Research Article

Physiological Effects of *Spirulina platensis* on Oxidative Stress and Liver Histopathological Changes in Alloxan-Induced Diabetic Rats

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Abstract | Diabetes mellitus results in high blood sugar levels and causes the body to produce injury to its' own tissues causing the body to produce more free radicals. This study investigated the effects of continuous ingestion of *Spirulina platensis* on free radical production and liver tissue injury in diabetic Wistar rats. Twenty-eight Wistar rats were randomly divided into four groups (n = 7 each): Spirulina, Diabetic, Diabetic + Spirulina, and Control. Diabetes was induced in the respective groups using alloxan at a dose of 150 mg/kg. To prevent hypoglycemia following alloxan administration, the rats were provided with 5% glucose solution. Throughout the 4-week experimental period, rats in the Spirulina-treated groups received *Spirulina* at a dose of 100 mg/kg body weight. At the end of the experiment, levels of free radicals, antioxidant enzyme activities, and liver histopathology were assessed. The diabetic group showed significant ($p < 0.05$) alterations, including hyperglycemia, increased oxidative stress, tissue injury, and elevated free radical levels. Histological examination of liver tissues revealed hepatocyte ballooning, necrosis, and cytoplasmic vacuolation. In contrast, treatment with *Spirulina* improved antioxidant status, reduced oxidative stress, and preserved liver structural integrity. These findings suggest that *Spirulina platensis* exerts hepatoprotective effects against diabetes-induced oxidative damage and may have potential as an adjunct therapy in the management of diabetes.

Keywords | Alloxan-induced diabullism, Antioxidative proteins, Liver microanatomy, *Spirulina platensis*, Experimental rats, Oxidative stress

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INTRODUCTION

Diabetes mellitus is characterized by hyperglycemia (elevated blood glucose levels) and is associated with systemic complications, including abnormalities in the metabolism of carbohydrates, lipids, and proteins (Abd El-Emam *et al*., 2024). The disease also induces molecular disturbances that lead to excessive production of reactive oxygen species (ROS), resulting in oxidative stress. This oxidative stress is implicated in the damage and dysfunction of various cellular and tissue components (Alum *et al*., 2022). The liver is one of the major organs that suffers from oxidative injury in this illness, which is because of the important role the liver plays in glucose control and as well as being the organ that is structurally and metabolically altered due to hyperglycemia (Alvarenga

et al., 2011). Studies have shown that the combination of chronic hyperglycemia and oxidative stress disrupts the normal structure of the cells of the liver, causing more damage to the liver in the case of diabetes (Aslam *et al*., 2023). Alloxan is one of the agents that causes diabetes experimentally because of its selective oxidative destruction of the pancreatic beta cells (Arıcan and Demirbaş, 2022). While alloxan's main effect is on the pancreas, diabetes resulting from alloxan also causes peripheral oxidative stress and injury to other organs, especially the liver (Aslam *et al*., 2023). Thus, the alloxan model is suitable to investigate the effects of antioxidant treatments on diabetes-related liver injury. Previous research have shown the oxidative stress and tissue injury correlation in diabetic models, however, the extent to which antioxidant supplementation may alleviate hepatic oxidative injury remains to be investigated. Phycocyanin, carotenoids, phenolic compounds, and trace elements are important constituents of *Spirulina platensis*. Spirulina has both anti-oxidant and cytoprotected related activities. These elements possess the ability to sweep neutralize free radicals and bolster the body's own anti-oxidant mechanisms. Thereby, the degree of tissue damage caused by oxidation may be reduced. Spirulina has been studied in diabetes models where supplementation has proven to lower levels of hyperglycemia along with the complications of oxidative stress and metabolic dysfunctions. Given these biological functions, *Spirulina platensis* may serve as the defence against diabetes related hepatic oxidative injury. The interest in Spirulina has been mainly due to its natural antioxidative properties, but its actual effect on oxidative stress and liver structural changes is almost completely uninvestigated on alloxan diabetic rats. This study intends to show the effects of *Spirulina platensis* on alloxan-induced diabetic rats in relation to oxidative stress, antioxidant enzyme activity, and structural changes in the liver.

MATERIALS AND METHODS

Experimental animals

The study included twenty-eight male Wistar rats aged 8 weeks and weighing between 200 to 250 grams. The subjects were kept in a laboratory setting at 22 ± 2 degrees Celsius and 12-hour light/12-hour dark cycles. The rats went through a week-long acclimation period with access to standard chow and water provided ad libitum.

Experimental design

The rats were randomly assigned into 4 experimental groups, with 7 animals in each group:

Group 1 (Control): Received normal diet and distilled water only.

Group 2 (*Spirulina platensis*): Received *Spirulina platensis* only.

Group 3 (Diabetic): Diabetes was induced by alloxan monohydrate.

Group 4 (Diabetic + *Spirulina platensis*): Diabetic rats received *Spirulina platensis* treatment.

Induction of diabetes

Rats that received an intraperitoneal alloxan monohydrate injection (150 mg/kg body weight) after 12 hours of fasting developed diabetes. In order to offset any risk of hypoglycemia after the alloxan injection, a 5% glucose solution was given. Diabetes was confirmed using a glucometer which showed a positive result after fasting blood glucose levels reached 250 mg/dL.

Administration of *Spirulina platensis*

The *Spirulina platensis* powder was obtained from the DXN Company in Najaf, Iraq. Treatment commenced after the diagnosis of diabetes. This was done through oral administration by gastric gavage at a rate of 100 mg/kg body weight once a day for 4 successive weeks.

Collection of blood samples and serum extraction

As part of the experiment, blood samples were collected from the inferior vena cava of the sedated rats after administration of sodium pentobarbital. Samples were allowed to clot, and the serum was separated from the clot and stored at -20 °C until the serum was analyzed biochemically after being centrifuged at 3000 rpm for 15 minutes.

Assessment of oxidative stress indicators

The manufacturer's instructions were followed for the quantitative colorimetric assay kits obtained from a vendor for the assessment of serum oxidative stress. The criteria included were thiobarbituric acid reactive species (TBARS), hydrogen peroxide (H_2O_2), and reduced glutathione (GSH).

Evaluation of antioxidant enzyme activities

Manufacturer provided instructions were followed concerning the serum oxidative stress indicators using the commercial colorimetric test kits. These pertain to the tests of thiobarbituric acid reactive substances (TBARS), hydrogen peroxide (H_2O_2), and reduced glutathione (GSH).

Histopathological Analysis

Livers were swiftly removed, chopped into tiny pieces, fixed in 10% buffered formalin for 24 to 48 hours, processed, sectioned at 5 µm, and stained with H & E using the Bancroft and Cook (2012) procedure.

Statistical analysis

Data are presented as mean ± standard deviation (SD), and

statistical analyses were performed using SPSS software. Group comparisons were conducted using one-way analysis of variance (ANOVA) and for multiple comparisons, Tukey's post hoc test was applied. A p-value of 0.05 or less was deemed statistically significant.

RESULTS

Effect of *Spirulina platensis* on total antioxidant status

Compared to the control group, the diabetic rats exhibited an oxidative imbalance, suggesting that oxidative damage was increased while the antioxidant defenses were decreased. Statistically significant differences ($p < 0.05$) were observed in oxidative stress markers, including TBARS, H_2O_2, and GSH (Table 1). The oxidative stress-related biochemical changes in the diabetic rats that were untreated, in contrast to the treated ones, showed paradoxically even more and significant ($p < 0.05$) increase of antioxidant enzymes. It is true that many parameters were not as high in this group as in the control group, nevertheless, these changes were largely positive.

Effect of *Spirulina platensis* on oxidative stress biomarkers and antioxidant enzyme activities

Statistically significant decreases ($p < 0.05$) were observed in the antioxidant enzymes measured in these rats viz., superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione reductase (GR), and glutathione-S-transferase (GST). In untreated diabetic rats, oxidative stress related biochemical alterations showed significant ($p < 0.05$) changes compared with the treated groups, including abnormal increases in the previously measured antioxidant-related parameters (Table 2). Although some parameters did not reach control levels, the treated groups demonstrated overall beneficial and improved biochemical profiles.

Histopathological findings

In the histological evaluation of the livers of the diabetic rats, there is still some preservation of lobular architecture and intrahepatic portal and sinusoidal vessels, as well as normal appearing hepatocytes and nuclei in the vessels (Figure 1). Ballooning and pronounced cytoplasmic vacuolation of hepatocytes. Additionally, there is some regional necrosis, periportal degeneration, and infiltration of mononuclear inflammatory cells. In comparison to the untreated diabetic rats, the livers of diabetic rats treated with Spirulina showed minimal deterioration and some stagnant inflammatory cells. *Spirulina platensis* is the only known substance that appears to reverse the diabetic pathological changes in the liver related to *Spirulina platensis*.

DISCUSSION

We investigated how alloxan-induced diabetes affects liver histology and changes the histology and oxidative biochemistry of the liver in rats. We also looked at oxidative stress and the role of antioxidant enzymes in diabetic individuals. The liver demonstrated pronounced structural changes, such as hepatocellular ballooning and vacuolar cytoplasmic degeneration, as well as necrosis with infiltrations by inflammatory cells. These studies provide

Table 1: Effect of *Spirulina platensis* extract and alloxan on total antioxidant status.

Experimental groups				Parameters
SP+D.M	**Diabetic**	***Spirulina platensis***	**Control**	
0.946 ±0.037^{b}	1.196±0.030^{a}	0.732 ±0.016^{d}	0.857±0.024^{c}	TBARS*
21.37±0.392^{b}	23.98±0.885^{a}	15.37±0.381^{d}	18.24±0.609^{c}	H_2O_2**
0.139±0.005^{c}	0.104±0.005^{d}	0.220±0.006^{a}	0.185±0.006^{b}	GSH***

Each treatment group comprised 7 participants, and values are shown as mean ± SE. Means with different superscript letters (a–d) are significantly different ($p < 0.05$). *nmol/ml, **mmol/l, ***U/ml. SP+D.M = *Spirulina platensis* + diabetic.

Table 2: Effect of *Spirulina platensis* extract and Alloxan on rat serum antioxidant enzymes.

Experimental groups				Parameters
SP+D.M	**Diabetic**	***Spirulina platensis***	**Control**	
0.97±0.021^{c}	0.68±0.021^{d}	1.30±0.034^{a}	1.13±0.038^{b}	SOD*
37.95±1.30^{c}	26.95±0.981^{d}	54.67±1.35^{a}	46.07±1.46^{b}	CAT*
20.92±0.766^{c}	15.05±0.628^{d}	29.48±0.915^{a}	24.71±0.744^{b}	GPx*
12.82±0.398^{c}	9.50± 0.347^{d}	18.03±0.542^{a}	15.35± 0.344^{b}	GR*
0.522±0.021^{c}	0.379±0.011^{d}	0.704±0.018^{a}	0.587±0.018^{b}	GST**

The values are shown as mean ± SE, with n=7 for each treatment group. Letters in superscript $^{(abcd)}$ show that there are significant differences ($p < 0.05$). *U/ml, **µmol/hr. SP+D.M = *Spirulina platensis* + diabetic.

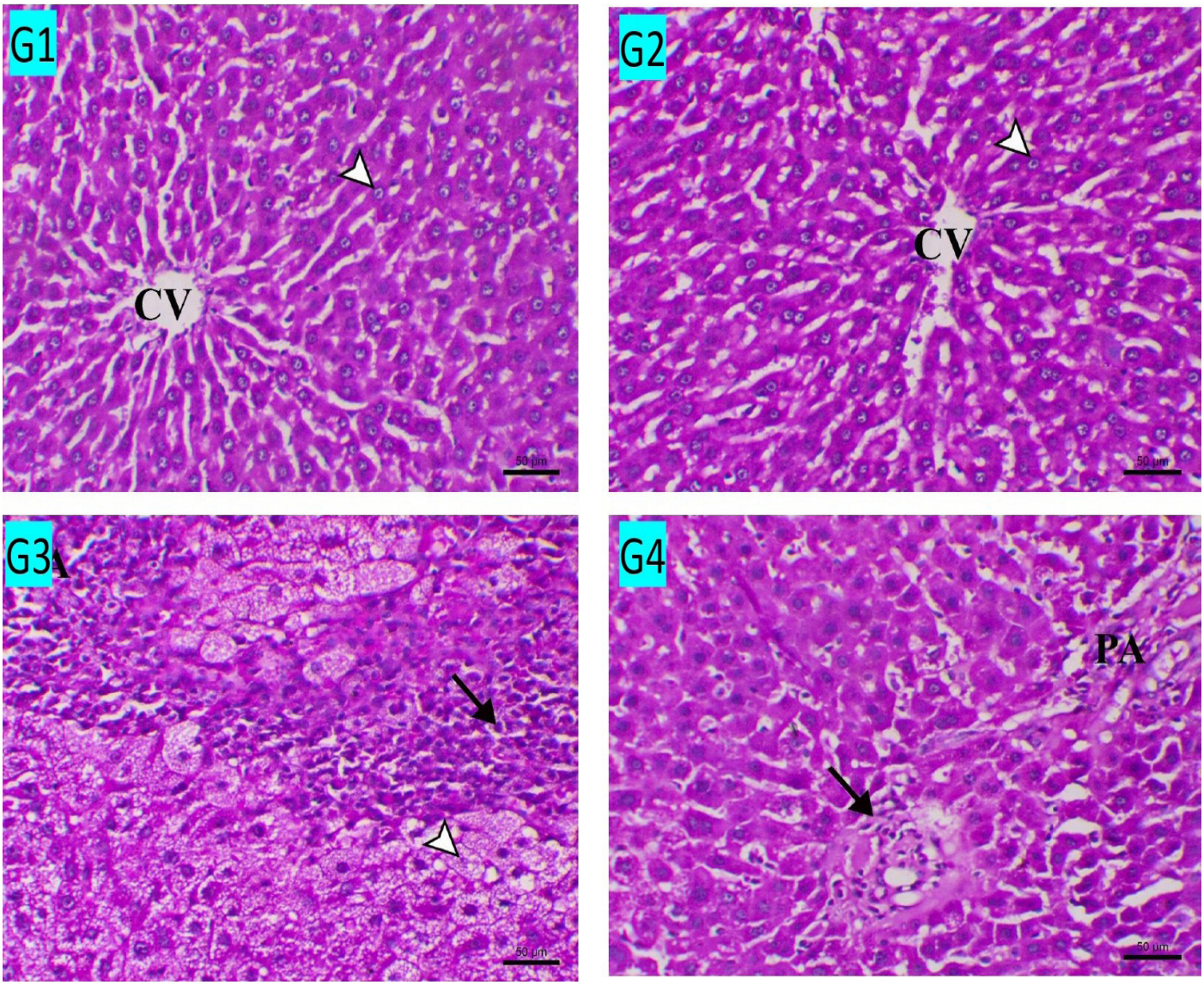

Figure 1: Photomicrographs of liver sections from the different experimental groups stained with hematoxylin and eosin (H&E). G1 and G2: normal hepatic architecture with normal hepatocytes, vesicular nuclei, preserved sinusoids, and central vein (CV). G3: severe periportal hepatic degeneration and necrosis with marked cellular ballooning and mononuclear inflammatory cell infiltration (arrow); PA indicates portal area. G4: marked reduction in hepatic degenerative changes with mild cytoplasmic vacuolation and mild periportal mononuclear inflammatory cell infiltration; PA indicates portal area. Scale bar = 50 µm.

evidence for the oxidative stress due to hyperglycemia and subsequent liver injury in diabetic experimental animals (Huang *et al*., 2023; Hussain *et al*., 2022; Hameed *et al*., 2025). The treatment with *Spirulina platensis* for four weeks significantly lessened these physiological and biochemical changes. The ability to reduce oxidative stress by (Seyidoglu *et al*., 2017; Al-Khuzaay *et al*., 2024; Tousson *et al*., 2024; Ibrahim *et al*., 20203) alludes to the positive oxidative stress modulation by *S. platensis* as evidenced by the increased activities of specific antioxidant enzymes and the restoration of some oxidative stress markers. The constituents of Spirulina like Phycocyanin, carotenoids and polyphenols might also explain those findings. The nutritional value of those constituents also support the theory that they can both decrease reactive oxygen species (ROS) and/or increase the antioxidant defense system (Liu *et al*., 2016; Madkour and Nasr, 2012; Al-Saeedi *et al*., 2026). Improved liver histology supports the idea that reduced oxidative stress helped preserve diabetic rats liver architecture (Hussain *et al*., 2022; Hameed *et al*., 2025). The greatest strength of this work is the combination of biochemistry and histology. This study shows that the relationship of oxidative stress and liver histomorphology provides a better understanding of the degree of hepatoprotection than studies using only one marker (Lupatini *et al*., 2017; Al-Obaidi *et al*., 2022; Alankooshi *et al*., 2023). There are problems associated with this study. Not including the evaluation of some blood biomarkers related to liver function, especially the enzymes ALT and AST, significantly lessens the importance of the changes

in the liver and the clinical significances of liver injury. This study focused on one dose and one duration of the intervention with *Spirulina platensis*, and one experimental model of diabetes. More studies with different dose/duration of treatment protocols and examination of the liver's biochemical, functional, and molecular aspects are necessary to provide a more thorough assessment of the clinical potential of *Spirulina platensis* in diabetes-related liver injury (Al-Obaidi *et al*., 2022; Al-Dulimi *et al*., 2025). The findings show that *Spirulina platensis* can protect liver tissue from oxidative stress and micro-and-macro structural hepatic injuries in the absence of the hepatic injury biomarkers ALT and AST and the histological and/or biochemical investigations supporting the hepatoprotective effects of *Spirulina platensis* demonstrate that It is due to *Spirulina platensis*'s ability to restore the cellular oxidative and antioxidative stress balance in liver hepatocytes (Seyidoglu *et al*., 2017; Hussain *et al*., 2022; Hameed *et al*., 2025). There is histological and biochemical evidence supporting the potential use of *Spirulina platensis* for hepatic injury associated with diabetes and additional studies focusing on those cellular and molecular aspects are needed to clarify the degree of hepatic tissue restoration and the duration of the treatment so that functional liver biomarkers can be evaluated.

CONCLUSION

These results suggest that *Spirulina platensis* may exert hepatoprotective effects against diabetes-induced oxidative damage and may have potential therapeutic value, particularly in combination with other treatments, for mitigating diabetic complications.

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NOVELTY STATEMENT

This study offers integrated biochemical and histopathological evidence that *Spirulina platensis* mitigates oxidative stress–associated hepatic harm in alloxan-caused diabetic rats. By combining oxidative stress biomarkers, antioxidant enzyme assessments, and direct liver histopathology, the present study strengthens the experimental evidence supporting *Spirulina platensis* as a potential adjunct antioxidant intervention for diabetes-induced liver damage.

AUTHOR'S CONTRIBUTION

Azhar Azher Al-Ankooshi, Zainab Alesawi: Study layout. Halah Flaeeih Hasan, Ahmed Flayyih Hasan: Methodology. Alaa A Akon, Hany M. El-Wahsh: Study design, supervision, manuscript writing and editing.

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Ethical approval

Ethical approval turned into acquired from Biotechnology Research Center, Al-Nahrain University.

Generative AI and AI-assisted technology statement

The authors well known that no synthetic intelligence equipment have been used inside the article.

Conflict of interest

The authors have declared no conflict of interest.

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