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Research Article
Hepatoprotective Effect of *Spirulina platensis* on Liver Functions of Diabetic Rats via TNF-α and IL-6 Pathway

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Abstract

Background and Objective: *Spirulina platensis* (SP) microalgae is a filamentous cyanobacterium with strong dietary anti-inflammatory and Phyto-antioxidant. This study aimed to investigate the potential hepatoprotective and antioxidants effect of phenolic compounds extracted from *Spirulina platensis* (SPP) in Albino diabetic rats. **Materials and Methods:** Thirty rats were randomly divided into five groups. The first group were received normal saline. The second group were received only STZ. The third group were received (STZ) and glibenclamide (600 μg kg^{-1} b.wt.). The fourth group received STZ and silymarin (100 mg kg^{-1} b.wt.). The fifth group were treated with (STZ) following administration with (SPP) at (50 mg kg^{-1} b.wt.). The blood samples were collected for estimation of the liver biomarkers and the hepatic tissues were isolated for assessment of the oxidative stress markers. The TNF-α and IL-6 were also evaluated and the histopathological were studied. **Results:** The results show that the predominant compounds of (SPP) were pyrogallol, E-vanillic, Ellagic, catechol and Benzoic (638.5, 31.53, 19.83, 7. 62 and 6.82 mg/100 g, respectively). The animal treatments with (STZ) lead to an increase in ALT, AST and lipid peroxidation. It significantly inhibits the activities of GPx, CAT and SOD. As well as, TNF-α and IL-6 were increased. **Conclusion:** The results conclude that SPP may be acting as a therapeutic protective agent against liver damage in diabetic rats.

Key words: *Spirulina platensis*, phenolic compounds, liver enzymes, diabetes, antioxidant, TNF-α, IL-6

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Diabetes Mellitus (DM) is a major disease affecting the pancreas and its major manifestations include disordered metabolism and hyperglycemia[1]. Management of DM includes exercise, diet and treatment with oral hypoglycemic medicine and/or insulin[2]. However, these treatments do not effectively prevent the different complications associated with diabetes such as hepatopathy, neuropathy, nephropathy, hypertension and cataract[3]. As well, DM help in the production of the free radical that also lead to several complications is caused by chronic hyperglycemia including failure, damage and the dysfunction of kidneys, blood vessels, eyes, cardiovascular and nerves[4]. Moreover, increases the risk of colon, liver, pancreatic, bladder and breast cancers[5]. The increasing numbers of chronic liver disease in patients with DM has encouraged interest to discover this relation and on the search of pathogenesis that promise to focus on the relationship between glucose homeostasis and hepatic metabolism[3].

The liver is a highly affected organ among diabetic patients. The liver function markers ALT and AST are found always elevated and are associated with nonalcoholic fatty liver disease in diabetic patients[6]. Pre-clinical and epidemiological evidence indicates a clinical link between DM and liver diseases such as nonalcoholic steatohepatitis, non-alcoholic fatty liver disease, liver cirrhosis and metastatic hepatocellular carcinoma[7]. The nonalcoholic fatty liver is a widespread co-morbidity in approximately 70% of patients with T2DM[8]. Moreover, Tumor Necrosis Factor-α (TNF-α) is a common cytokine in liver injury, which control the immune system development, subsistence signalling pathways of cell, generation and different metabolic processes regulation[9,10]. Other studies also mentioned that TNF-α plays important role in the induction of hepatocyte apoptosis by lipopolysaccharide or concanavalin A leading to hepatotoxicity[11], also in regeneration and repair of liver tissues after hepatotoxicity[12]. Additionally, TNF-α acts as a protective, host defense and deleterious agent in a toxic shock. Therefore, TNF-α has double functions in liver damage, either intensive or relieving injury, it offers trials for designing medicine to protect the liver from injury[13]. In addition to, the Interleukin 6 (IL-6) are a family of cytokines that are characterized by their widespread use of expressed signal-transducing receptor, glycoprotein 130 (gp130) and applies many body functions. It plays a pathological important role in heart failure, cancers and inflammatory diseases including asthma, rheumatoid arthritis and systemic lupus erythematosus[14,15]. In liver tissue, IL-6 is considered the important initiator of the acute stage of response and infection defense. Furthermore, IL-6 is essential for hepatocyte homeostasis and is an effective hepatocyte mitogen. The IL-6 is not only embroiled in liver regeneration, but also liver metabolic function. As well as, the preserve initiation of the IL-6 signalling pathway is injurious to the liver and might lead to liver tumour development[16].

Recently, natural food products have been used as herbal medicine for thousands of years for treating chronic hyperglycemia[17]. These products have fewer side effects than synthetic products such as glibenclamide and metformin are used as antihyperglycemic agents for regulating DM or silymarin that regulate liver injury[18]. Many algal products have been reported to improve the nutritional value of foods because of their capability to prevent cell oxidative damage[19].

Spirulina platensis is one such natural product that is consumed as a food supplement by humans and animals or as whole food. It is rich in protein[20], vitamins such as C, A, E and B-complex, essential amino acids, γ-linolenic acid and antioxidants such as phycocyanin and β-carotenoids[21], in addition to minerals such as selenium and chromium[22]. Improvement of appropriate antioxidant molecules is achievement more significance as it plays an important role, in avoiding or deferring hepatotoxicity. Butyl hydroxyanisole (BHA) and butylhydroxytoluene (BHT) are commonly used as synthetic antioxidants but they may be accompanied by toxicity and health risks. Therefore, using phenolic compounds of SP is considered a good source of natural antioxidants. The present study was aimed to illuminate the potential use of SPP against oxidative stress and liver injury in diabetic rats.

MATERIALS AND METHODS

Study area: The study was carried out at Department of Biochemistry, Faculty of Agriculture, Ain Shams University, Egypt and Department of Pharmacology and Toxicology, College of Pharmacy, Prince Sattam Bin Abdulaziz University, Al-Kharj, Saudi Arabia, from November, 2020 to December, 2021.

Chemicals and solvents: All analytical grade chemicals and solvents were used in this study. Alanine transaminase (ALT), aspartate transaminase (AST), glucose, total cholesterol, triglyceride, alkaline phosphatase and Gamma-glutamyl transferase kits were obtained from the Egyptian Company of Biotechnology, Cairo, Egypt. The oxidative stress markers as superoxide dismutase (SOD), malondialdehyde (MDA), catalase (CAT) and glutathione peroxidase (GPX), the inflammatory markers IL-6 and TNF-α were purchased from BioDiagnostic, Egypt.

***In vivo* experiment**

Animals and housing conditions: A total of 54 male Albino rats weighing 150-190 g were obtained from the animal house of the Agriculture Research Center, Cairo, Egypt. The rats were housed in plastic cages and maintained on a standard diet and water ad libitum. All rats were adapted for 7 days under the laboratory conditions at 25°C and 12 hrs light/dark succession. All protocols and procedures followed were in agreement with the Institutional Animal Ethics Committee of ARC and recommendation of the appropriate care and use of laboratory animals.

Induction of DM in rats: The animals were induced for DM by intraperitoneal injecting of streptozotocin (STZ) freshly prepared solution (45 mg kg^{-1} b.wt.) in 100 mM citrate buffer. The blood samples were collected 72 hrs after injection and the blood glucose levels were estimated. The animals with high blood glucose levels of more than 350 mg dL^{-1} were selected as type 2 diabetic rats[23].

Experimental design: After establishing the diabetic model, 30 rats were divided into 5 groups with 6 animals with each group, orally administered daily for 21 days as follows:

Group I: Received only normal saline and served as the normal control group (NC)

Group II: Received STZ-induced diabetes and served as +ve control group

Group III: Diabetic rats have orally received glibenclamide (600 µg kg^{-1} b.wt.)

Group IV: Diabetic rats were administered with silymarin (100 mg kg^{-1} b.wt.)

Group V: Diabetic rats were administered with SPP (50 mg kg^{-1} b.wt.)

Identification and quantification of phenolic and flavonoid of *Spirulina platensis* biomass by HPLC: Total soluble phenolic of spirulina biomass was identified by HPLC and described in our earlier study[24].

Biochemical estimations: The liver function marker AST and ALT in serum, as well as, triglyceride, total cholesterol, Gamma-glutamyltransferase and alkaline phosphatase were determined according to the recommendations of the manufacturer.

Lipid peroxidation and antioxidant enzymes estimation: The liver tissue of all animals were homogenized in 10% w/v phosphate buffer (100 mM, pH 7.4) followed by centrifugation at 12000 rpm for 30 min at 4°C. The oxidative stress parameters: MDA, CAT, GPx and SOD were determined in the obtained supernatants according to the recommendations of the manufacturer.

Liver TNF-α and IL-6 determination: Animals liver tissue was thawed on ice with the addition of 1 ml of phosphate-buffered saline containing protease inhibitors to prevent protein degradation. The samples of liver tissue were homogenized three times for 5 min at 4°C. The homogenates were centrifuged and the supernatants were kept at -80°C. The protein content of liver samples was measured, TNF-α and IL-6 were detected according to the instructions of the ELISA kits (Abcam, Cambridge, MA, USA).

Histopathological examination: Tissue samplings were collected from the liver and immediately fixed in 10% formalin, after appropriate fixation, the thin sections of paraffin were prepared and stained with Hematoxylin and Eosin stain (H&E) for the subsequent histopathological lesions in the hepatic tissue. The sections were graded numerically to assess the degree of the histopathological countenance of the hepatic damage. Hepatocyte necrosis, hyaline degeneration, fatty change and infiltration of Kupffer cells were distinguished in the histological findings[25].

Statistical analysis: All data were presented as Mean±SEM ($n = 6$). Statistical significance was determined by one-way analysis of variance (ANOVA) using GraphPad Prism (GraphPad Software, San Diego, CA, USA). The comparison of the individuals was achieved by Tukey's test. The values were considered statistically significant at $p<0.05$.

RESULTS AND DISCUSSION

Recently, *Spirulina* has been concerned with more attention as a good alternative source of pharmaceutical beneficial compounds. Recently, research studies reported that *Spirulina* has several health benefits such as antioxidants immunomodulatory, anti-microbial activities, anti-viral, anticancer and anti-inflammatory. Now-a-days, many researchers are focusing on *Spirulina* because it helps in preventing lipid peroxidation, scavenges free radicals and DNA damage.

Table 1: Identification of the phenolic contents (mg/100 g) by HPLC analysis

Compounds	*Spirulina platensis* (mg/100 g)	*Spirulina platensis* (min)
Pyrogallol	638.50	6.875
Gallic	4.64	7.011
4-Amino-benzoic	0.32	8.246
Protocatchuic	1.26	8.694
Catechol	7.62	8.636
P-OH- benzoic	1.90	9.136
Caffeine	0.75	9.762
Chlorogenic	3.06	9.859
Vanillic	2.62	9.875
E-Vanillic	31.53	12.107
Caffeic	5.97	10.324
P-Coumaric	0.28	10.900
salicylic	3.89	16.258
Benzoic	6.82	13.268
Ferulic	0.39	11.657
Iso-Ferulic	0.22	11.938
Alpha-Coumaric	1.26	12.524
Ellagic	19.83	13.488
3,4,5-methoxy-cinnamic	1.40	14.183
Rosmarinic	0.18	14.282
Coumarin	1.08	14.552
Cinnamic	0.12	15.285

Table 2: Identification of the flavonoid contents (mg/100 g) by HPLC analysis

Compounds	*Spirulina platensis* (mg/100 g)	*Spirulina platensis* (min)
Catechin	3.77	8.246
Epicatechin	1.68	10.324
Naringin	4.74	12.340
Hesperidin	9.01	12.438
Rutin	1.23	12.575
Quercetin	2.26	13.482
Quercetin	0.92	14.887
Naringenin	0.89	15.034
Hesperetin	5.16	15.324
Kaempferol	1.20	16.243
Apigenin	0.54	16.600

Identification and quantification of phenolic and flavonoid of *Spirulina platensis* by HPLC: From the results of our earlier study[24], *Spirulina platensis* were exposed to HPLC analyses to examine phenolic and flavonoids that have the capability as antioxidants. We identified and quantified thirty-three of phenolic compounds. The phenols compounds including pyrogallol, E-vanillic, ellagic, catechol, benzoic, caffeic, gallic and chlorogenic were recorded as 638.5, 31.53, 19.83, 7.62, 6.82, 5.97, 4.64 and 3.06 (mg/100 g), respectively (Table 1, 2). Pyrogallol was the major phenolic (638.50 mg/100 g) and hesperidin was the major flavonoid (9.013 mg/100 g).

Effect of SPP on liver function: The results show that serum levels of ALT and AST were higher in diabetic groups compared with a normal control group ($p<0.05$), indicating impairment of liver functions (Fig. 1a, b). In the same way, the activities of ALP and Gamma-glutamyl transferase (GGT) (Fig. 1c, d), as well as TC, TG were found also to be significantly increased compared with the normal control group ($p<0.05$) (Fig. 1e, f). Meanwhile, in diabetic rats treated with SPP, the enzyme levels were reversed significantly decreased ($p<0.05$) (Fig. 1a-f).

Effects of treatment with SPP on lipid peroxidation and antioxidant enzymes: The results show that MDA in the hepatic tissue was significantly increased (Fig. 2a), while the antioxidant enzyme activities of SOD, GPx and CAT were significantly decreased in the diabetic group compared with normal control ($p<0.05$) (Fig. 2b-d). The SPP treatment of diabetic rats was decreased MDA values and increased the activities of antioxidant enzymes ($p<0.05$).

SPP effects on cytokine concentrations: The results show that levels of cytokines (IL-6 and TNF-α) were higher in diabetic control compared with normal control (Fig. 3a, b). Treatment of diabetic rats with SPP showed a significant reduction in IL-6 and TNF-α levels ($p<0.05$) (Fig. 3a, b).

Histopathological examination of liver tissue: Liver of normal control shown hepatic lobule with normal histological structures (Fig. 4a). In opposing, the liver tissue diabetic group show focal hepatic necrosis accompanied with Kupffer cells activation, cholangitis and inflammatory cells infiltration (Fig. 4b). Some examined sections in the third group showed slight Kupffer cells activation and binucleation of hepatocytes (Fig. 4c). However, the liver of the fourth group showed sinusoidal leukocytosis and Kupffer cells activation (Fig. 4d). Finally, the liver of the fifth group treated with (SPP) showed the liver histopathological structure became almost like the control group (Fig. 4e).

The liver is the first protection line against damage, which might result in apoptosis and hepatic necrosis, induced by free radicals, oxidative stress, drugs and xenobiotics[26]. The reactive oxygen species (ROS) induced by diabetes not only cause indirect liver damage but also, promote inflammation via activation of several cytokines[27]. Oxidative stress is defined as an imbalance between production and the removal of ROS. The increase of these oxidative stress that contributes ultimately to diabetic complications pathogenesis, is the result of either improved ROS production or decreased ROS scavenging capacity. The process generates ROS, including hydroxyl radicals and hydrogen peroxide. The accumulation of ROS by the liver may be lead to functional defects in the cell membrane, oxidation of DNA and protein and finally to

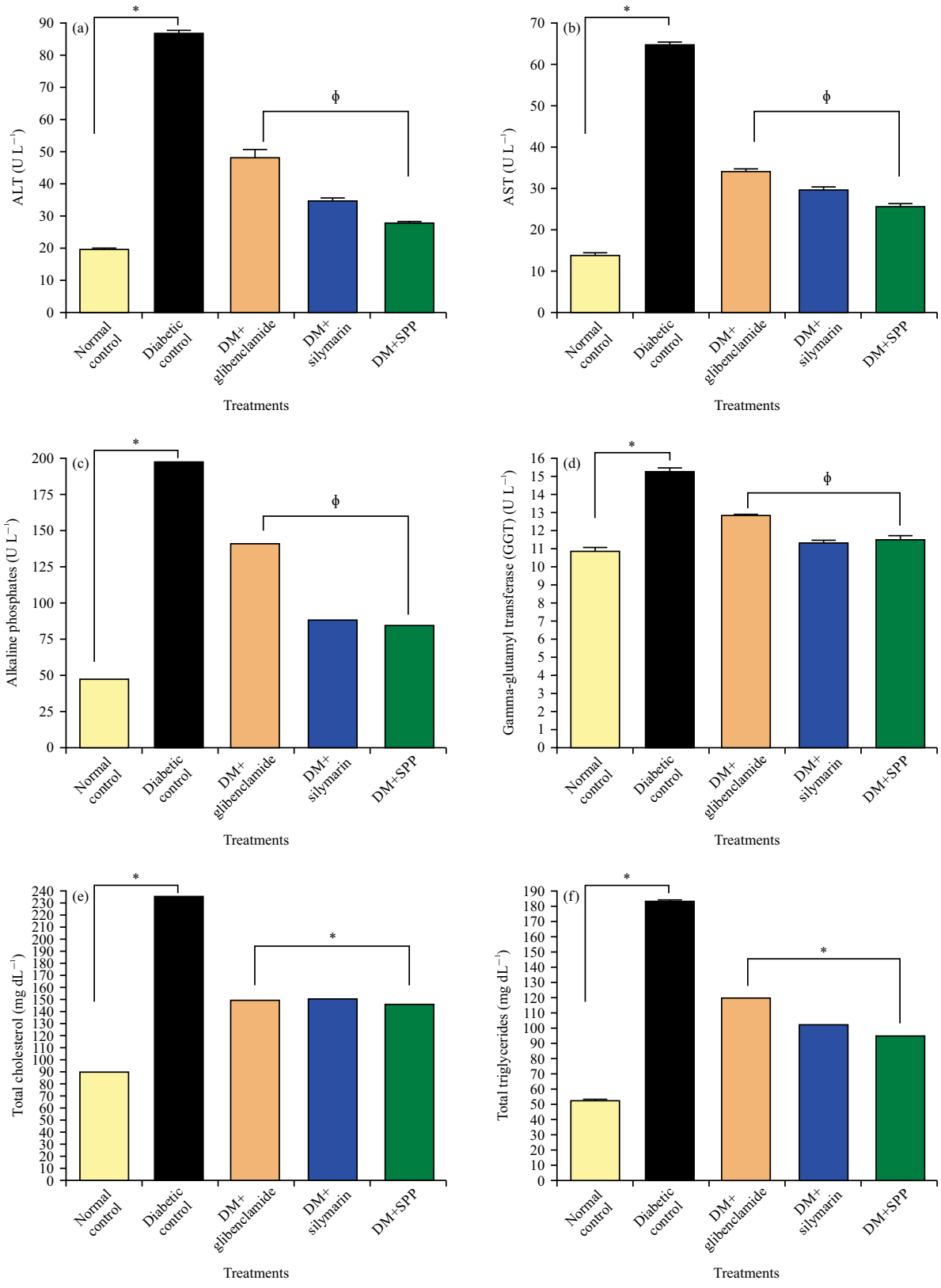

Fig. 1(a-f): Effect of SPP on the level of serum marker enzymes against diabetic-induced hepatotoxicity in rats, (a) ALT, (b) AST, (c) ALP, (d) GGT, (e) Total cholesterol and (f) Total triglycerides

Data are Means±SD of six rats in each group, the values among groups are significant value at *p<0.05

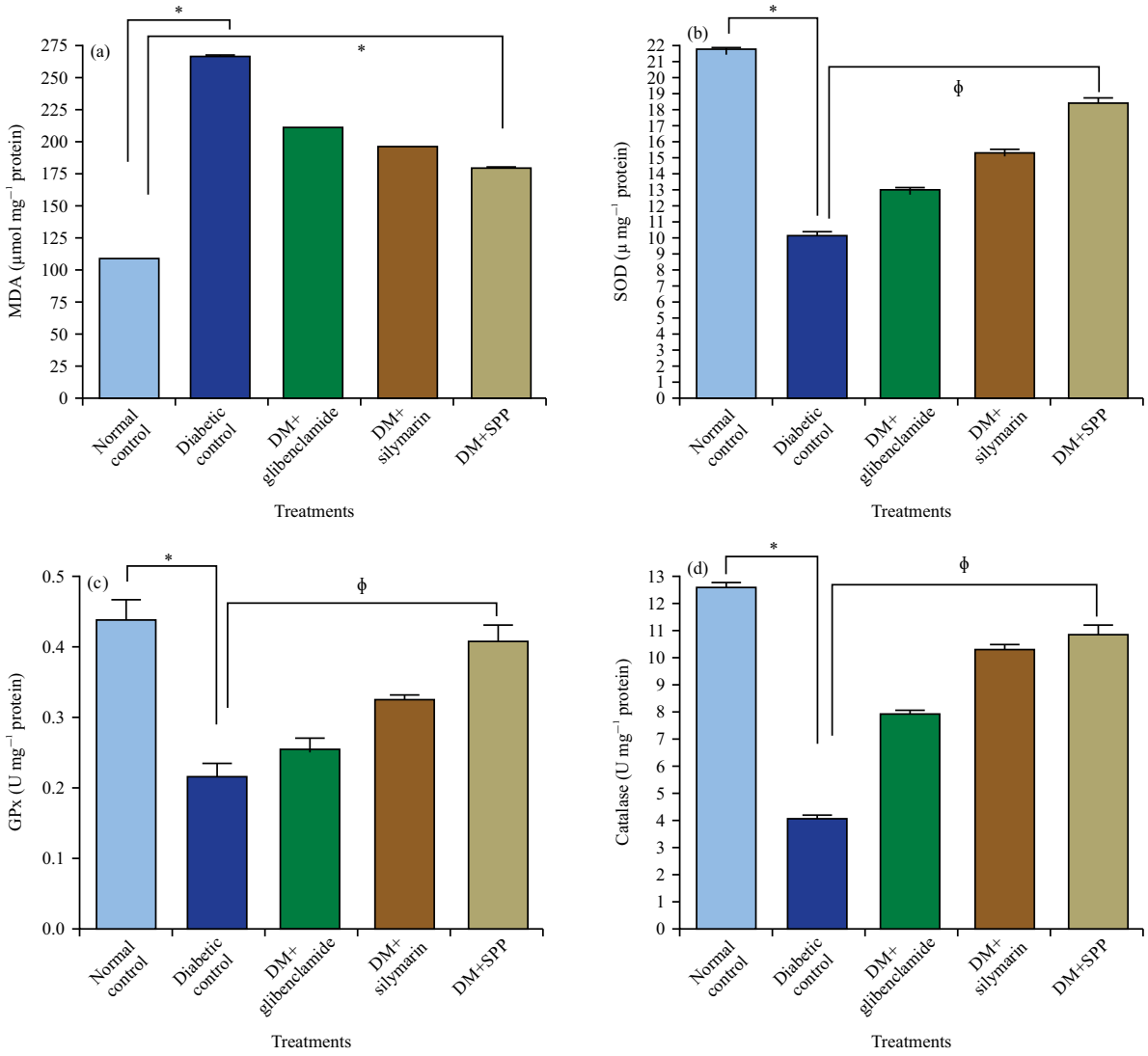

Fig. 2(a-d): Antioxidant enzymes in different groups (N = 6), (a) MDA, (b) SOD, (c) GPx and (d) Catalase

Data are expressed as Mean±SD, GPx, MDA, SOD and CAT, *p<0.05: Treatment compared with NC and ϕp<0.05: Treatment compared with DM control

hepatocellular damage[28]. Firstly, the oxidative stress induced by diabetes leads to ROS increase and fat accumulation in the liver. Therefore, the oxidative stress results in increasing of lipid peroxidation and inflammatory cytokines, followed by consequences of inflammation, liver necrosis and finally fibrosis[29].

The antioxidant enzymes such as SOD, GPx and catalase that work as antioxidative defence systems showed low activities in different organ tissues during diabetes[30] and this may be due to the increased production of hydrogen peroxide and superoxide via auto-oxidation of the excessive amounts of glucose and glycation of proteins non-enzymatically[31]. These antioxidant enzymes activities were decreased in diabetic group rats, while administration with SPP increased its activities, therefore, controlling ROS production[31]. Moreover, the high levels of serum ALT, AST and ALP in diabetic rats, indicate a deficiency of liver functions and tissue damage. Treatment of the diabetic rats with SPP could inhibit these enzymes activity in comparison with diabetic control. The production of the cytokine in liver tissues depends mainly on the induction of initial response to them[32], IL-6 is one of the cytokines that have a role in hepatic protection through the initiation of the recovery of the liver and protection from damage[33]. The first step in the IL-6 pathway started by IL-6 binding to its receptor (IL-6R) which initiate the activation of the STAT3 pathway by binding to the glycoprotein 130

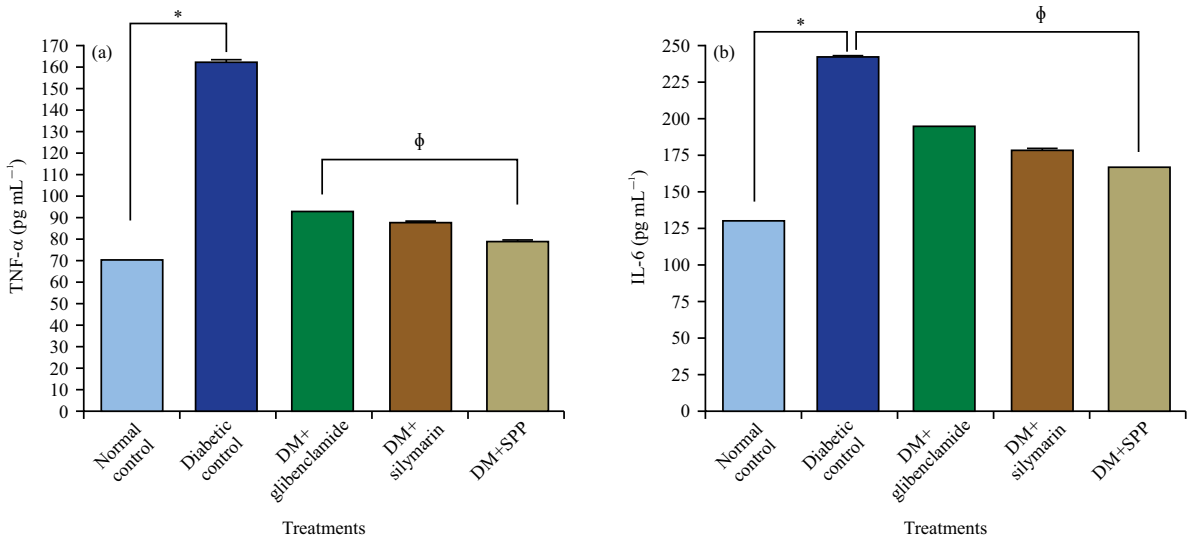

Fig. 3(a-b): Cytokines (IL-6 and TNF-α) levels in different groups (N = 6), (a) TNF-α and (b) IL-6
Data are expressed as Mean±SD, *p<0.05: Treatment compared with NC and ᵠp<0.05: Treatment compared with DM control

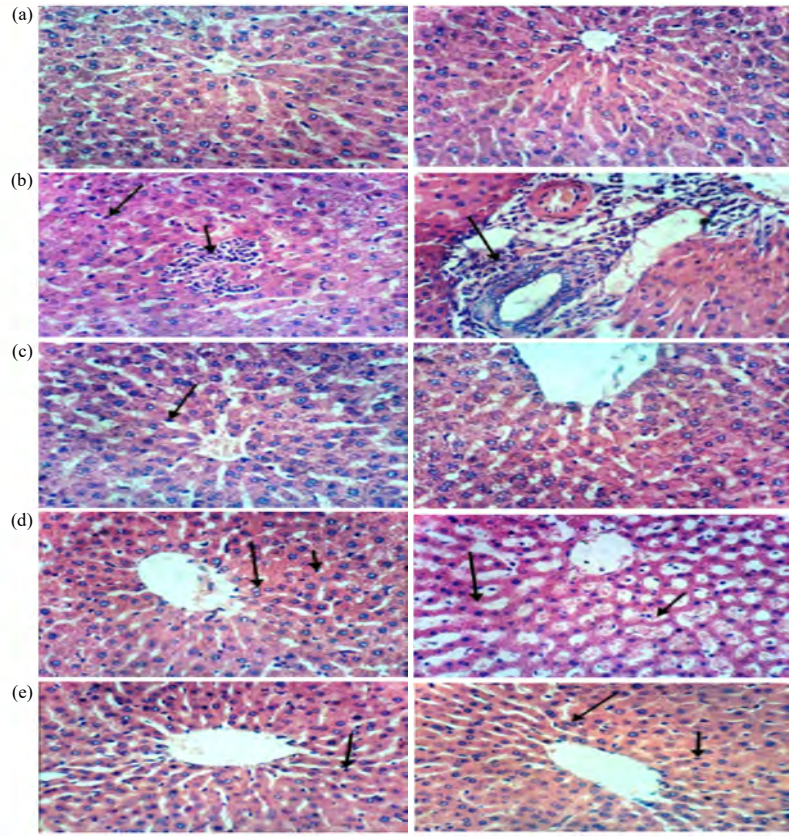

Fig. 4(a-e): Photomicrograph of hematoxylin and eosin-stained section, (a) Liver of normal control rats show the normal, well defined histological structure of hepatic lobule, (b) Diabetic control rats liver show hepatic toxicity signs included focal hepatic necrosis accompanied with inflammatory cells infiltration and Kupffer cells activation, (c) Third group liver treated with glibenclamide showing slightly Kupffer cells activation and binucleation of hepatocytes, (d) Fourth group rats liver treated with silymarin show improvement in the histological structure of hepatic lobule and (e) Fifth group rats liver treated with SPP showing Kupffer cells activation
H&E×400

(gp130). The other pathway is through the IL-6 signal (IL-6 trans-signalling)[34]. Therefore, IL-6 may regulate liver damage by two pathways because it acts as a pro- and anti-inflammatory cytokine. The results indicated, diabetes help in upregulating the expression of IL-6, while treatment with SPP act as the opposite effects by suppression of these changes because it acts as an anti-inflammatory agent. The pro-inflammatory cytokines TNF-α activate different intracellular pathways regarding the regulation of cell inflammation, proliferation and cell death. The TNF-α is a hepatotoxicity mediator and contributes to the restoration of functioning liver tissues by encouraging hepatocyte proliferation and liver tissues regeneration.

Our results showed that diabetes contributes to lowering the concentration of the antioxidant enzyme in liver tissues and increased the malondialdehyde levels, lipid profile, liver function enzymes and the pro-inflammatory markers TNF-α and IL-6. While, at oral administration with SPP (50 mg kg^{-1} b.wt.), effects on lowering malondialdehyde levels, lipid parameters, liver functional enzymes, IL-6 and TNF-α ($p<0.05$). The previous study stated, the phenolic compounds in *Spirulina* including salicylic, caffeic, chlorogenic, quimic and synaptic trans-cinnamic act as antioxidants individually or synergistically[35], also, other study reported, cinnamic acid inhibit diabetic complications by inhibiting the tyrosine phosphatases-1β gene[35]. Moreover, Naringenin found in *Spirulina* has also inhibitory effects on the pro-inflammatory cytokine (IL-6, IL-8, IL-1β and TNF-α) response activated by lipopolysaccharide in the whole blood[36]. From the results, we conclude that the serum levels of AST, ALT, ALP, TG, TC and MDA were downregulated, while those of SOD, CAT and GSH-Px were upregulated. The SPP also reduced IL-6 and TNF-α levels, in diabetic rats.

CONCLUSION

The SPP can be capable of reducing diabetic-induced liver oxidative injury through its anti-inflammatory and antioxidative effects. Although, its antidiabetogenic action requires further investigation, our results suggested that the use of SPP as a supplement may be considered as a promising therapeutic molecule for the protection or early treatment of diabetic disorders.

SIGNIFICANCE STATEMENT

The study presents important information about the role of phenolic compounds extracted from *Spirulina platensis* microalgae as a protective agent against oxidative stress and liver damage in diabetic rats, also its role against the pro-inflammatory markers TNF-α and IL-6, by the authors (Gamal AG, Salwa M, Khalid MA, Vidya DS and Nahla MH). Therefore, this paper is significant.

REFERENCES

1. Zahratunnisa, N., B. Elya and A. Noviani, 2017. Inhibition of alpha-glucosidase and antioxidant test of stem bark extracts of *Garcinia fruticosa* lauterb. Pharmacogn. J., 9: 273-275.
2. Gembillo, G., Y. Ingrasciotta, S. Crisafulli, N. Luxi, R. Siligato, D. Santoro and G. Trifirò, 2021. Kidney disease in diabetic patients: From pathophysiology to pharmacological aspects with a focus on therapeutic inertia. Int. J. Mol. Sci., Vol. 22. 10.3390/ijms22094824.
3. Muthuraman, P., R. Senthilkumar and K. Srikumar, 2009. Alterations in beta-islets of Langerhans in alloxan-induced diabetic rats by marine *Spirulina platensis*. J. Enz. Inhib. Med. Chem., 24: 1253-1256.
4. Dewi, R.T. and F. Maryani, 2015. Antioxidant and α-glucosidase inhibitory compounds of *Centella asiatica*. Procedia Chem., 17: 147-152.
5. Shahid, R.K., S. Ahmed, D. Le and S. Yadav, 2021. Diabetes and cancer: Risk, challenges, management and outcomes. Cancers, Vol. 13. 10.3390/cancers13225735.
6. Mykhalchyshyn, G., N. Kobyliak and P. Bodnar, 2015. Diagnostic accuracy of acyl-ghrelin and it association with non-alcoholic fatty liver disease in type 2 diabetic patients. J. Diabetes Metab. Disord., Vol. 14. 10.1186/s40200-015-0170-1.
7. Mantovani, A. and G. Targher, 2017. Type 2 diabetes mellitus and risk of hepatocellular carcinoma: spotlight on nonalcoholic fatty liver disease. Ann. Transl. Med., Vol. 5. 10.21037/atm.2017.04.41.
8. Dharmalingam, M. and P. Yamasandhi, 2018. Nonalcoholic fatty liver disease and type 2 diabetes mellitus. Indian J. Endocrinol. Metab., 22: 421-428.
9. Lai, W.Y., J.W. Wang, B.T. Huang, E.P.Y. Lin and P.C. Yang, 2019. A novel TNF-α-targeting aptamer for TNF-α-mediated acute lung injury and acute liver failure. Theranostics, 9: 1741-1751.
10. Varfolomeev, E. and D. Vucic, 2018. Intracellular regulation of TNF activity in health and disease. Cytokine, 101: 26-32.
11. Wroblewski, R., M. Armaka, V. Kondylis, M. Pasparakis and H. Walczak *et al*., 2016. Opposing role of tumor necrosis factor receptor 1 signaling in T cell–mediated hepatitis and bacterial infection in mice. Hepatology, 64: 508-521.
12. Chiu, H., C.R. Gardner, D.M. Dambach, S.K. Durham, J.A. Brittingham, J.D. Laskin and D.L. Laskin, 2003. Role of tumor necrosis factor receptor 1 (p55) in hepatocyte proliferation during acetaminophen-induced toxicity in mice. Toxicol. Appl. Pharmacol., 193: 218-227.

13. Grivennikov, S.I., A.V. Tumanov, D.J. Liepinsh, A.A. Kruglov and B.I. Marakusha *et al*., 2005. Distinct and nonredundant *in vivo* functions of TNF produced by T cells and macrophages/neutrophils: Protective and deleterious effects. Immunity, 22: 93-104.
14. Rose-John, S., 2017. Interleukin-6 family cytokines. Cold Spring Harbor Perspect. Biol., Vol. 10. 10.1101/cshperspect.a028415.
15. Garbers, C. and J. Scheller, 2013. Interleukin-6 and interleukin-11: Same same but different. Bio. Chem., 394: 1145-1161.
16. Schmidt-Arras, D. and S. Rose-John, 2016. IL-6 pathway in the liver: From physiopathology to therapy. J. Hepatol., 64: 1403-1415.
17. Vinayagam, R. and B. Xu, 2015. Antidiabetic properties of dietary flavonoids: A cellular mechanism review. Nutr. Metabol., Vol. 12. 10.1186/s12986-015-0057-7
18. Aissaoui, O., M. Amiali, N. Bouzid, K. Belkacemi and A. Bitam, 2017. Effect of *Spirulina platensis* ingestion on the abnormal biochemical and oxidative stress parameters in the pancreas and liver of alloxan-induced diabetic rats. Pharm. Biol., 55: 1304-1312.
19. Zhou, Z.P., L.N. Liu, X.L. Chen, J.X. Wang, M. Chen, Y.Z. Zhang and B.C. Zhou, 2005. Factors that effect antioxidant activity of c-phycocyanins from *Spirulina platensis*. J. Food Biochem., 29: 313-322.
20. Oliveira, E.G., G.S. Rosa, M.A. Moraes and L.A.A. Pinto, 2009. Characterization of thin layer drying of *Spirulina platensis* utilizing perpendicular air flow. Bioresour. Technol., 100: 1297-1303.
21. Nege, A.S., E.D. Masithah and J. Khotib, 2020. Trends in the uses of *Spirulina microalga*: A mini-review. Sci. J. Fish. Mar., 12: 149-166.
22. Huang, Z., B.J. Guo, R.N.S. Wong and Y. Jiang, 2007. Characterization and antioxidant activity of selenium-containing phycocyanin isolated from *Spirulina platensis*. Food Chem., 100: 1137-1143.
23. Etuk, E.U., 2010. Animals models for studying diabetes mellitus. Agric. Biol. J. North Am., 1: 130-134.
24. Gabr, G.A., S.M. El-Sayed and M.S. Hikal, 2020. Antioxidant activities of phycocyanin: A bioactive compound from *Spirulina platensis*. J. Pharm. Res. Int., 32: 73-85.
25. Hamad, A.M. and H.G. Ahmed, 2016. Association of connective tissue fibers with estrogen expression in breast lesions among sudanese females. Int. Clin. Pathol. J., 2: 97-102.
26. Tacke, F., T. Luedde and C. Trautwein, 2009. Inflammatory pathways in liver homeostasis and liver injury. Clin. Rev. Allergy Immunol., 36: 4-12.
27. Zhang, J.M. and J. An, 2007. Cytokines, inflammation and pain. Int. Anesthesiol. Clin., 45: 27-37.
28. Koyuturk, M., O. Sacan, S. Karabulut, N. Turk, S. Bolkent, R. Yanardag and S. Bolkent, 2015. The role of ghrelin on apoptosis, cell proliferation and oxidant-antioxidant system in the liver of neonatal diabetic rats. Cell Biol. Int., 39: 834-841.
29. Pomacu, M., M. Traşcă, V. Pădureanu, A. Bugă and A. Andrei *et al*., 2021. Interrelation of inflammation and oxidative stress in liver cirrhosis. Exp. Ther. Med., Vol. 21. 10.3892/etm.2021.10034.
30. Karthivashan, G., M.T. Fard, P. Arulselvan, F. Abas and S. Fakurazi, 2013. Identification of bioactive candidate compounds responsible for oxidative challenge from hydro-ethanolic extract of *Moringa oleifera* leaves. J. Food Sci., 78: C1368-C1375.
31. Colla, L.M., C.O. Reinehr, C. Reichert and J.A.V. Costa, 2007. Production of biomass and nutraceutical compounds by *Spirulina platensis* under different temperature and nitrogen regimes. Bioresour. Technol., 98: 1489-1493.
32. Adisakwattana, S., J. Pongsuwan, C. Wungcharoen and S. Yibchok-anun, 2013. *In vitro* effects of cinnamic acid derivatives on protein tyrosine phosphatase 1B. J. Enzyme Inhib. Medic. Chem., 28: 1067-1072.
33. Ramadori, G. and T. Armbrust, 2001. Cytokines in the liver. Eur. J. Gastroenterol. Hepatol., 13: 777-784.
34. Saile, B., C. Eisenbach, H. El-Armouche, K. Neubauer and G. Ramadori, 2003. Antiapoptotic effect of interferon-α on hepatic stellate cells (HSC): A novel pathway of IFN-α signal transduction via janus kinase 2 (JAK2) and caspase-8. Eur. J. Cell Biol., 82: 31-41.
35. Wolf, J., S. Rose-John and C. Garbers, 2014. Interleukin-6 and its receptors: A highly regulated and dynamic system. Cytokine, 70: 11-20.
36. Bodet, C., V.D. La, F. Epifano and D. Grenier, 2008. Naringenin has anti inflammatory properties in macrophage and *ex vivo* human whole blood models. J. Periodontal Res., 43: 400-407.