insight*, vol. 7, no. 6, 2022.

[27] N. W. Tietz, *Clinical Guide to Laboratory Tests*, 3rd ed. Philadelphia: Saunders, 1995.

[28] V. S. Vaidya *et al.*, "Kidney injury molecule-1 outperforms traditional biomarkers," *Nature Biotechnology*, vol. 28, no. 5, pp. 478–485, 2010.

[29] C. Gopu *et al.*, "GC-MS analysis of bioactive compounds in plant extracts," *Journal of Medicinal Plants Studies*, vol. 9, no. 3, pp. 209–218, 2021.

[30] [30] C. Srimaroeng *et al.*, "Antidiabetic and renoprotective effects of *Cladophora glomerata* extract," *Journal of Diabetes Research*, 2015.

[31] A. Ghasemi and S. Jeddi, "Streptozotocin as a tool for induction of rat models of diabetes," *EXCLI Journal*, vol. 22, pp. 274–292, 2023.

[32] H. U. Rehman, K. Ullah, and A. Rasool, "Comparative impact of streptozotocin on glucose homeostasis," *Scientific Reports*, vol. 13, 7921, 2023.

[33] A. Ghasemi, S. Khalifi, and S. Jeddi, "Streptozotocin-nicotinamide-induced rat model of type 2 diabetes," *Acta Physiologica Hungarica*, vol. 101, pp. 408–420, 2014.

[34] N. Rahman *et al.*, "Molecular docking of isolated alkaloids for α-glucosidase inhibition," *Biomolecules*, vol. 9, no. 10, 544, 2019.

[35] J. Kellogg, M. H. Grace, and M. A. Lila, "Phlorotannins from Alaskan seaweed inhibit carbolytic enzyme activity," *Marine Drugs*, vol. 12, no. 10, pp. 5277–5294, 2014.

[36] A. C. Boschero and E. Delattre, "Mechanism of gentamicin-inhibited insulin release," *Archives Internationales de Pharmacodynamie et de Thérapie*, vol. 273, pp. 167–176, 1985.

[37] A. A. Alrasheedi, A. I. Basnawi, and M. A. Althaiban, "Effects of *Spirulina platensis* on renal function and antioxidant defence," *Journal of Functional Foods*, vol. 122, 106485, 2024.

[38] B. Yongkhamcha and N. Buddhakala, "Phytochemical compositions and anti-inflammatory activity of *Spirogyra neglecta* extracts," *Trends in Sciences*, vol. 20, no. 4, 2023.

[39] K. Kolefer, D. Miaffo, and R. Ponka, "Evaluation of antidiabetic properties of *Ficus vallis-choudae*," *The Scientific World Journal*, 2021.

[40] S. Kumari *et al.*, "Nephroprotective effect of vanillic acid in diabetic rats," *Journal of Diabetes & Metabolic Disorders*, vol. 20, pp. 571–582, 2021.

[41] G. L. King and M. R. Loeken, "Hyperglycemia-induced oxidative stress in diabetic complications," *Histochemistry and Cell Biology*, vol. 122, no. 4, pp. 333–338, 2004.

[42] S. V. Suryavanshi and Y. A. Kulkarni, "NF-κB: a potential target in diabetic vascular complications," *Frontiers in Pharmacology*, vol. 8, 798, 2017.

[43] M. Boozari and H. Hosseinzadeh, "Natural medicines for acute renal failure," *Phytotherapy Research*, vol. 31, no. 12, pp. 1824–1835, 2017.

[44] L. K. Al-Halaseh *et al.*, "Nephroprotective activity of green microalgae," *Journal of Pure and Applied Microbiology*, vol. 16, pp. 2775–2782, 2022.

[45] A. A. Sosa, S. H. Bagi, and I. H. Hameed, "Analysis of bioactive compounds using GC-MS," *Journal of Pharmacognosy and Phytotherapy*, vol. 8, no. 5, pp. 109–126, 2016.

[46] Y. Chtourou *et al.*, "Renal protective effect of dietary polyphenols in diabetic rats," *Nutrients*, vol. 14, no. 14, 2867, 2022.

[47] H. Wang *et al.*, "TNF-α deficiency prevents renal inflammation and oxidative stress," *Kidney and Blood Pressure Research*, vol. 42, no. 3, pp. 416–427, 2017.

[48] K. Nagao and T. Yanagita, "Functional lipids in metabolic syndrome," *Journal of Nutritional Science and Vitaminology*, vol. 61, pp. S159–S161, 2015.

[49] O. Aizzat *et al.*, "Modulation of oxidative stress by *Chlorella vulgaris* in STZ-induced diabetic rats," *Advances in Medical Sciences*, vol. 55, no. 2, pp. 281–288, 2010.

[50] T. Matsunami *et al.*, "Oxidative stress and gene expression of antioxidant enzymes in diabetic rats," *International Journal of Clinical and Experimental Pathology*, vol. 3, no. 2, pp. 177–184, 2009.

[51] L. Rayapu *et al.*, "Protective role of marine macroalgae extracts against STZ-induced diabetic rats," *Journal of Coastal Life Medicine*, vol. 5, pp. 521–530, 2017.

[52] A. Ontawong *et al.*, "Cladophora glomerata extract exhibits antioxidant and anti-inflammatory effects," *Nutrition Research and Practice*, vol. 18, no. 5, pp. 633–646, 2024.

[53] K. Pourkhalili *et al.*, "Renoprotective effect of fucoidan from seaweed on gentamicin-induced nephrotoxicity," 2022.

[54] A. M. A. Nahdi, A. John, and H. Raza, "Molecular mechanisms of streptozotocin-induced oxidative stress," *Oxidative Medicine and Cellular Longevity*, 2017.

[55] V. Udupa and V. Prakash, "Gentamicin induced acute renal damage evaluated using urinary biomarkers," *Toxicology Reports*, vol. 6, pp. 91–99, 2019.

[56] Q. H. Luo *et al.*, "Evaluation of KIM-1 and NGAL as early indicators for nephrotoxicity," *Kidney and Blood Pressure Research*, vol. 41, no. 6, pp. 911–918, 2016.

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