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Spirulina Unleashed: A Pancreatic Symphony to Restore Glycemic Balance and Improve Hyperlipidemia and Antioxidant Properties by Transcriptional Modulation of Genes in a Rat Model

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Abstract: Hyperlipidemia is the root cause of numerous chronic conditions, leading to high mortality rates around the globe. Spirulina (*Arthrospira platensis*) microalgae serve as a promising reservoir of bioactive compounds with diverse pharmacological properties. The current study examined the nutritional profile of spirulina powder in relation to strict glycemic control, specifically focusing on its potential to lower lipid levels. In an in vivo investigation, normal healthy male Wistar albino rats (n = 60) were divided into two groups: a negative control group (NC) of ten rats and a high-fat diet group (n = 50) that were fed a cholesterol-rich diet until their cholesterol levels reached or exceeded 250 mg/dL. Subsequently, the hypercholesterolemic rats were then randomly allocated to several treatment groups: a positive control (PC); a standard treatment diet (STD) involving fenofibrate at a dose of 20 mg/kg body weight; and three experimental groups (T1, T2, and T3) that received spirulina powder supplementation at doses of 300, 600, and 900 mg per kg body weight, respectively, for the period of 12 weeks. Blood samples were analyzed for oxidative stress biomarkers, insulin levels, lipid profiles, liver function, and expression of gene levels in the diabetogenic pathway. The study utilized spectrophotometric colorimetric methods to identify oxidative stress biomarkers, serum kit methods to measure lipid profiles and liver enzymes, and the assessment of qPCR for mRNA quantity. According to the research findings, spirulina powder has certain noteworthy features. It had the greatest quantity of chlorogenic acid (4052.90 µg/g) among seven phenolics and two flavonoid compounds obtained by HPLC-UV analysis. Furthermore, the proximate analysis demonstrated that spirulina is high in protein (16.45 ± 0.8%) and has a significant energy yield of 269.51 K-calories per 100 g. A maximal spirulina dose of 900 mg/kg/wt significantly lowered oxidative stress, cholesterol, triglyceride, low-density lipoproteins (LDL), and insulin levels ($p \leq 0.05$). In contrast, high-density lipoprotein (HDL) and total antioxidant capacity (TAC) levels increased significantly ($p \leq 0.05$) compared to all other groups, except the NC group. The study provides remarkable proof about the pharmacological impact of spirulina powders. Significant reductions ($p \leq 0.05$) in liver enzymes {alanine aminotransferase (ALT) and aspartate aminotransferase (AST)} were observed across all treatment groups, with the exception of the NC, compared to the positive control. The treatment groups had significantly greater gene expression levels of *INS-1*, *PDX-1*, *IGF-1*, and *GLUT-2* than the positive control group ($p \leq 0.05$). These findings highlight spirulina's potential as a long-term regulator of hyperglycemia in rat models with induced hyperlipidemia, owing to its phenolic bioactive components that serve as antioxidants.

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1. Introduction

Hyperlipidemia is an international health problem that primarily affects people in the Western Hemisphere. It is a group of genetic and acquired illnesses that are defined by elevated lipid levels in the body [1]. High levels of lipoproteins and plasma lipids are indicative of the medical disorder known as hyperlipidemia. Triglycerides (TG), cholesterol esters, phospholipids, and cholesterol make up plasma lipids. The three lipoproteins that make up plasma are decreased high-density lipoprotein (HDL), low-density lipoprotein (LDL), and very-low-density lipoprotein (VLDL) [2].

Cholesterol is an essential part of cellular structure and serves as a precursor for steroid hormone synthesis. It is found in different lipoprotein classes, including HDL, LDL, and VLDL, facilitating its transport at the systemic and cellular levels [3]. The correlation between non-HDL and LDL cholesterol and the incidence of a significant adverse cardiovascular event is well-established [4]. However, it is worth noting that elevated LDL and VLDL levels serve as the primary independent risk factors for cardiovascular events. Concurrently, lower levels of HDL and higher TG are recognized as additional risk factors. Several pharmacological interventions have demonstrated effectiveness in reducing the prevalence of cardiovascular disease. These interventions target the modulation of lipid profiles by reducing total cholesterol, TG, LDL, and VLDL, as well as increasing HDL [5].

Spirulina, a cyanobacterium known for its nutritional density, is commonly used as a dietary supplement and in the creation of functional foods and beverages. Its popularity in health products underscores its superfood status, attributed to its rich protein, vitamin, and antioxidant content. Its use is particularly prominent in Europe, North America, select regions of Asia, and Oceania, where its numerous health benefits have been recognized [6]. This substantial nutritional content provides spirulina with multiple health benefits, including antioxidant, anti-inflammatory, immunomodulatory, and insulin-sensitizing characteristics, as well as favorable impacts on various disorders [7]. Initially, spirulina's potential was harnessed for its rich vitamin and protein content [8]. It is a great source of gamma-linolenic acid (GLA) and has a protein content of 60–70 percent. It also provides essential vitamins like B12 and pro-vitamin A (beta-carotene), along with minerals like iron, showcasing its nutritional value and versatility [9]. Spirulina's remarkable composition, including its rich gamma-linolenic acid and high protein content, continues to captivate researchers [10].

Consuming spirulina as a dietary supplement offers various health benefits, such as preventing and treating hypercholesterolemia, hyperglycemia, inflammatory diseases, allergies, cancer, drug-induced and environmental toxins, viral infections, cardiovascular disease, diabetes, and other metabolic disorders. Spirulina is often called a "superfood" because of its rich and concentrated nutritional content [11].

Spirulina has long been recognized for its ability to enhance immunity and bolster resistance against viral infections. Anecdotal evidence over the years has correlated spirulina consumption with a reduction in symptoms associated with colds and flu [12]. Users of spirulina have reported lowered levels of triacylglycerol and total cholesterol, along with elevated HDL and reduced systolic and diastolic blood pressure [13].

Numerous spirulina species are abundant in antioxidants, including phenolic compounds, beta-carotene, phycocyanin, vitamin E, micronutrients, and gamma-linolenic acid. Importantly, spirulina is recognized for its safety profile, being free of serious adverse effects [10,12]. In our present study, we aimed to assess the effectiveness of Spirulina platensis as a functional dietary intervention for managing diabetes in a high-fat diet-induced hyperlipidemia animal model by evaluating the whole nutritional composition and antioxidant profile of spirulina by using HPLC-UV and as well as the transcriptional factors of gene expressions of *INS-1*, *PDX-1*, *IGF-1*, and *GLUT-2*.

2. Materials and Methods

2.1. Procurement of Herbal Material

Spirulina (Golden Greens Organic, Rendlesham, Woodbridge, UK) powder microalgae were bought from an online market, and the quality of the spirulina was identified by a botanist from the Botany Department at Government College University Faisalabad using voucher no. 381-bot-23.

2.2. Proximate Analysis

The proximate analysis of spirulina powder was determined according to the standard procedures of AOAC [14]. The moisture, ash, and fat of the sample were estimated using the method 930.15, method 08–01, and method 30–10. For moisture content, samples (5 g) were dried in a hot-air oven (Memmert, Äußere Rittersbacher, Schwabach, Germany) at 105 °C for 1 h. Further, samples were placed in an electric muffle furnace (FHX–12, Daihan Scientific, Largo, FL, USA) to calculate the ash content at ~500–550 °C for 6 h. The crude protein was estimated using the Kjeldahl method 64–50. Furthermore, crude fiber was obtained using method no. 978.10.

2.3. Phenolic Acid Profile of Spirulina Powder by Using HPLC

The analysis of individual phenolic contents within the methanolic extract of spirulina was conducted using specialized instrumentation. This setup included a detector (SPD-10AV), a C18 column (25 cm × 4.6 mm, 5.0 µM), an oven, and a SIL-20A autosampler, all sourced from Shimadzu Scientific Instruments in Kyoto, Japan. The analytical procedure followed a specific sequence. Firstly, 0.1 g of the extract was dissolved in 1 mL of methanol, and a 10 µL volume of this solution was injected into the HPLC system. The analytical approach incorporated a linear gradient system, utilizing (solvent A) 1% (v/v) acetic acid in water and (Solvent B) methanol. The gradient elution was initiated at a concentration of 15% for the initial 00–15 min, increased to 45% during the subsequent 15–30 min, and reached full strength at 100% after 30–45 min. A consistent flow rate of 1 mL per minute was maintained, and absorbance measurements were made at a wavelength of 280 nm. To confirm the identity of analytic peaks, their retention times and UV spectra were compared with those of established reference standards, as per the methodology outlined by Soni et al. [15].

2.4. Experimental Trial and Induction of Hyperlipidemia

In the in vivo study, healthy male *Wistar albino* rats (n = 60) aged six weeks weighed (200 ± 250 g) were utilized and subsequently divided into two groups: one receiving a high-fat diet (n = 50) and the other serving as the negative control (NC) (n = 10). The rats in the high-fat diet group were subjected to a regimen that included 35% vegetable fat mixed into their regular chow rodent diet for a period of two months, resulting in the development of hyperlipidemia with a cholesterol level of 250 mg/dL. In contrast, the NC group continued to receive a standard rodent chow diet, known as CMD, as used previously. Following the development of hyperlipidemia, the rats were randomly assigned to five equal groups for treatments. These groups were a positive control (PC) group, which received no treatment; the standard treatment diet group (STD), which received fenofibrate orally at a dosage of 20 mg/kg body weight; and three experimental groups labeled T1 (administered spirulina powder at 300 mg/kg body weight), T2 (administered spirulina powder at 600 mg/kg body weight), and T3 (administered spirulina powder at 900 mg/kg body weight). All treatments were provided orally over a period of 12 weeks. Blood samples were taken from each group at the start of the experiment (day 0) and again at weeks 4, 8, and 12. In addition, liver and pancreatic tissue samples were collected and preserved in 10% formalin for histopathological examination, and pancreatic tissue was preserved in water for RNA extraction. In contrast, serum samples were stored at −20 °C for further analysis. It is essential to note that all experimental procedures were conducted with the approval of the

ethical review board at Government College University Faisalabad, under Reference No. GCUF/ERC/311.

2.5. Oxidative Stress Parameters

2.5.1. Total Oxidant Status (TOS)

The biochemistry analyzer (Biolab-310; Biobase, Jinan, China) was utilized for spectrophotometry to determine the total oxidants in serum (105 µL). In reagent 1, 150 µM of xylenol orange, 140 mM of NaCl, and 1.35 M glycerol were poured into 25 mM of H_2SO_4, and the pH of the solution was 1.75. Reagent was prepared by dissolving 2, 5 mM of Fe3+ and 5 mM of 4-(4-amino-3-methoxyphenyl)-2-methoxyaniline in 25 mM of sulfuric acid. This measurement was calibrated using hydrogen peroxide (105 µL) as a standard of reference and depended on the oxidation of ferrous ions to ferric ions in a solution of various oxidant species. The absorbance was calculated at 650 nm against blank after 4 min, and the results are expressed in terms of micromolar hydrogen peroxide equivalent per liter (µmol H_2O_2 equiv/L) [16].

2.5.2. Total Antioxidant Capacity (TAC)

To assess the TAC in serum samples (35 µL), a spectrophotometric analysis was conducted employing the BIOLAB-310 instrument. Briefly, the solution was prepared by stirring 25 mM sulfuric acid in 140 mM sodium chloride solution. After that, 10% glycerol was mixed in the main solution, and then 250 µM Xlenol orange was dissolved. A novel automated colorimetric method was utilized, with 10 mM o-dianisidine dihydrochloride serving as the substrate. The TAC of the samples was quantified by establishing equivalence to Trolox standards, providing a reliable measure of antioxidant activity. The absorbance of the sample was read at 444 nm against blank after 3 min [16].

2.5.3. Malondialdehyde (MDA)

The concentration of MDA in serum was determined using the method and BIOLAB-310 instrument as described in the research work of Bird and Draper [17], with some modifications. Briefly, 5 mL of sample was poured into test tubes. Then, the tubes were placed in a centrifuge machine to spin at 1500 rpm for 10 to 15 min at 4 °C, and the serum was removed for MDA determination. The amount of MDA in a serum sample was then measured spectrophotometrically at a wavelength of 532 nm, providing an accurate estimate of lipid peroxidation levels [17].

2.5.4. Catalase

Catalase levels were estimated in serum samples by using a spectrophotometer described previously by Hadwan [18]. Briefly, serum sample was poured in a cuvette containing 50 mmol L^{-1} phosphate-buffered saline (pH 7.2) and 30 mmol L^{-1} hydrogen peroxide at room temperature. After being mixed vigorously and incubated for 3 min at 37 °C, ammonium molybdate was added to each tube, and the absorbance was measured at a wavelength of 240 nm for 1 min against the blank using BIOLAB-310. The following Equation (1) of first order reaction (k) was used to measure the concentration of catalase [19]:

$$kU = 2.303/t \times [\log(S^{\circ}/S - M] \times Vt/Vs \quad (1)$$

where

S° = optical density of standard;
S = optical density of test sample;
M = optical density of control sample;
Vt = total volume of all components;
Vs = total volume of serum sample.

2.6. Lipid Profile and Insulin

In this research work, the Crescent Diagnostic (Jeddah, Saudi Arabia) reagent kit process was utilized to calculate total cholesterol, triglycerides (TG), and high-density lipoprotein–cholesterol (HDL) spectrophotometrically (BIOLAB-310) [20,21]. The measurement of LDL was conducted using a specific formula as provided below in Equation (2) [22].

$$\text{LDL} - \text{Cholesterol (mg/dL)} = \text{Total cholesterol} - (\text{TG}/5) - (\text{HDL} - \text{Cholesterol}) \quad (2)$$

Serum insulin level was calculated using an ELISA (Calbiotech; Catalog No. IN374S; El Cajon, CA, USA) [23].

2.7. Serum Liver Enzymes

2.7.1. Alanine Aminotransferase (ALT)

The serum ALT levels were measured on a semi auto analyzer BIOLAB® 320 at 340 nm by using Bio-active's ALT (GPT) SR kit (catalogue # 10498-99-93-183). We mixed 100 µL of sample, standard, or distilled water with 500 µL of R1 buffer and incubated the mixture at 37 °C for 30 min. A tube of distilled water was used as a reagent blank, which was tested alongside the standard and samples. Next, a pipette of 500 µL of reagent R2 was inserted into each tube and was kept at 25 °C for 20 min. After the second incubation, the reaction was stopped by adding diluted R3 (1:10 R 3 by distilled water). The absorbance of samples and standards was measured [24].

2.7.2. Aspartate Aminotransferase (AST)

Serum AST levels were determined on a semi auto analyzer BIOLAB® 320 at 340 nm by using a commercially available liquiform technique kit (Crescent® Diagnostic kit, Jeddah, Saudi Arabia, catalogue #15204C). A 100 µL sample was gently combined with 500 µL buffer R1 and vortexed. After 30 min of incubation at 37 °C, a reagent blank (100 µL distilled water) was utilized. Then, 500 µL of reagent R2 was pipetted into an Eppendorf tube and incubated for 20 min at 25 °C. The reaction was stopped by adding 5.0 mL of R3 (sodium hydroxide), which was then incubated for an additional 5 min. The sample absorbance was then measured at a wavelength of 546 nm against the reagent blank [25].

2.8. Histopathology

Liver and pancreatic tissue were fixed in 10% paraformaldehyde for 24 h and dried with ethanol at varied concentrations. The tissues were sectioned into 2–4 micrometer thick slices using a microtome and encased in paraffin wax. The slices were subsequently deparaffinized, stained with eosin and hematoxylin, and examined under a light microscope [26].

2.9. Gene Expression

The mRNA expression levels in pancreatic tissue were determined using the RT-qPCR technique. TRIzol reagent (Invitrogen, Waltham, MA, USA) was used to extract total RNA, and the Nanodrop 2000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA) was used to assess the concentration and purity of the RNA that was extracted. The RevertAid cDNA synthesis kit (Thermo Fisher Scientific, Waltham, MA, USA) was used to reverse transcribe total extracted mRNA to cDNA in accordance with the manufacturer's instructions. Maxima SYBR Green/ROX qRT-PCR Master Mix (Thermo Fisher Scientific, Waltham, MA, USA) was analyzed using BIO-RAD iQ5 Multicolor Real-Time PCR Optical Module equipment. INS1, PDX1, IGF1, and Glut-2 expression profiles were measured in the pancreas. The housekeeping/reference genes GAPDH were used for standardization [16,27]. Specific primer sequences were used to amplify the genes (INS1, PDX1, IGF1, and Glut-2 genes) as described in Table 1.

Table 1. Primer sequences for gene expression analysis.

Genes	Sequence	
INS-1	Forward	*AGGCTCTGTACCTGGTGTGTG*
	Reverse	*CGGGTCCTCCACTTCACGAC*
PDX-1	Forward	*TCCCGAATGGAACCGAGACT*
	Reverse	*TTCATCCACGGGAAAGGGAG*
IGF-1	Forward	*ACCCGGGACGTACCAAAATG*
	Reverse	*CCGAGCTGGTAAAGGTGAGC*
GLUT-2	Forward	*GGCATGTTTTTCTGTGCCGT*
	Reverse	*AAGAACACGTAAGGCCCGAG*

2.10. Statistical Analysis

The data collected from various parameters are presented as mean ± SEM. To analyze these data, a two-way analysis of variance was conducted using Prism Pad 9.2.0 software. Subsequently, post hoc significant Duncan multiple range tests were applied through the use of Costat 2.0, especially when groups showed significant differences from each other, with the level of significance set at $p \leq 0.05$.

3. Results

3.1. Proximate Analysis of Spirulina Powder

The examined spirulina powder included large amounts of important nutrients. Carbohydrates (38.17 ± 1.7%), proteins (16.45 ± 0.8%), and crude fiber (16.46 ± 0.8%) of spirulina powder were the primary components. Additionally, the crude fat (5.67 ± 0.2%) and ash content (15.69 ± 0.7%) were measured. Notably, the energy yield for 100 g of spirulina powder was 269.51 K calories. These findings provide important information on the nutritional quality of the analyzed material, as shown in Table 2.

Table 2. Percentages of proximate components found in spirulina powder.

Parameters	Result (%)
Moisture	7.56 ± 0.3
Ash	15.69 ± 0.7
Crude fat	5.67 ± 0.2
Protein (N*6.25)	16.45 ± 0.8
Carbohydrates	38.17 ± 1.7
Crude fiber	16.46 ± 0.8
K-Calories/100 g	269.51

3.2. Phenolic and Flavonoid Contents Through HPLC

Several phenolic compounds were found in a chromatographic study of spirulina powder (Figure 1, Table 3). Chlorogenic acid had the greatest concentration at 4052.90 µg/g, indicating its abundance in the sample. P-coumaric acid was found in the lowest concentration at 55.03 µg/g. The study found two flavonoid molecules, with quercetin having the greatest quantity at 107.15 µg/g. Rutin had the lowest concentration, measured at 12.39 µg/g. These findings add to the understanding of the bioactive elements of spirulina powder, which may influence its health-related aspects, as shown in Figure 1.

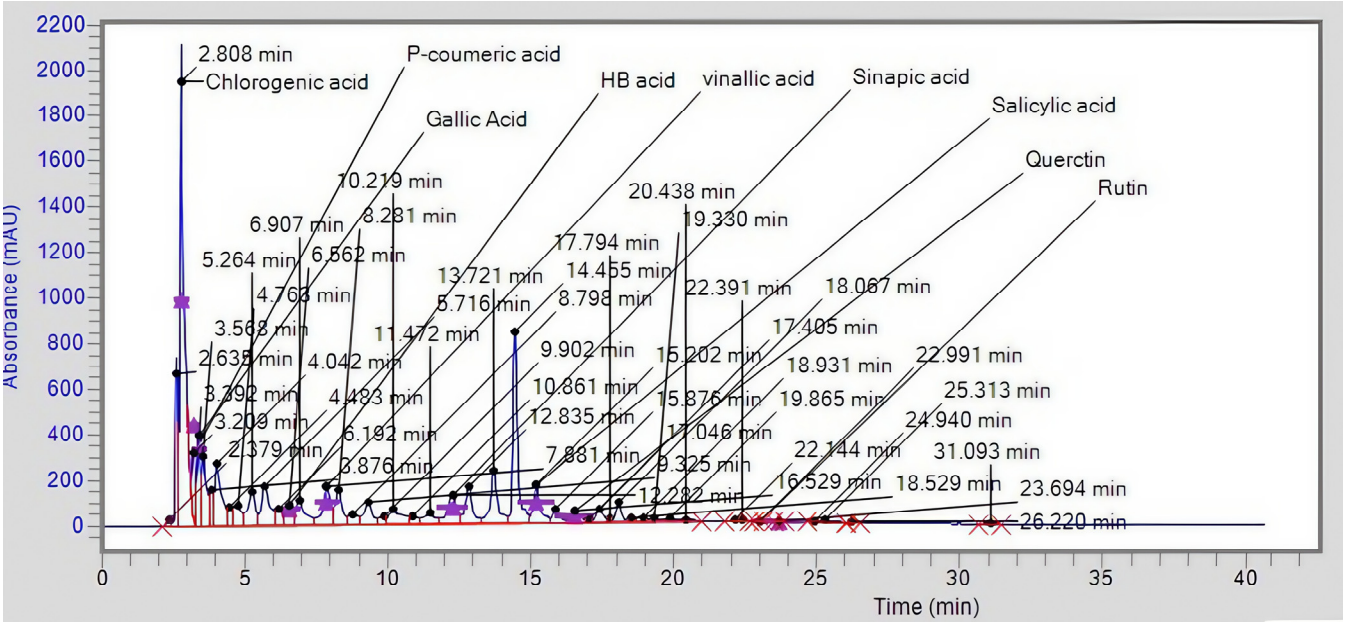

Figure 1. Quantitative analysis of various phenolic and flavonoid compounds in spirulina powder by HPLC-UV chromatogram.

Table 3. Quantitative analysis of various phenolic and flavonoid compounds in spirulina powder by HPLC-UV chromatogram.

No.	Compound	Peak Area (mAU/min)	K Factor	Conc. (µg/g)
1	Chlorogenic acid	21,331,085.5	0.00019	4052.906
2	P-Coumaric acid	1,941,105.9	0.0003	582.3318
3	Gallic Acid	3,514,736.1	0.000079	277.6642
4	Hydroxybenzoic acid-acid	1,507,741.8	-	-
5	Vinallic acid	3,237,237.3	0.00044	1424.384
6	Sinapic acid	2,798,676.8	0.00005	139.9338
7	Salicylic acid	2,730,988.6	0.000377	1029.583
8	Querctin	1,127,940.7	0.000095	107.1544
9	Rutin	31,310.1	-	-

3.3. Body Weight

During the baseline period (zero weeks), there was no significant change in all groups, except the negative control (NC) given free access to a standard diet. The body weight of NC rats was increased with increasing age. Body weight declined significantly ($p \leq 0.05$) in the treatment groups compared to the PC (positive control) in the fourth and eighth weeks, while remaining constant in the standard treatment diet (STD) group. In the 12 weeks, body weight eventually considerably decreased ($p \leq 0.05$) in treatment groups T1 (309 ± 6.95 g), T2 (291 ± 4.92 g), and T3 (288 ± 1.51) compared to STD (284 ± 1.49), but declined significantly ($p \leq 0.05$) when compared to PC (348 ± 7.91), as shown in Table 4.

Table 4. Body weight (g ± SEM) of rats in NC, PC, STD, and different spirulina powder treated groups within the 12 weeks of the experimental protocol.

Group	0 Day	4 Weeks	8 Weeks	12 Weeks	BWG (%)
NC	154 ± 2.79 ^{d}	172 ± 2.54 ^{c}	196 ± 3.95 ^{b}	204 ± 1.89 ^{a}	33.0
PC	236 ± 3.21 ^{d}	278 ± 5.55 ^{c}	312 ± 7.91 ^{b}	348 ± 8.31 ^{a}	47.6
STD	235 ± 3.19 ^{d}	254 ± 4.14 ^{c}	273 ± 5.53 ^{b}	284 ± 6.49 ^{a}	20.7
T1	236 ± 3.21 ^{d}	254 ± 4.14 ^{c}	266 ± 4.52 ^{b}	309 ± 6.95 ^{a}	30.9
T2	234 ± 3.18 ^{d}	254 ± 4.14 ^{c}	266 ± 4.53 ^{b}	291 ± 5.92 ^{a}	24.1
T3	235 ± 3.19 ^{d}	256 ± 4.19 ^{c}	261 ± 4.48 ^{b}	288 ± 6.51 ^{a}	22.5

NC (normal control), PC (positive control), STD group (Fenoget oral dose of 20 mg/kg), and different spirulina-powder-treated groups: T1 (300 mg/kg body weight), T2 (600 mg/kg body weight) and T3 (900 mg/kg body weight), for a 12-week period in high-fat-diet-induced hyperlipidemic rats considering $p \leq 0.05$). $^{a–d}$ Different superscripts on bars show a significant difference between different groups (Duncan's test).

3.4. Oxidative Stress Parameters

All test individuals who received spirulina powder showed a significant reduction in their overall levels of total oxidative stress (TOS) following the completion of the 12-week treatment period. This reduction was statistically significant ($p \leq 0.05$) when compared to the positive control (PC) group. Though the change did not reach statistical significance ($p > 0.05$) when compared to the NC group, the TOS levels were comparable between the spirulina-treated subjects and the NC group at the end of treatment after 12 weeks. Overall, TOS was decreased significantly ($p \leq 0.05$) in all spirulina-powder-treated groups as compared to the PC (positive control) but non-significant ($p \geq 0.05$) to the NC (Figure 2a). Overall total antioxidant capacity (TAC) status was increased significantly at the end of 12 weeks ($p \leq 0.05$) in all spirulina-powder-treated groups as compared to the PC but decreased compared with the NC, except T3, which was non-significant ($p \geq 0.05$) compared to NC (Figure 2b). Malondialdehyde (MDA) levels were significantly ($p \leq 0.05$) decreased in spirulina-treated groups but were found to be increased as compared to the NC group (Figure 2c). Catalase levels were significantly ($p \leq 0.05$) increased as compared to the PC group but were found to be decreased ($p \leq 0.05$) as compared to the NC at the end of treatment at 12 weeks (Figure 2d). These findings demonstrate spirulina's potential as a modulator of oxidative stress and antioxidant defense mechanisms.

3.5. Lipid Profile

In the 12-week clinical research, spirulina powder administration was linked to a noteworthy reduction in serum cholesterol levels in all treatment cohorts (T1, T2, and T3) when compared to the comparator groups, except the NC. Significantly, compared to the positive control (PC) group, the spirulina regimen resulted in a drop in cholesterol concentrations; the T3 group showed the greatest significant reduction, measured at (68.8\pm 1.4) mg/dL. These results highlight spirulina's potential as a powerful regulator of lipid metabolism, as seen in Figure 3a.

Since spirulina has a high content of antioxidants, it is possible to explain the observed drop in total triglyceride levels between the groups receiving therapy (T1, T2, and T3), except for the NC. These antioxidants are essential for reducing inflammation in the body. It is well recognized that chronic inflammation interferes with regular metabolic functions, such as lipid metabolism. Spirulina may improve the body's capacity to metabolize lipids effectively, preferring the creation of energy over the storage of fat, by lowering inflammation. The observed considerable drop in triglyceride levels ($p \leq 0.05$) in comparison to other groups is explained by this shift in metabolic preference, whereby the body uses the available lipids for energy instead of storing them as fat.

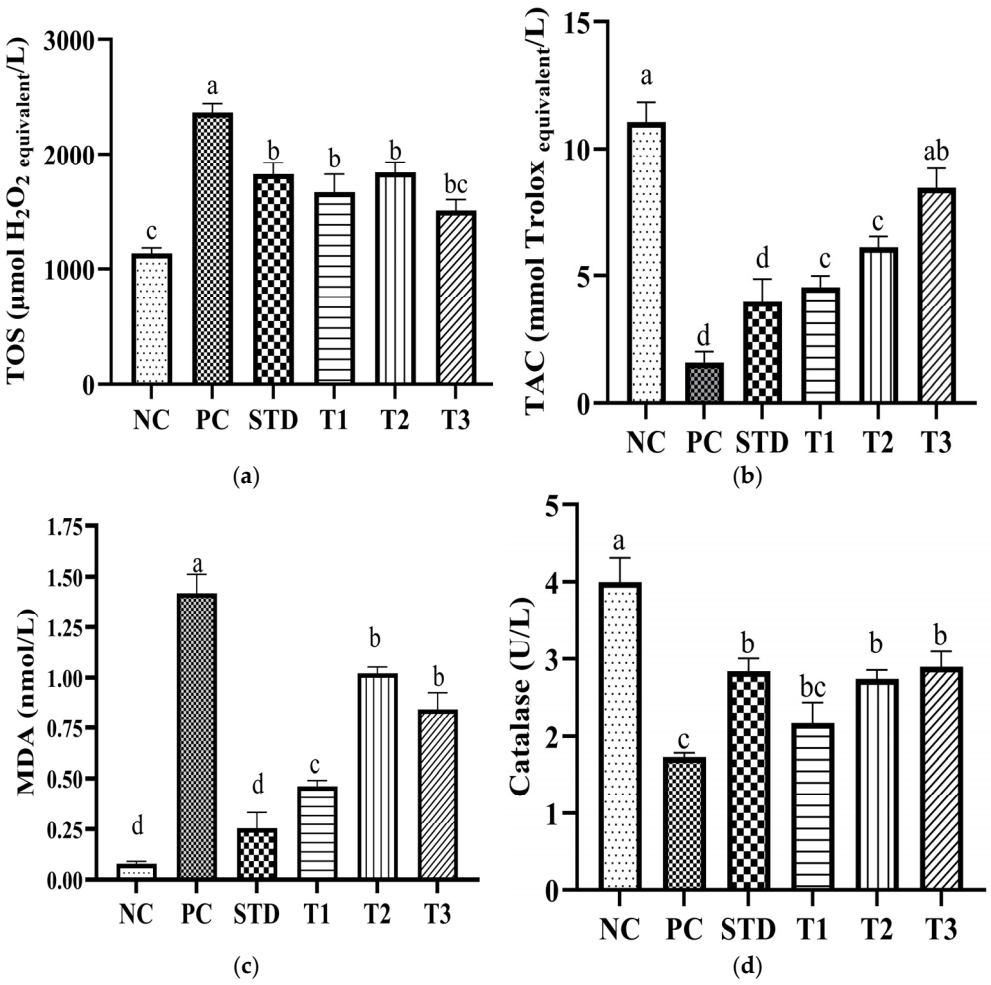

Figure 2. Oxidative stress biomarkers. (**a**) Total oxidative stress (TOS), (**b**) total antioxidant capacity (TAC), (**c**) malondialdehyde, and (**d**) catalase levels in the NC (normal control), PC (positive control), STD group (Fenoget oral dose of 20 mg/kg), and different spirulina-powder-treated groups: T1 (300 mg/kg body weight), T2 (600 mg/kg body weight), and T3 (900 mg/kg body weight), for a 12 week period in high-fat-diet-induced dyslipidemic rats. This means values with different superscripts are significantly different from each other ($p \leq 0.05$). $^{a–d}$ Different superscripts on bars show a significant difference between different groups (Duncan's test). Various bars represent concentration gradient variations; higher concentrations correlate with higher percent inhibition.

The low-density lipoprotein values in all three therapy subgroups (T1, T2, and T3) were significantly lower ($p \leq 0.05$) than those in the other groups (NC excluded). Low-density lipoprotein levels were considerably lower ($p \leq 0.05$) in the STD (29.7 ± 0.9 mg/dL) and treatment groups T1 (29.5 ± 0.9 mg/dL), T2 (29 ± 0.9 mg/dL), and T3 (25.7 ± 0.7 mg/dL) compared to the PC (40.3 ± 0.86 mg/dL). Except for the NC, all groups showed no significant change at zero weeks ($p \leq 0.05$). Low-density lipoprotein levels in the PC rose dramatically between the fourth and eighth weeks when compared to other groups. Low-density lipoprotein levels decreased significantly ($p \leq 0.05$) in the STD (27.9 ± 1.43 mg/dL) and treatment groups T1 (26.8 ± 1.27 mg/dL), T2 (25.7 ± 1.12 mg/dL), and T3 (20 ± 0.82 mg/dL) compared to the PC (43.7 ± 0.93 mg/dL), as shown in Figure 3c.

In our 12-week trial, spirulina powder treatment resulted in a substantial increase in LDL levels in groups T1, T2, and T3 compared to the other groups, with the exception of the NC. Notably, the spirulina-treated groups had increased LDL levels compared to the positive control (PC) group. LDL levels remained higher in spirulina-treated groups after 12 weeks, with group T3 showing the highest significant effect (46.8 ± 2.46 mg/dL), as shown in Figure 3d. These findings emphasize spirulina's potential impact on lipid metabolism.

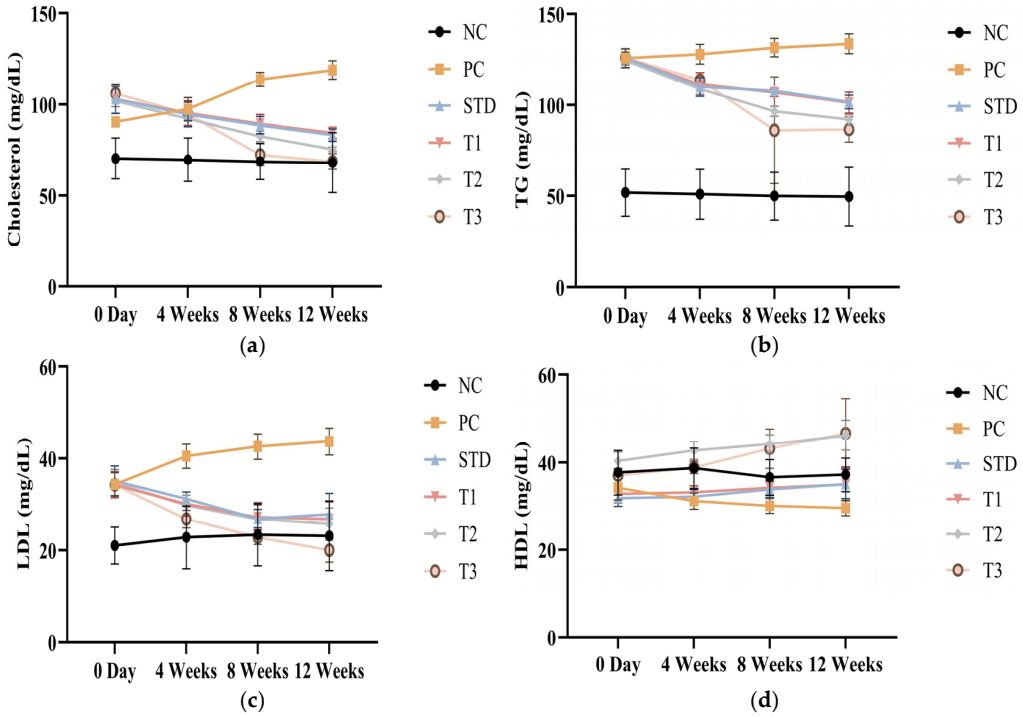

Figure 3. Lipid profile showing (**a**) cholesterol (mg/dL), (**b**) triglycerides (TG) (mg/dL), (**c**) low-density lipoprotein (LDL) (mg/dL), and (**d**) high-density lipoprotein (HDL) (mg/dL) levels in the NC (normal control), PC (positive control), STD group (Fenoget oral dose of 20 mg/kg body weight), and different spirulina-powder-treated groups: T1 (300 mg/kg body weight), T2 (600 mg/kg body weight), and T3 (900 mg/kg body weight), for a 12 week period in high-fat-diet-induced hyperlipidemic rats considering $p \leq 0.05$. Positive partial correlation—orange color, negative partial correlation—black color.

3.6. Serum Insulin

Serum insulin was decreased significantly ($p \leq 0.05$) in T1 (19.24 ± 0.62 µ lU/mL), T2 (17.45 ± 0.5 µ lU/mL), and T3 (20 ± 0.61 µ lU/mL), as compared to the PC (22.77 ± 1.02 µ lU/mL), but increased as compared to the NC (15.45 ± 0.40 µ lU/mL) at the end of 12 weeks and treatment, as shown in Figure 4.

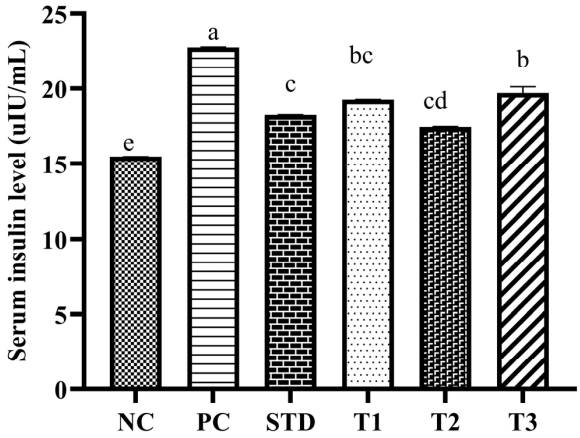

Figure 4. Serum insulin in the NC (normal control), PC (positive control), STD group (Fenoget oral dose of 20 mg/kg body weight), and different spirulina-powder-treated groups: T1 (300 mg/kg body weight), T2 (600 mg/kg body weight), and T3 (900 mg/kg body weight), for a three-month period in high-fat-diet-induced hyperlipidemic rats. $^{a–e}$ Different superscripts on bars show a significant difference between different groups (Duncan's test).

3.7. Serum ALT and AST

ALT was significantly reduced ($p \leq 0.05$) in the STD (68.8 ± 1.09 U/L) and treatment groups T1 (74.4 ± 1.3 U/L), T2 (71 ± 1.41 U/L), and T3 (62 ± 0.95 U/L) as compared to the PC (86 ± 1.8 U/L). In the fourth and eighth weeks, ALT level significantly rose in the PC as compared to other groups. At 12 weeks, the ALT level was a noteworthy ($p \leq 0.05$) decrease in the STD (60.8 ± 0.39 SGPT) and treatment groups T1 (64.1 ± 1.62 U/L), T2 (57 ± 1.45 U/L), and T3 (42.6 ± 0.65 U/L) as compared to the PC (90.7 ± 2.48 U/L) group at 0, 4, 8, and 12 weeks, as shown in Table 5.

AST was significantly reduced ($p \leq 0.05$) in the STD (162 ± 5.6 U/L) and treatment groups T1 (144 ± 1 U/L), T2 (494 ± 1.6 U/L), and T3 (133.5 ± 1.3 U/L) as compared to the PC (176 ± 3.8 U/L). The AST levels were non-significant ($p \leq 0.05$) in treatment groups (T1, T2, and T3) as compared to other groups except the NC. At 0 weeks, there was a non-significant ($p \leq 0.05$) change in all groups except the NC. In the fourth and eighth weeks, AST level significantly rose in the PC as compared to other groups. At 12 weeks, the AST level was significantly ($p \leq 0.05$) decreased in the STD (148 ± 2.24 U/L) and treatment groups T1 (110 ± 1.02 U/L), T2 (107 ± 0.77 U/L), and T3 (96.3 ± 0.79 U/L) as compared to the PC (177 ± 1.45 U/L) group at 0, 4, 8, and 12 weeks, as shown in Table 5.

3.8. Histopathology Evaluation of the Liver and Pancreas

3.8.1. Pancreatic Tissues

Photomicrographs (400X) displaying the histology of pancreatic tissue from rats stained with H&E are shown in Figure 5, where blue stars show the beta cells, the yellow arrow shows the exocrine portion, and the straight line shows the length of islets of Langerhans. In the NC group, the pancreas of rats showed normal pancreatic cells and fully functional islets of Langerhans. However, the pancreas of the positive control (PC) group depicted a smaller number of beta cells as well as the shrinkage of islets of Langerhans. Rats in the STD, standard treatment group (200 mg/kg Fenoget), had pancreas tissue with a lesser number of islets associated with deformities compared to a normal structure. Treatment groups (T1-T3) and T1 (300 mg/kg body weight) demonstrated loss of cellular contents, small-sized islets of Langerhans, and apoptosis of β-cells. The histological architecture was restored in T2 and T3 (600 mg/kg and 900 mg/kg NC (normal control), the PC (positive control), the STD group (Fenoget oral dose of 20 mg/kg body weight), and different spirulina-powder-treated groups: T1 (300 mg/kg body weight), T2 (600 mg/kg body weight), and T3 (900 mg/kg body weight), for a three-month period in high-fat-diet-induced hyperlipidemic rats considering $p \leq 0.05$, displaying normal pancreatic parenchyma with fully functional β-cells in the islets of Langerhans, as illustrated in Figure 5.

Table 5. Serum ALT and AST levels in the NC, PC, STD, and different spirulina-powder-treated groups within the 12 weeks of the experimental protocol.

Groups	Alanine Transaminase (U/L)					Aspartate Aminotransferase (U/L)				
	0 Day	**4 Weeks**	**8 Weeks**	**12 Weeks**	**Mean ± SEM**	**0 Day**	**4 Weeks**	**8 Weeks**	**12 Weeks**	**Mean ± SEM**
NC	35.1 ± 0.5	35.2 ± 1.2	37.9 ± 0.3	35.9 ± 0.7	36.02 ± 0.7	95.1 ± 0.8	93.9 ± 0.7	94.5 ± 0.87	94.2 ± 0.75	94.42 ± 0.79
PC	84.1 ± 1.0	87.5 ± 1.6	91.7 ± 1.6	92.2 ± 1.5	88.87 ± 1.4	178.7 ± 0.5	177.9 ± 0.4	178.2 ± 0.4	180.8 ± 0.79	178.9 ± 0.54
STD	87.2 ± 1.5	82.9 ± 1.6	73.9 ± 1.9	66 ± 1.88	77.5 ± 1.7	178.7 ± 0.5	166.4 ± 0.9	153.2 ± 1.5	141 ± 1.11	159.82 ± 1.0
T1	85 ± 1.87	78.5 ± 1.2	72.9 ± 1.4	68.9 ± 1.3	76.32 ± 1.4	177.9 ± 0.4	159.6 ± 1.5	129.8 ± 1.1	111.7 ± 1.80	144 ± 1.22
T2	86.4 ± 0.8	76.7 ± 0.8	65.3 ± 0.9	59.7 ± 0.8	72.02 ± 0.8	178.2 ± 1.4	159.3 ± 1.3	134.7 ± 1.7	106.2 ± 0.70	144.6 ± 1.31
T3	85.8 ± 1.2	78.5 ± 1.2	67 ± 1.40	37.9 ± 1.7	67.3 ± 1.4	180.8 ± 0.7	149.7 ± 2.9	118.9 ± 1.8	94.1 ± 0.74	94.42 ± 0.8

NC (normal control), PC (positive control), STD group (Fenoget oral dose of 20 mg/kg body weight), and different spirulina-powder-treated groups: T1 (300 mg/kg body weight), T2 (600 mg/kg body weight), and T3 (900 mg/kg body weight), for a three-month period in high-fat-diet-induced hyperlipidemic rats considering $p \leq 0.05$.

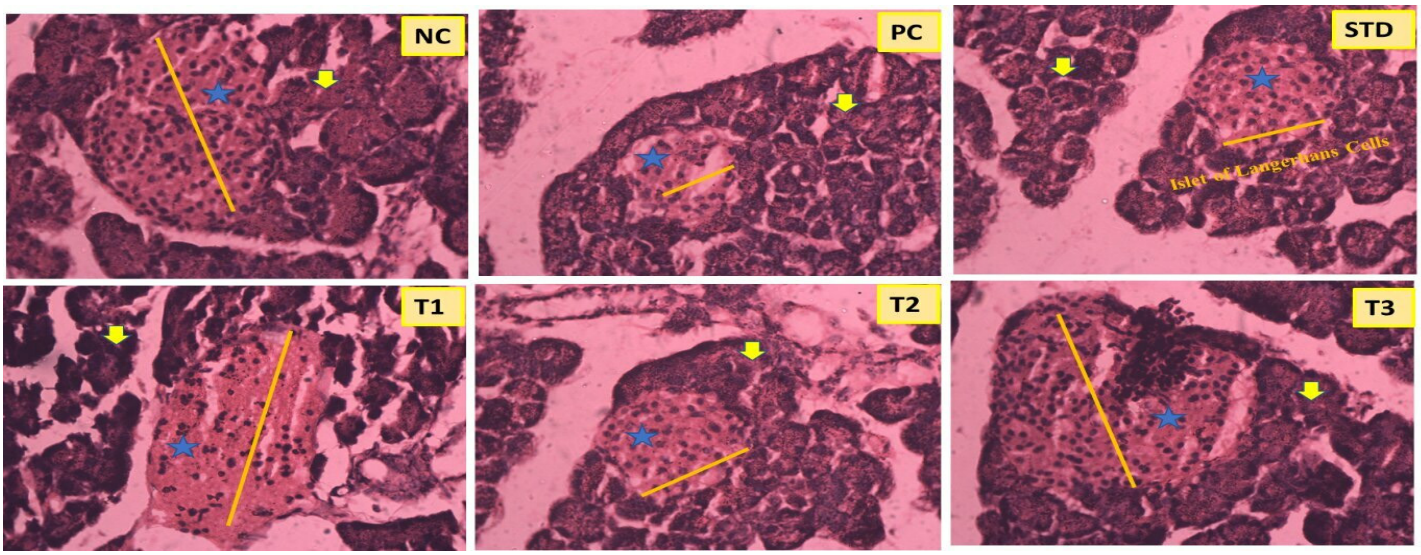

Figure 5. Histo-micrograph of pancreatic tissues collected from the NC, PC, STD, and different spirulina-powder-treated groups: T1 (300 mg/kg body weight), T2 (600 mg/kg body weight), and T3 (900 mg/kg body weight), for three months in high-fat-diet-induced dyslipidemic rats. Blue stars show the beta cells, the yellow arrow shows the exocrine portion, and the straight line shows the length of islets of Langerhans.

3.8.2. Liver Tissue

The hepatocyte architecture and portal vein size were found to be normal in the NC group. Additionally, there was no fat accumulation, and the structures in the histogram were arranged in a well-organized manner in the NC group. However, histological analysis of the PC showed cellular infiltration, hepatocyte death, portal vein deformation, and fat buildup in the liver, in addition to the loss of cellular contents, which are visible due to hyperlipidemia, shown in Figure 6.

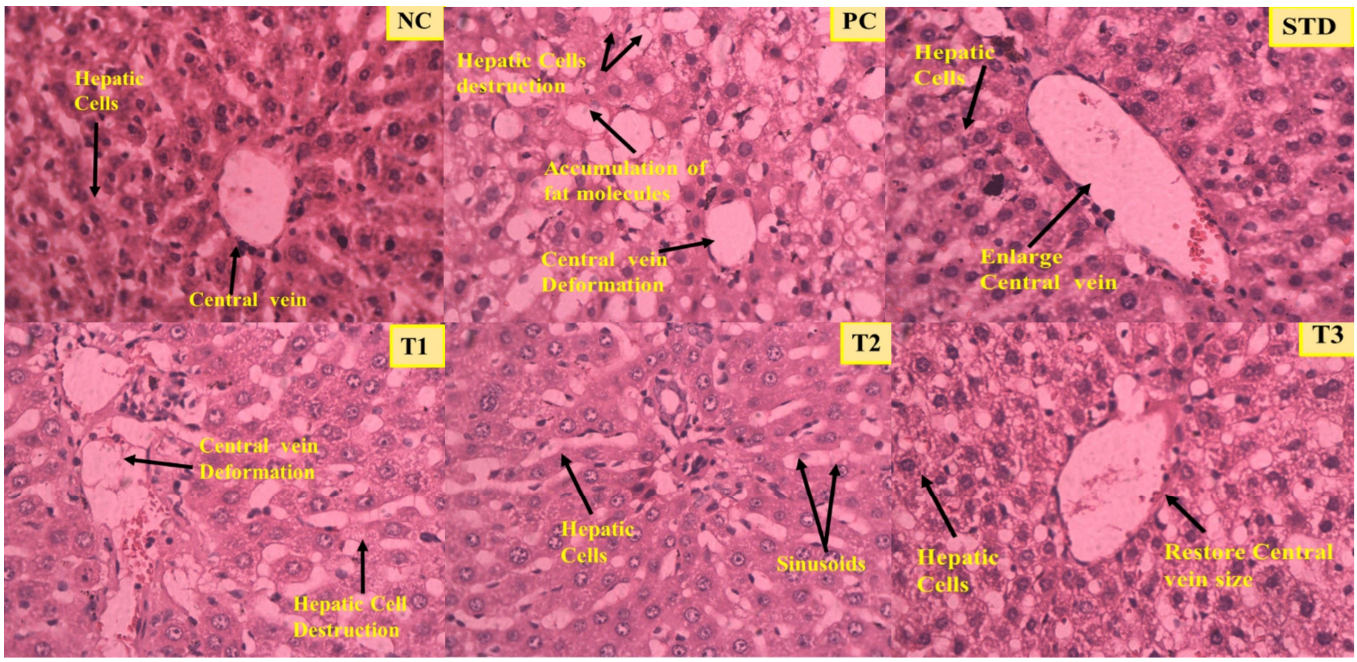

Figure 6. Histo-micrograph of liver tissues collected from the NC, PC, STD, and different spirulina-powder-treated groups: T1 (300 mg/kg body weight), T2 (600 mg/kg body weight), and T3 (900 mg/kg body weight), for three months in high-fat-diet-induced dyslipidemic rats.

Additionally, there was cellular infiltration and larger portal vein diameters in the livers of the STD group. Within the treatment group, T1 exhibited cellular infiltration or destruction along with structural abnormalities as compared to T2 and T3 (600 mg/kg body weight and 900 mg/kg body weight) spirulina-powder-treated groups. In the T2 and T3 (600 mg/kg body weight and 900 mg/kg body weight) groups, there was a regrowth of the normal histological structure exhibiting normal hepatic parenchyma, as shown in Figure 6.

3.9. Gene Expression

INS-1 expression was significantly ($p \leq 0.05$) upregulated in the STD (1.93 ± 0.02) and treatment groups T1 (1.94 ± 0.01), T2 (1.98 ± 0.01), and T3 (1.92 ± 0.01) as compared to the PC (0.66 ± 0.01) at the end of 12 weeks of treatment (Figure 7a). *IGF-1* expression was significantly ($p \leq 0.05$) downregulated in the STD (0.79 ± 0.14) and treatment groups T1 (0.80 ± 0.15), T2 (0.82 ± 0.15), and T3 (0.79 ± 0.15) as compared to the PC (0.27 ± 0.04) at the end of 12 weeks of treatment (Figure 7b). *PDX-1* expression was significantly ($p \leq 0.05$) upregulated in the STD (0.40 ± 0.20) and treatment groups T1 (0.40 ± 0.20), T2 (0.41 ± 0.20), and T3 (0.40 ± 0.19) as compared to the PC (0.27 ± 0.04) at the end of 12 weeks of treatment (Figure 7c). *GLUT-2* expression was significantly ($p \leq 0.05$) downregulated in the STD (0.66 ± 0.63) and treatment groups T1 (0.66 ± 0.63), T2 (0.67 ± 0.65), and T3 (0.65 ± 0.63) as compared to the PC (0.23 ± 0.21) at the end of 12 weeks of treatment (Figure 7d).

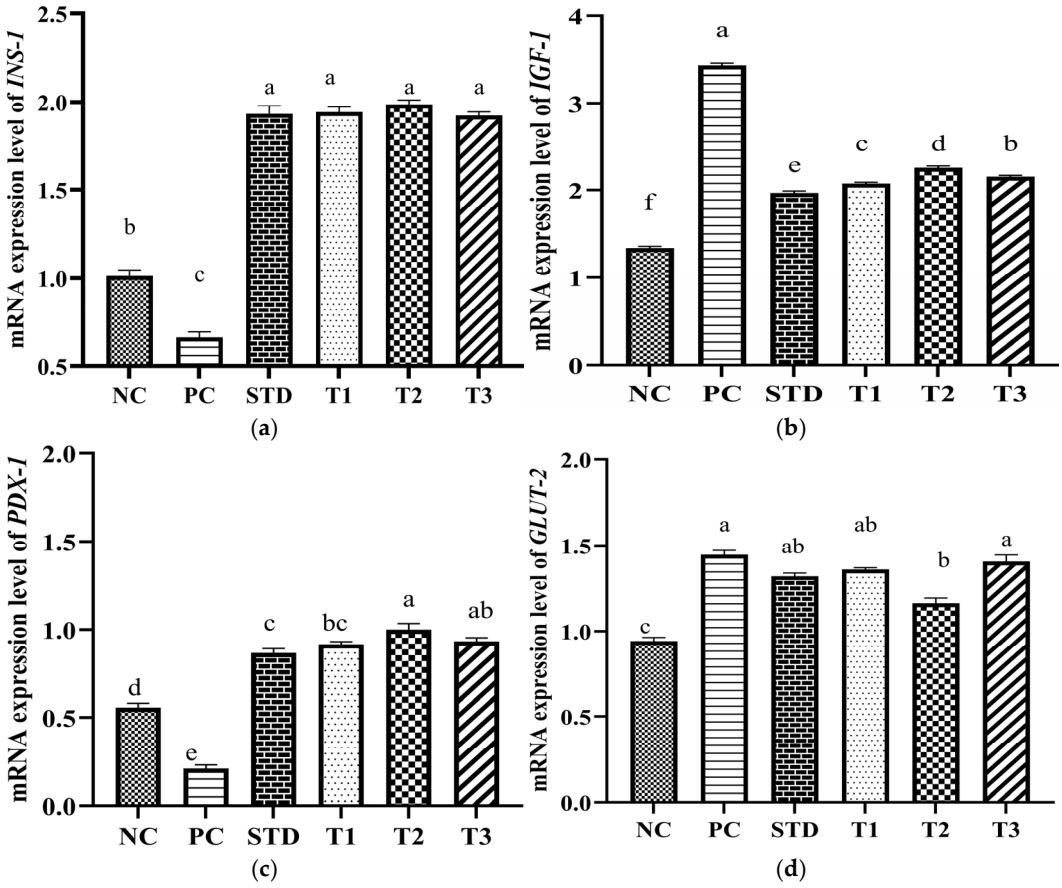

Figure 7. Gene expression of *INS-1* (**a**), *IGF-1* (**b**), *PDX-1* (**c**), and *GLUT-2* (**d**) (mRNA expression levels ± SEM) in the NC (normal control), PC (positive control), STD group (Fenoget oral dose of 20 mg/kg body weight), and different spirulina-powder-treated groups: T1 (300 mg/kg body weight), T2 (600 mg/kg body weight), and T3 (900 mg/kg body weight), for the three-month period in high-fat-diet-induced dyslipidemic rats. $^{a–f}$ Different superscripts on bars show a significant difference between different groups (Duncan's test). Various bars represent concentration gradient variations; higher concentrations correlate with higher percent inhibition.

Statistical analysis showed the major contribution of Dim 1 (57.3%) and Dim 2 (25.7%). Catalase (CAT), TAC, and HDL had positive correlation with each other, while negatively co-relating with body weight, TG, TOS, MDA, insulin, LDL, and TL (Figure 8).

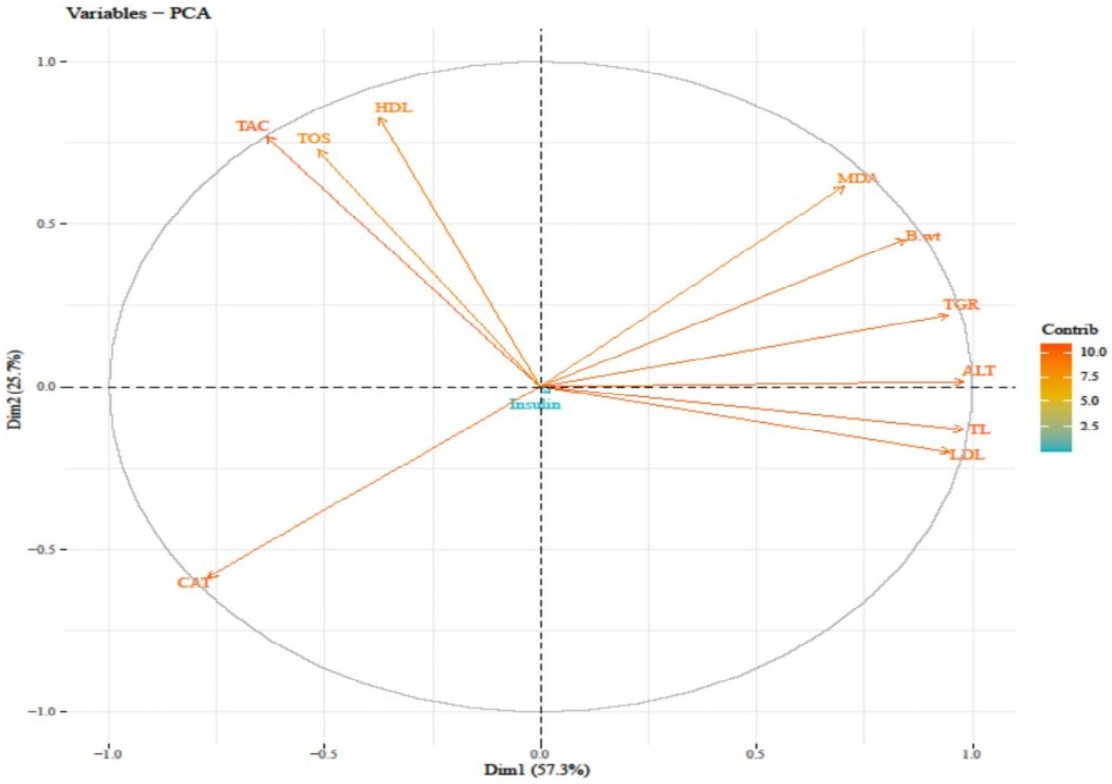

Figure 8. Principal component analysis showing the relation among various attributes studied over 12-week periods in high-fat-diet-induced dyslipidemic rats treated with spirulina powder.

4. Discussion

Hyperlipidemia increases the risk of the development of diabetes [28]. In hyperlipidemia conditions, cells contain as much cholesterol as a pool that leads to lipid peroxidation of the cell membrane. Such altered cell membranes leak peroxide and free radicals from the cell. Such an imbalance between antioxidants and free radicals develops into oxidative stress [29]. In the current study, hyperlipidemia rats developed glucolipid alteration, which promotes oxidative stress. Fatty acid and triglyceride production is driven by excess glucose in the liver, and the rate at which the liver secretes substantial amounts of triglyceride particles causes insulin sensitivity [30]. Significantly increased levels of *INS-1*, *IGF-1*, *PDX-1*, and *GLUT-2* transcriptional factors in PC indicate glucose dysregulation, which ultimately disrupts the homeostasis of the lipid metabolism by our recent findings of increased levels of TC, TGR, and LDL, which led to lipid peroxidation of pancreatic β-cells, resulting in cellular damage. β-Cells are reduced in number, thereby reducing the production of insulin and the onset of diabetes type 2. However, the level of serum insulin in the PC group is increased due to increased resistance to insulin sensitivity due to lipid peroxidation of insulin receptors present throughout the body. Such a condition leads to persistent hyperglycemia. Oxidative stress plays a crucial role in the new onset of diabetes [30] and the development of cardiovascular complications [31]. Production of higher levels of reactive oxygen species (ROS) worsens the pancreatic β-cells' activity and results in decreased insulin secretion [32]. However, serum glucose and insulin levels decreased in spirulina-treated groups at different dose levels. We speculate that total oxidative stress was reduced due to increased total antioxidant capacity (TAC) in spirulina-treated groups in a dose-dependent manner, maximally with a dose of 900 mg/kg body weight (Figure 3b). Phenolic compounds like

chlorogenic acid, p-coumaric acid, synaptic acid, gallic acid, salicylic acid, HB acid, and vanillic acid present in spirulina powder as secondary metabolites of the plant and possess the natural tendency to neutralize the free radicals produced during lipid peroxidation [33]. The antidiabetic effects of spirulina powder were augmented due to the presence of the flavonoids quercetin, having antioxidant and anti-inflammatory properties, and rutin, by decreasing absorption of carbohydrates from the small bowel, inhibiting gluconeogenesis and stimulating insulin section from pancreatic β-cells [7].

The level of MDA was also increased in the PC group but decreased in spirulina-treated groups at different levels. MDA is a marker of lipid peroxidation and is increased in diabetic groups [34]. It is speculated that the antioxidants present as phenolic compounds such as p-numeric acid, gallic acid, and chlorogenic acid may neutralize the MDA, thereby reducing lipid peroxidation. The level of catalase as an antioxidant was increased in spirulina-treated groups as compared to hyperlipidemia rats. Catalase functions as an antioxidant, which scavenges the free radical as well as breaking down free radical hydrogen peroxide into water and molecular oxygen. Previously, serum catalase levels were found to be lower in diabetic individuals as compared to healthy persons [35]. In spirulina-treated groups at different dose levels, TC, TGR, and LDL were reduced. Phycocyanin, a water-soluble protein in spirulina powder, potentially reduces blood cholesterol and atherogenic index while increasing the level of HDL [36]. The activity of these medicines in decreasing serum cholesterol may be strongly related to the inhibition of cholesterol absorption in the jejunum caused by micellar solubility of cholesterol [37]. Among lipoproteins as a transporter of lipids between various cells and tissues are apolipoproteins. Apolipoproteins, as lipid carriers, increase binding affinity with lipoprotein receptor sensitivity, thereby increasing cellular consumption of lipids to help in the regulation of lipid homeostasis [38].

Serum alanine transaminase (ALT) and aspartate aminotransferase (AST) were increased in the hyperlipidemia group but decreased in spirulina in different dose-treated groups at the same level. The elevated plasma activity of the AST, ALT, and ALP enzymes in rats fed a diet high in fat shows that oxidative damage to tissues also results in hepatocyte injury. These enzymes are regarded as indicators of liver dysfunction. These enzymes are typically transferred to the plasma as a result of hepatocyte injury. Rats fed a high-fat diet had higher plasma levels of triglycerides and cholesterol, which led to the development of lipotoxicity and lipid buildup in the liver [39].

In the current study, body weight gain was reduced in hyperlipidemic rats when they were administered spirulina powder at 300, 600, and 900 mg/kg body weight due to the capacity of spirulina powder that decreased the food intake. Mechanistically, the gut-derived hormone ghrelin increased appetite in the PC hyperlipidemia group. At the same time, GLP-1 (glucagon-like peptide-1) and cholecystokinin decreased appetite in spirulina powder treated at different dose level groups. Previously, spirulina's active ingredient may also have reduced body weight gain by decreasing glucose levels and promoting insulin sensitivity [40]. This confirms that serum insulin levels in different dose levels were decreased and sustained as compared to the hyperlipidemia-induced group at the end of 12 weeks. Moreover, proximate analysis of spirulina powder in the current study showed the presence of higher fiber content (16.46%), which functionally provides strict glycemic control by reducing gastric emptying; amylase inhibition leading to delayed breakdown of starch; and further reduction in the diffusion of carbohydrate metabolites through microvilli, enhancing the absorptive barrier of amylolytic product through the mucosa. Such characteristics of dietary fiber present in functional food provide physiological bases for the strict control of hyperglycemia [41].

Along with fiber, spirulina powder contains seven different phenolic acids as bioactive compounds, among which, chlorogenic acid (4052.90 μg/g) at the highest amount reduced hyperglycemia in hyperlipidemic rats. Spirulina contains some phytochemicals that prevent the enzyme glucose-6-phosphatase from working. Those who have diabetes along with other related illnesses may benefit from this inhibition. Plant-based foods naturally contain p-coumaric acid, which adds to dietary diversity. Rutin and quercetin are two examples

of flavonoids that have anti-inflammatory and antioxidant qualities. These substances can prevent chronic illnesses. Regular ingestion of these chemicals is needed to maximize their beneficial effects in disease prevention. Quercetin interacts with molecular targets in the pancreas, small intestine, skeletal muscles, liver, and adipose tissue to control glycemic homeostasis of the whole body. Quercetin is an intriguing alternative for diabetes management because of its pleiotropic effects on glucose metabolism and insulin dynamics. Quercetin facilitates the translocation of GLUT4 to the cell membrane, which aids in the absorption of glucose by muscle and adipocytes [42].

In the current study, spirulina powder supplementation significantly enhanced mRNA levels of *INS-1*, *IGF-1*, *PDX-1*, and *GLUT-2* transcriptional factors involved in the synthesis of insulin by pancreatic β-cells, resulting in a reduction of blood glucose levels. Upregulation of insulin regulatory enzymes favors the hypochloremic, antioxidative, and antidiabetic agents as bioactive polyphenols present in the crude powder of spirulina as a functional food.

5. Conclusions

A natural product with strong antioxidant qualities, spirulina powder contains phenolic and flavonoid components. In the long-term feeding study, rats were provided with varying levels of spirulina powder supplementation for the period of 12 weeks in which the highest dose of spirulina powder at 900 mg/kg body weight was reported to possess lipid-lowering properties and antioxidant properties due to its high concentration of bioactive substances that may interact with lipid metabolic pathways along with its high potential to enhance the insulin sensitivity and glucose uptake in tissues by transcriptional modulation of different genes. Research has shown that specific bioactive compounds in spirulina can impact *INS-1* gene transcription, leading to increased insulin production. It may also show its effects on *PDX-1* transcriptional activity by enhancing insulin production and β-cell function, as well as on the *GLUT-2* gene that is responsible for glucose uptake in the liver and pancreatic β-cells. The interaction between spirulina's bioactive elements and cellular signaling pathways could result in the upregulation or downregulation of key genes involved in glucose and lipid metabolism, leading to better safety profile in terms of toxicity. These preliminary findings are promising, conclusively showing that spirulina appears to have beneficial effects on hyperlipidemia and glycemic balance, which may be mediated through the transcriptional modulation of genes and means it can be used as an ideal functional food for the treatment of these metabolic conditions and others that can be validated through rigorous clinical trials.

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References

1. Hill, S.A.; Bordoni, S.; Mitchell, J.L. A theory for the Hadley cell descending and ascending edges throughout the annual cycle. *J. Atmos. Sci.* **2022**, *79*, 2515–2528. [CrossRef]
2. Corbett, K.S.; Edwards, D.K.; Leist, S.R.; Abiona, O.M.; Boyoglu-Barnum, S.; Gillespie, R.A.; Himansu, S.; Schäfer, A.; Ziwawo, C.T.; DiPiazza, A.T.; et al. SARS-CoV-2 mRNA vaccine design enabled by prototype pathogen preparedness. *Nature* **2020**, *586*, 567–571. [CrossRef] [PubMed]
3. Feldman, D.; Pamboukian, S.V.; Teuteberg, J.J.; Birks, E.; Lietz, K.; Moore, S.A.; Morgan, J.A.; Arabia, F.; Bauman, M.E.; Buchholz, H.W.; et al. The 2013 International Society for Heart and Lung Transplantation Guidelines for mechanical circulatory support: Executive summary. *J. Heart Lung Transplant.* **2013**, *32*, 157–187. [CrossRef] [PubMed]
4. Deng, R.; Chow, T.J. Hypolipidemic, antioxidant, and antiinflammatory activities of microalgae Spirulina. *Cardiovasc. Ther.* **2010**, *28*, e33–e45. [CrossRef] [PubMed]
5. Sonmez, A.; Ercin, C.N.; Cesur, M.; Dogru, T. Nutraceuticals for non-alcoholic fatty liver disease. In *Nutraceuticals and Cardiovascular Disease*; Humana: Cham, Switzerland, 2021; pp. 141–165.
6. Masten Rutar, J.; Jagodic Hudobivnik, M.; Nečemer, M.; Vogel Mikuš, K.; Arčon, I.; Ogrinc, N. Nutritional quality and safety of the spirulina dietary supplements sold on the Slovenian market. *Foods* **2022**, *11*, 849. [CrossRef]
7. Mohammadi, M.; Soltanzadeh, M.; Ebrahimi, A.R.; Hamishehkar, H. Spirulina platensis protein hydrolysates: Techno-functional, nutritional and antioxidant properties. *Algal Res.* **2022**, *65*, 102739. [CrossRef]
8. Kulshreshtha, A.; Jarouliya, U.; Bhadauriya, P.; Prasad, G.; Bisen, P. Spirulina in health care management. *Curr. Pharm. Biotechnol.* **2008**, *9*, 400–405. [CrossRef]
9. Sharoba, A.M. Nutritional value of spirulina and its use in the preparation of some complementary baby food formulas. *J. Food Dairy Sci.* **2014**, *5*, 517–538. [CrossRef]
10. Tang, G.; Suter, P.M. Vitamin A, nutrition, and health values of algae: Spirulina, Chlorella, and Dunaliella. *J. Pharm. Nutr. Sci.* **2011**, *1*, 111–118. [CrossRef]
11. Attia, Y.A.; Hassan, R.A.; Addeo, N.F.; Bovera, F.; Alhotan, R.A.; Al-Qurashi, A.D.; Al-Baadani, H.H.; Al-Banoby, M.A.; Khafaga, A.F.; Eisenreich, W.; et al. Effects of Spirulina platensis and/or Allium sativum on Antioxidant Status, Immune Response, Gut Morphology, and Intestinal Lactobacilli and Coliforms of Heat-Stressed Broiler Chicken. *Vet. Sci.* **2023**, *10*, 678. [CrossRef]
12. AlFadhly, N.K.; Alhelfi, N.; Altemimi, A.B.; Verma, D.K.; Cacciola, F.; Narayanankutty, A. Trends and technological advancements in the possible food applications of Spirulina and their health benefits: A Review. *Molecules* **2022**, *27*, 5584. [CrossRef] [PubMed]
13. Trotta, T.; Porro, C.; Cianciulli, A.; Panaro, M.A. Beneficial effects of spirulina consumption on brain health. *Nutrients* **2022**, *14*, 676. [CrossRef] [PubMed]
14. AOAC. *Official Methods of Analysis*, 18th ed.; Association of Official Analytical Chemists: Gaithersburgs, MD, USA, 2006.
15. Soni, R.A.; Sudhakar, K.; Rana, R. Spirulina–From growth to nutritional product: A review. *Trends Food Sci. Technol.* **2017**, *69*, 157–171. [CrossRef]
16. Anwar, H.; Navaid, S.; Muzaffar, H.; Hussain, G.; Faisal, M.N.; Ijaz, M.U.; Riđanović, S. Analyzing cross-talk of EPO and EGF genes along with evaluating therapeutic potential of Cinnamomum verum in cigarette-smoke-induced lung pathophysiology in rat model. *Food Sci. Nutr.* **2023**, *11*, 1486–1498. [CrossRef] [PubMed]
17. Bird, R.P.; Draper, H.H. Comparative studies on different methods of malonaldehyde determination. *Method Enzymol.* **1984**, *105*, 299–305.
18. Hadwan, M.H. Simple spectrophotometric assay for measuring catalase activity in biological tissues. *BMC Biochem.* **2018**, *19*, 7. [CrossRef]
19. Krishna, H.; Avinash, K.; Shivakumar, A.; Al-Tayar, N.G.S.; Shrestha, A.K. A quantitative method for the detection and validation of catalase activity at physiological concentration in human serum, plasma and erythrocytes. *Spectrochim. Acta Part A Mol. Biomol. Spectrosc.* **2021**, *251*, 119358. [CrossRef]
20. Mustafa, I.; Anwar, H.; Irfan, S.; Muzaffar, H.; Ijaz, M.U. Attenuation of carbohydrate metabolism and lipid profile by methanolic extract of Euphorbia helioscopia and improvement of beta cell function in a type 2 diabetic rat model. *BMC Complement. Med. Ther.* **2022**, *22*, 23. [CrossRef]
21. Yano, Y.; Irie, N.; Homma, Y.; Tsushima, M.; Takeuchi, I.; Nakaya, N.; Goto, Y. High density lipoprotein cholesterol levels in the Japanese. *Atherosclerosis* **1980**, *36*, 173–181. [CrossRef]
22. Hoffmann, G.; Hiefinger, R.; Weiss, L.; Poppe, W. Five methods for measuring low-density lipoprotein cholesterol concentration in serum compared. *Clin. Chem.* **1985**, *31*, 1729–1730. [CrossRef]
23. Albadawy, R.; Agwa, S.H.; Khairy, E.; Saad, M.; El Touchy, N.; Othman, M.; Matboli, M.J.B. Clinical Significance of HSPD1/MMP14/ITGB1/miR-6881-5P/Lnc-SPARCL1-1: 2 RNA Panel in NAFLD/NASH Diagnosis: Egyptian Pilot Study. *Biomedicines* **2021**, *9*, 1248. [CrossRef] [PubMed]

24. Liu, Z.; Que, S.; Xu, J.; Peng, T. Alanine aminotransferase-old biomarker and new concept: A review. *Int. J. Med. Sci.* **2014**, *11*, 925. [CrossRef] [PubMed]
25. Schemitt, E.G.; Colares, J.R.; Hartmann, R.M.; Morgan-Martins, M.I.; Marroni, C.A.; Tuñón, M.J.; Marroni, N.P. Effect of glutamine on oxidative stress and inflammation in a rat model of fulminant hepatic failure. *Nutr. Hosp.* **2016**, *33*, 210–219.
26. Singh, G.; Senapati, S.; Satpathi, S.; Behera, P.K.; Das, B.; Nayak, B. Establishment of decellularized extracellular matrix scaffold derived from caprine pancreas as a novel alternative template over porcine pancreatic scaffold for prospective biomedical application. *FASEB J.* **2022**, *36*, e22574. [CrossRef] [PubMed]
27. Sultan, M.; Amstislavskiy, V.; Risch, T.; Schuette, M.; Dökel, S.; Ralser, M.; Balzereit, D.; Lehrach, H.; Yaspo, M.-L. Influence of RNA extraction methods and library selection schemes on RNA-seq data. *BMC Genom.* **2014**, *15*, 675. [CrossRef]
28. Farooq, A.; Rhee, G.H.; Shim, H.; Valizadeh, B.; Lee, J.; Khan, M.; Jeon, B.-H.; Jang, S.-H.; Choi, Y.J.; Park, Y.-K. Enhanced mono-aromatics production by the CH4-assisted pyrolysis of microalgae using Zn-based HZSM-5 catalysts. *Chemosphere* **2024**, *351*, 141251. [CrossRef]
29. Pandey, V.K.; Singh, P.; Srivastava, S.; Zanwar, S.; Dar, A.H.; Singh, R.; Lal, A. Box–Behnken design based statistical modelling to study the effects of spirulina (*Arthrospira platensis*) incorporation on nutritional standards of vegan snack product. *J. Agric. Food Res.* **2023**, *14*, 100700. [CrossRef]
30. Sibiya, T.; Ghazi, T.; Mohan, J.; Nagiah, S.; Chuturgoon, A.A. Spirulina platensis ameliorates oxidative stress associated with antiretroviral drugs in hepg2 cells. *Plants* **2022**, *11*, 3143. [CrossRef]
31. Palmegiano, G.B.; Agradi, E.; Forneris, G.; Gai, F.; Gasco, L.; Rigamonti, E.; Sicuro, B.; Zoccarato, I. Spirulina as a nutrient source in diets for growing sturgeon (*Acipenser baeri*). *Aquac. Res.* **2005**, *36*, 188–195. [CrossRef]
32. Zheng, J.; Inoguchi, T.; Sasaki, S.; Maeda, Y.; McCarty, M.F.; Fujii, M.; Ikeda, N.; Kobayashi, K.; Sonoda, N.; Takayanagi, R. Phycocyanin and phycocyanobilin from Spirulina platensis protect against diabetic nephropathy by inhibiting oxidative stress. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **2013**, *304*, R110–R120. [CrossRef]
33. Li, K.; Duan, X.; Zhou, L.; Hill, D.R.; Martin, G.J.; Suleria, H.A. Bioaccessibility and bioactivities of phenolic compounds from microalgae during in vitro digestion and colonic fermentation. *Food Funct.* **2023**, *14*, 899–910. [CrossRef] [PubMed]
34. Bitam, A.; Aissaoui, O. *Spirulina Platensis, Oxidative Stress, and Diabetes*; Elsevier: Amsterdam, The Netherlands, 2020; pp. 325–331.
35. Damessa, F. Nutritional and Functional Values of Microalgae (Spirulina) Naturally Found in East Africa. Ph.D. Thesis, NM-AIST, Arusha, Tanzanian, 2021.
36. Nagaoka, S.; Shimizu, K.; Kaneko, H.; Shibayama, F.; Morikawa, K.; Kanamaru, Y.; Otsuka, A.; Hirahashi, T.; Kato, T. A novel protein C-phycocyanin plays a crucial role in the hypocholesterolemic action of Spirulina platensis concentrate in rats. *J. Nutr.* **2005**, *135*, 2425–2430. [CrossRef] [PubMed]
37. Ohmori, M.; Ehira, S. Spirulina: An example of cyanobacteria as nutraceuticals. In *Cyanobacteria: An Economic Perspective*; John Wiley & Sons, Ltd.: Chichester, UK, 2014; pp. 103–118.
38. Coué, M.; Tesse, A.; Falewée, J.; Aguesse, A.; Croyal, M.; Fizanne, L.; Chaigneau, J.; Boursier, J.; Ouguerram, K. Spirulina liquid extract protects against fibrosis related to non-alcoholic steatohepatitis and increases ursodeoxycholic acid. *Nutrients* **2019**, *11*, 194. [CrossRef] [PubMed]
39. Rajak, U.; Nashine, P.; Verma, T.N.; Pugazhendhi, A. Performance and emission analysis of a diesel engine using hydrogen enriched n-butanol, diethyl ester and Spirulina microalgae biodiesel. *Fuel* **2020**, *271*, 117645. [CrossRef]
40. Guldas, M.; Ziyanok-Demirtas, S.; Sahan, Y.; Yildiz, E.; Gurbuz, O. Antioxidant and anti-diabetic properties of Spirulina platensis produced in Turkey. *Food Sci. Technol.* **2020**, *41*, 615–625. [CrossRef]
41. Reynolds, E. *Natural Product Therapeutics and COVID-19: The Case for Clinical Development of Spirulina Extracts*; Back of the Yards Algae Sciences Inc.: Chicago, IL, USA, 2020; Available online: https://www.algaesciences.com/post/natural-product-therapeutics-and-covid-19-the-case-for-clinical-development-of-spirulina-extracts (accessed on 27 October 2024).
42. Salama, E.-S.; Kurade, M.B.; Abou-Shanab, R.A.; El-Dalatony, M.M.; Yang, I.-S.; Min, B.; Jeon, B.-H. Recent progress in microalgal biomass production coupled with wastewater treatment for biofuel generation. *Renew. Sustain. Energy Rev.* **2017**, *79*, 1189–1211. [CrossRef]