

# Role of *Spirulina platensis* in the control of glycemia in DM2 rats

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**Abstract**—It is well documented that oxidative stress is a basic mechanism behind the development of diabetic state. The current study was undertaken to elucidate the hypoglycemic role of *Spirulina platensis* in comparison with the antidiabetic drug glibenclamide. Male Wistar rats weighing  $250 \pm 50$  g were made diabetic by injection with a single i.p. dose of streptozotocin (STZ) (60 mg/kg body weight). Serum glucose level significantly elevated ( $> 300$ ,  $p \leq 0.05$ ). Diabetic group was orally administered for 45 days with crude extract of *Spirulina platensis* (15mg/kg). Another group was orally treated daily with glibenclamide drug (5 mg/kg) for 45 days. Blood and tissue samples were collected at day 3 post STZ injection (from diabetic group), and at day 45 post-treatment in other groups. Liver function enzymes, blood glucose level, lipid profile, nitric oxide (NO), malondialdehyde (MDA), phosphoenol pyruvate carboxykinase (PEPCK) were significantly increased, while superoxide dismutase (SOD), glutathione (GSH), total protein, lactate dehydrogenase (LDH), pyruvate kinase (PK) and hexokinase (HK) were inhibited after STZ treatment. Kidney function, showed significant elevation in total urea and creatinine in STZ diabetic rats, while significant amelioration in *Spirulina platensis* treated diabetic rats. Histological examination of diabetic liver and kidney showed degenerative changes of hepatocytes. Proliferation of mesangial cells and matrix of glomeruli, thickened arteriole and Tubular dilatation of nephrocytes. Treatment of diabetic rats with ethanol extract of *Spirulina platensis* blunted the increment in serum glucose induced by STZ, preserved liver architecture and ameliorated all the previous mentioned biochemical parameters. It could be concluded that, the oral administration of ethanol extract of *Spirulina platensis* in STZ diabetic rats clearly indicated the beneficial effects of it in controlling hyperglycemia.

**Keywords:** Blood glucose, carbohydrate metabolizing enzymes, lipid profile, STZ, diabetes mellitus, *Spirulina platensis*.

## 1 INTRODUCTION

**D**iabetes mellitus (DM) is a serious health problem being the third greatest cause of death all over the world, and if not treated, it is responsible for many complications affecting various organs in the body. *Diabetes mellitus* is a disease results from abnormality of carbohydrate metabolism and characterized by absolute (type 1) or relative (type 2) deficiencies in insulin secretion or receptor insensitivity to endogenous insulin, resulting in hyperglycemia. Hyperglycemia that is initiating from unregulated glucose level is widely recognized as the causal link between diabetes and diabetic complications. It was found that, hyperglycemia cause tissue damage by mechanisms involving repeated changes in cellular metabolism. One of the key metabolic pathways as being major contributors to hyperglycemia induced cell damage, is the non enzymatic reaction between excess glucose and several proteins (as hemoglobin and albumin) to form advanced glycosylated end product (AGE). Production of AGE interferes with cell integrity by modifying protein function or by inducing receptors mediated production of reactive oxygen species (ROS) (1).

Various agents have been utilized in an attempt to prevent or

delay the onset of type 1 diabetes in prediabetic patients or animals. In addition to immunosuppressive therapy, an early prophylactic treatment of diabetic rats with insulin has been shown to delay the onset of diabetes while preserving the structure and function of the pancreas (2). The mechanism by which exogenous insulin exerts its beneficial effect in preventing the onset of diabetes is believed to be due to a feedback inhibition of pancreatic insulin secretion. This "resting" of the pancreatic beta cells may slow their destruction and help preserve their function. Investigation of the pathophysiology of the secondary complications of diabetes is focusing increasingly on the role of oxidative stress in their initiation and progression. Hence, a large number of studies are in progress to find natural sources, as they effective in reducing the intensity of diabetes (3). *Spirulina platensis*, is a blue green microalga, has been used since ancient times as a source of food because of its high nutritional value (4). The cyanobacterium *Spirulina platensis* is rich in nutrients, such as proteins, vitamins, minerals, carbohydrates, and  $\gamma$ -linolenic acid. It is gaining more and more attention, not only for the foods aspects but also for the development of potential pharmaceuticals (5). This alga is being widely studied, not only for nutritional reasons but also for its reported medicinal properties; thus, several studies have shown that *Spirulina* or its extracts could prevent or inhibit cancer in humans and animals, and recent works have indicated that this species has immuno-promoting effects (6). *Spirulina* contains a whole spectrum of natural mixed carotene and xanthophyll phytopigments which, together with phy-

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cocyanin, seem to be related to its antioxidant activity (7). It was reported that *Spirulina platensis* could be used as a matrix for the production of selenium-containing compounds and proved to be successful in transforming inorganic selenium to organic selenium in vivo when cultivated in selenium-rich medium (8). The antioxidant activities of selenium-containing phycocyanin and its different aggregates (monomer, trimer, and hexamer) against free radicals of superoxide, hydrogen peroxide, and 2,2-diphenyl-1-picryl-hydrazyl (DPPH) were found to be variable (9). Furthermore, it was found that *spirulina* showed hypocholesterolemic, hypolycemic and health improvement (10,11), effects which is gaining attention as a nutraceutical and a source of potential pharmaceutical.

The present study is undertaken to evaluate the antidiabetic effect of *spirulina platensis* on streptozotocin induced diabetes in male albino Wistar rats. Liver function enzymes, blood glucose level, lipid profile, NO, MDA, PEPCK, SOD, GSH, total protein, LDH, PK and HK were estimated. Also, kidney markers; total urea and creatinine were determined. In addition, histological examination of diabetic, treated liver and kidney was performed as compared to normal control.

## 2 MATERIALS AND METHODS

### 2.1 CHEMICALS

All chemicals in the present study were of analytical grade, products of Sigma, Merck and Aldrich. All kits were the products of Biosystems (Alcobendas, Madrid, Spain), Sigma Chemical Company (St. Louis, MO, USA), Biodiagnostic Company (Cairo, Egypt).

### 2.2 Microalgae strain

#### Collection of *Spirulina platensis* samples and Preparation for Extraction

The growth of the algal material was in the Fertilization technology Dept., National Research Centre, Dokki-Cairo, Egypt. Method of collection employed the techniques used by Abou-Elela et al. (12).

#### Preparation of *Spirulina platensis* for extraction

After collection, *Spirulina platensis* algae were washed with fresh water to remove salts and debris, air dried. 500 grams of the powdered samples were macerated and added with 2000 ml of 95% ethanol solvent until such time all materials were submerged and allowed to stand for 24 hours with occasional shaking in a dark condition. After 24 hours, it was filtered. The crude residue was soaked again in a fresh ethanol solvent for another 48 hours. The filtrate from the first and second soaking was then mixed together. The combined filtrates were concentrated using rotary evaporator at 50°C. The resulting concentrated extract was further concentrated by the use of a vacuum oven to remove residual solvent (13).

### 2.3 Animals

Male Wistar albino rats (250±50 g) were obtained from animal

house of National Research Centre, Dokki, Giza, Egypt. Rats were fed on a standard diet and free access to tap water. They were kept for one week to be acclimatized to the environmental conditions.

### 2.4 Experimental design

Sixty male albino rats were selected for this study and divided to five groups (ten rats in each group) as follows:

Group 1: normal healthy control rats, Groups 2: normal rats orally treated with 15mg/kg body weight of total extract of *Spirulina platensis*, for 45 days (11). Group 3: considered as diabetic groups; where type 2 diabetes was induced by STZ. Each rat was injected intraperitoneally with a single dose of STZ (60 mg/kg body weight dissolved in 0.01 M citrate buffer immediately before use (14). After injection, animals had free access for food and water and were given 5% glucose solution to drink overnight to encounter hypoglycaemic shock. Animals were checked daily for the presence of glycosuria (15). Animals were considered to be diabetic if glycosuria was present for 3 consecutive days. After 3 days of STZ injection fasting blood samples were obtained and fasting blood sugar was determined (>300 mg/dl). Hyperglycemic rats were used for the experiment and classified as follows:

Group 4: diabetic Rat's oral administered 15 mg/Kg body weight of total ethanol extract of *Spirulina platensis* for 45 Day, Group 5: diabetic rats administered orally antidiabetic glibenclamide reference drug 5 mg /kg body weight daily for 45 days (16)

### 2.5 Sample preparations

After 45 days of treatments, rats were fasted overnight (12-14 hours), anesthetized by diethylether and blood collected by puncture of the sublingual vein in clean and dry test tube, left 10 minutes to clot and centrifuged at 3000 rpm for serum separation. The separated serum was used for biochemical analysis of liver function enzymes, blood glucose level, lipid profile and total protein content. After blood collection, rats of each group were sacrificed, the livers were removed immediately (a part was fixed in 10% formalin for histopathological examination), weighed and homogenized in 5-10 volumes of appropriate medium using electrical homogenizer, centrifuged at 4000 rpm for 15 min, the supernatants were collected and placed in Eppendorff tubes and stored at -20°C and used for determination of oxidative stress markers (nitric oxide: NO and malondialdehyde: MDA), antioxidant (GSH and SOD) and carbohydrate metabolizing enzymes (HK, LDH, pyruvate PK and PEPCK). The homogenization was carried out as described by Newsholme et al. (17).

### 2.6 Blood biochemical analyses

Glucose was determined in serum by colorimetric assay according to Trinder ([18]; Alkaline phosphatase enzyme activity was measured by the method of Belfield and Goldberg(19); AST and ALT were measured by the method of Reitman and Frankel (20); Total protein was assayed in serum according to Bradford(21), lipid profile and kidney markers; total urea

and creatinine were carried out using diagnostic kits.

### 2.7 Liver tissue biochemical analyses

HK was assayed in liver tissue homogenate according to Abrahao-Neto et al. (22). LDH enzyme activity was determined in liver tissue homogenate according to the method of Bergmeyer et al. (23). PK enzyme activity was determined in liver tissue homogenate according to Bucher and Pfeleiderer (24). PEPCK was determined in liver tissue homogenate according to the method of Suarez et al. (25). Lipid peroxidation was determined in tissue liver homogenate according to Ruizlarre et al. (26). NO was determined in liver tissue homogenate according to Moshage et al. (27). GSH was assayed in liver tissue homogenate according to Beutler et al. (28). SOD was assayed in tissue liver homogenate according to Paoletti et al. (29).

### 2.8 Histopathology

Liver and kidney specimens were fixed in 10% formalin, processed to paraffin blocks, sectioned (4 μm thick) and stained with hematoxyline and eosin. They were examined using light microscopy for demonstration of pathological changes including degeneration, atrophy, cell destruction and necrosis and the efficiency of micronutrients to ameliorate these pathological features (30).

### 2.9 Statistical Analyses

Data were analyzed by comparing values for different treatment groups with the values for individual controls. Results are expressed as mean ± SD. The significant differences among values were analyzed using analysis of variance (one way Anova) coupled with Co stat Computer Program ,where unshared letters indicate significant correlation at P <0.05.

## 3 RESULTS

The present results demonstrate the biochemical effects of *Spirulina platensis* treatment in comparison with the current available antidiabetic glibenclamide reference drug against liver disorders induced by reactive oxygen species in diabetic model. Table (1) manipulated liver function enzyme activities, blood glucose level and total protein content in normal, STZ and treated groups. Insignificant change was observed in normal rats treated with *Spirulina platensis* extract as compared to normal untreated rats. In STZ group a significant increase was observed in blood glucose level (with percentage increase reached to 225.79%), and in liver function enzyme activities; AST, ALT and ALP with percentage increase 170.75, 185.19 and 110.56%, respectively. However, significant decreases was detected in total protein content amounting to 31.62%, as compared to the normal control. Significant normalization was noticed in blood glucose level, liver enzymes and total protein content in diabetic group treated with *Spirulina platensis* with simultaneous results for glibenclamide-treated diabetic rats.

The data obtained in Table (2) show that, insignificant change in lipid profile, total cholesterol, triglycerides, HDL-cholesterol, LDL-cholesterol and total lipids in normal control treated rats with ethanol extract of *Spirulina platensis*. On the other hand, diabetic rats showed significant increase in lipid profile; total cholesterol, triglycerides, LDL-cholesterol and total lipids with percentages increase amounting to 262.90, 254.36, 1118.09 and 81.35%, respectively. While significant decrease in HDL-cholesterol was observed (76.99%), as compared to the normal control group.

Treatments of diabeticrats with *Spirulina platensis* crude extract significantly reversed these elevation and controlled the reduced LDH- cholesterol level with percentages of improvement recorded 248.58, 291.81, 70.61, 978.22 and 90.43 %, respectively for total cholesterol , triglycerides , HDL-cholesterol , LDL -cholesterol and total lipid .In addition , standard glibenclamide drug declared more or less similar results .

Considering antioxidant markers, insignificant change was observed in all antioxidant parameters post treatment of normal control rats with ethanol extract of *Spirulina platensis*. Significant increase was noticed in NO and MDA post STZ injection with percent of elevation amounting to 76.58 and 550.35%, respectively. However, significant reduction was recorded in GSH and SOD with percentages decrease amounting to 64.41 and 114.27%, respectively. It has been easily noticed that , significant amelioration in NO, MDA, GSH and SOD levels post treatment of diabetic rats with *Spirulina platensis* extract with percentages of amelioration amounting to 59.71,514.89, 49.71 and 56.37%, respectively as compared to antidiabetic standard drug which recorded significant improvement amounting to 61.63 , 525.31,42.94 and 57.79 % , respectively (Table 3).

Table 1: Table 1: Effect of *Spirulina platensis* on blood glucose level, liver function enzymes and total protein content in STZ induced diabetes in rats.

Groups	Normal control	Spirulina Treated normal rats	STZ	STZ +Spirulina	Glibenclamide
Blood glucose level	110.50 ± 3.21 <sup>a</sup>	116.58 ± 4.00 <sup>a</sup>	360.00 ± 5.20 <sup>b</sup>	151.20 ± 2.30 <sup>a</sup>	151.50 ± 2.10 <sup>a</sup>
AST	2.53 ± 0.16 <sup>a</sup>	2.93 ± 0.22 <sup>a</sup>	6.85 ± 0.05 <sup>b</sup>	3.00 ± 0.01 <sup>a</sup>	2.85 ± 0.05 <sup>a</sup>
ALT	1.62 ± 0.038 <sup>a</sup>	1.92 ± 0.05 <sup>a</sup>	4.62 ± 0.08 <sup>b</sup>	1.88 ± 0.26 <sup>a</sup>	1.76 ± 0.06 <sup>a</sup>
ALP	3.41 ± 0.08 <sup>a</sup>	3.61 ± 0.09 <sup>a</sup>	7.18 ± 0.25 <sup>b</sup>	4.29 ± 0.04 <sup>a</sup>	4.03 ± 0.058 <sup>a</sup>
Total protein	117.60 ± 2.0 <sup>a</sup>	119.60 ± 3.22 <sup>a</sup>	80.42 ± 0.43 <sup>b</sup>	100.77 ± 2.01 <sup>a</sup>	107.00 ± 2.64 <sup>a</sup>

Data are means ± SD of ten rats in each group. Liver function enzyme activities are expressed in μ mole/ml and blood glucose level in mg /dL. Total protein is expressed in mg/ml. Statistical analysis is carried out using Costat computer program coupled with post-hoc (least significance difference LSD).Unshared letters indicate significant correlation at P <0.05.

**Table 2: Effect of *Spirulina platensis* on lipid profile in STZ induced diabetes in rats.**

Groups Parameters	Normal control	Spirulina Treated normal rats	STZ	STZ +Spirulina	Glibenclamide
TC	49.60±10.95 <sup>a</sup>	53.03±5.45 <sup>a</sup>	180.00 ± 16.08 <sup>f</sup>	56.7±5.23 <sup>a</sup>	84.69 ± 8.24 <sup>b</sup>
TG	24.72±7.16 <sup>b</sup>	23.29±5.09 <sup>b</sup>	87.60±10.89 <sup>c</sup>	15.45±1.90 <sup>b</sup>	38.18 ± 1.57 <sup>b</sup>
HDL	30.21±2.48 <sup>c</sup>	29.21±3.76 <sup>c</sup>	6.95±2.27 <sup>h</sup>	28.28±1.97 <sup>c</sup>	18.82±5.62 <sup>c</sup>
LDL	12.49±9.24 <sup>d</sup>	13.07±2.69 <sup>d</sup>	152.14±11.56 <sup>i</sup>	29.96±13.05 <sup>d</sup>	58.23±4.65 <sup>d</sup>
Total lipids	1100.00±52.6 <sup>e</sup>	1105.26±52.5 <sup>e</sup>	1994.9±109.55 <sup>j</sup>	1000.2±60.79 <sup>e</sup>	1421.00±52.65 <sup>e</sup>

Data are means ± SD of ten rats in each group. Lipid profile parameters are expressed in ug /dl except HDL which is expressed in mg/dl. Statistical analysis is carried out using Costat computer program coupled with post-hoc (least significance difference LSD). Unshared letters indicate significant correlation at P <0.05.

**Table 3: Effect of *Spirulina platensis* on NO, MDA,GSH levels and antioxidant enzyme(SOD) in STZ induced diabetes in rats.**

Groups Parameters	Normal control	Spirulina Treated normal rats	STZ	STZ +Spirulina	Glibenclamide
NO	43.68 ± 0.71 <sup>a</sup>	44.55 ± 2.11 <sup>a</sup>	77.13 ± 1.00 <sup>b</sup>	51.05 ± 3.21 <sup>a</sup>	50.21 ± 1.91 <sup>a</sup>
MDA	18.53 ± 1.19 <sup>a</sup>	18.53 ± 1.19 <sup>a</sup>	120.51 ± 1.47 <sup>b</sup>	25.10 ± 1.53 <sup>a</sup>	23.17 ± 2.63 <sup>a</sup>
GSH	3.40 ± 0.30 <sup>a</sup>	3.93 ± 0.14 <sup>a</sup>	1.21 ± 0.03 <sup>b</sup>	2.90 ± 0.34 <sup>a</sup>	2.67 ± 0.58 <sup>a</sup>
SOD	9.88 ± 0.67 <sup>a</sup>	8.98 ± 0.67 <sup>a</sup>	1.41 ± 0.16 <sup>b</sup>	6.98 ± 1.07 <sup>a</sup>	7.12 ± 1.3 <sup>a</sup>

Data are means ± SD of ten rats in each group. MDA is expressed in μmole/min/g tissue. GSH and SOD are expressed in μ mole/mg protein/min. NO is expressed in μg/g tissue. Statistical analysis is carried out using one way analysis of variance (ANOVA) using Costat computer program. Unshared letters indicate significant correlation at P <0.05.

**Table 4: Effect of *Spirulina platensis* crude extract on some glucoytic and gluconeogenic enzymes in STZ induced diabetes in rats**

Groups Parameters	Normal control	Spirulina Treated normal	STZ	STZ +Spirulina	Glibenclamide
HK	105.12 ± 1.67 <sup>a</sup>	112.10 ± 2.07 <sup>a</sup>	21.19 ± 1.69 <sup>b</sup>	57.18 ± 0.95 <sup>c</sup>	86.99 ± 4.89 <sup>d</sup>
PK	62.60 ± 2.45 <sup>a</sup>	65.69 ± 3.41 <sup>a</sup>	20.43 ± 1.17 <sup>b</sup>	47.47 ± 2.03 <sup>c</sup>	54.10 ± 1.15 <sup>d</sup>
LDH	39.27 ± 0.80 <sup>a</sup>	39.27 ± 0.80 <sup>a</sup>	14.41 ± 0.62 <sup>b</sup>	29.52 ± 0.90 <sup>c</sup>	33.47 ± 1.48 <sup>d</sup>
PEPCK	3.40 ± 0.30 <sup>a</sup>	3.93 ± 0.14 <sup>a</sup>	1.21 ± 0.03 <sup>b</sup>	2.90 ± 0.34 <sup>c</sup>	2.67 ± 0.58 <sup>d</sup>

Data are means ± SD of ten rats in each group. HK, PK, LDH and PEPCK are expressed in μ mole/mg protein min. Statistical analysis is carried out using Costat computer program coupled with post-hoc (least significance difference LSD). Unshared letters indicate significant correlation at P <0.05.

**Table 5 : Effect of *Spirulina platensis* on total urea and creatinine in STZ induced diabetes in rats.**

Groups Parameters	Normal control	Spirulina Treated normal	STZ	STZ +Spirulina	Glibenclamide
Total urea	32.00 ± 4.1 <sup>a</sup>	34.00 ± 3.80 <sup>a</sup>	89.00 ± 2.8 <sup>b</sup>	31.00 ± 1.97 <sup>a</sup>	33.00 ± 1.9 <sup>a</sup>
Creatinine	0.80±0.01 <sup>a</sup>	0.85 ±0.10 <sup>a</sup>	3.90 ± 0.20 <sup>b</sup>	0.96±0.15 <sup>a</sup>	0.90± 0.05 <sup>a</sup>

Data are means ± SD of ten rat in each group. Total urea and creatinine are expressed in mg/dl, tissue. Statistical analysis is carried out using one way analysis of variance (ANOVA) using Costat computer program unshared letters indicate significant correlation at P <0.05.

With respect to glucolytic and gluconeogenic enzymes, insignificant change was observed in all enzymes level resulting from treatment of the normal control rats with *Spirulina platensis*. However in diabetic group, significant inhibition in HK, PK, LDH enzyme activities (with percentages of inhibition reached to 79.84, 67.36, 63.30 %, respectively accompanied with significant increase in PEPCK (64.41%) (Table 4). Significant enhancement was noticed in all demonstrated enzymes in treated-diabetic groups either with the ethanol extract of *Spirulina platensis* (34.23, 43.19 , 38.48 and 49.71 %, respectively ), or standard drug (62.59, 53.78 , 48.54 and 42.94% , respectively), as compared to the normal control.

Regarding to, total urea and creatinine , insignificant change was noticed in their levels in normal treated rats with total ethanol extract of *Spirulina platensis* as compared to normal control group. Diabetic rats showed significant elevation in total urea and creatinine levels with percentages increase amounting to 178.13 and 387.8%, respectively, as compared to normal control group. Treatment of STZ diabetic rats with *Spirulina platensis* restored the elevated levels with percentage of improvement reached to 181.25 and 367.5 %, for total urea and creatinine respectively (Table 5). Similar results were obtained for reference drug.

Histopathological examination showed that normal structure of liver and kidney in both normal control rats and normal rats treated with crude extract of *Spirulina platensis* (Figs. 1 and 2).Diabetic liver exhibited foci of inflammatory cells in between hepatocytes and surrounding a central vein, necrosis and degenerative changes of hepatocytes (Fig. 3).

Treatment with *Spirulina platensis* total ethanol extract and glibenclamide drug showing similarly ordinary hepatic strands radiating from central vein, normal hepatocytes (some hepatocytes are slightly vacuolated) with normal nuclei and sinusoids (Fig. 4). On the other hand, histopathological examination of kidney in STZ diabetic rats showed proliferation of mesangial cells and matrix of glumeroli , thickened arteriole and Tubular dilatation (Figure 5), while treatment of diabetic rats with total extract of *Spirulina platensis* showed normal mesangial cells and matrix of glumeroli , focal thickened arteriole and mild tubular dilatation (blue arrow) (Fig. 6)

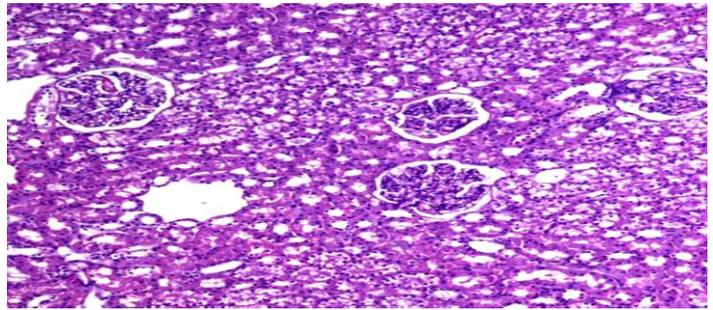


Fig 2: Normal kidney section showing normal orientation of nephrons with adequate glomeruli and well-spaced tubules (H&E,x100)

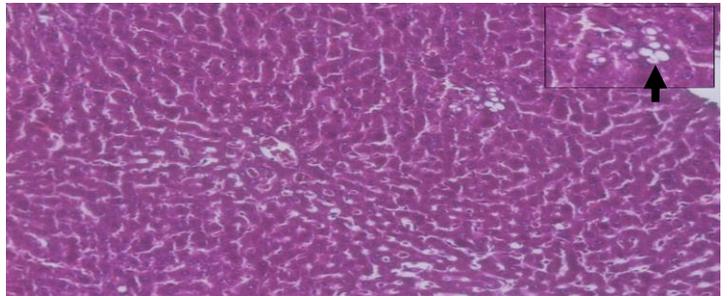


Fig 3. Liver section of STZ diabetic rats showing vacuolar degeneration of hepatocytes (steatosis) (black arrow)(H&E, x200).

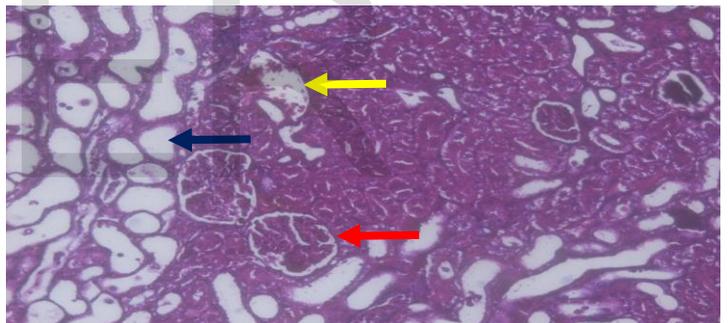


Fig 4: Kidney section of STZ diabetic rats showing proliferation of mesangial cells and matrix of glumeroli (red arrow); thickened arteriole (yellow arrow); Tubular dilatation (blue arrow) (H&E,x200).

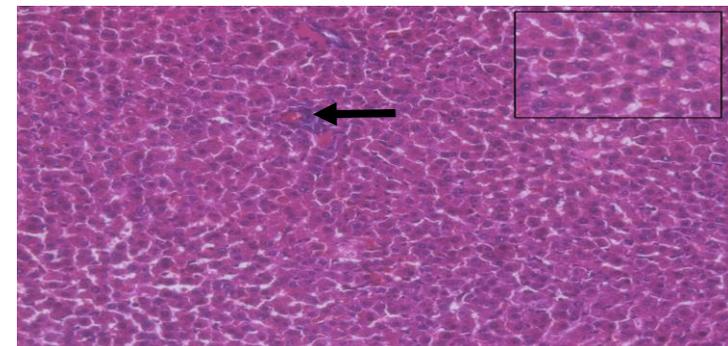


Fig 5: liver section of STZ diabetic rats treated with *Spirulina platensis* crude extract, showing normal hepatic lobular architecture, with scattered inflammatory cells (black arrow) (H&E,x100).

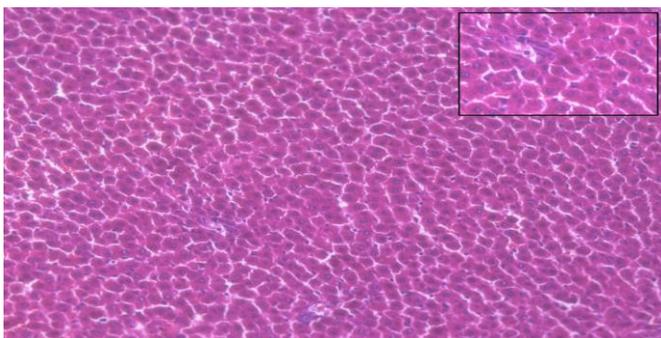


Fig 1: Normal liver section showing normal hepatic lobular architecture (H&E,x200)

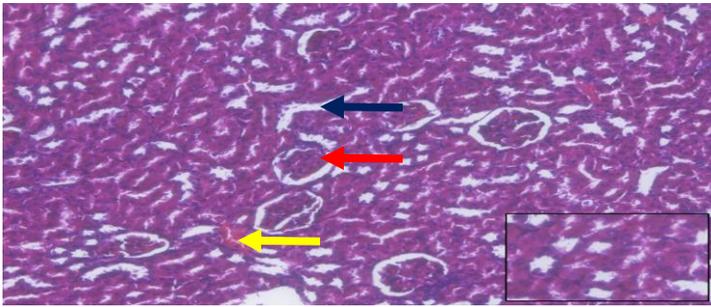


Fig 6: kidney section of STZ diabetic rats treated with *Spirulina platensis* crude extract showing: normal mesangial cells and matrix of glomeroli (red arrow); focal thickened arteriole (yellow arrow) and mild tubular dilatation (blue arrow) (H&E, x200).

#### 4 Discussion

Oxidative stress has been found to play an important role in the pathogenesis of diabetes. In turn, the generation of reactive oxygen species (ROS), has been shown to play an integral and possibly a causative part in the pathogenesis of diabetic retinopathy (31). This hypothesis is supported by evidence that many biochemical pathways strictly associated with hyperglycemia (glucose autooxidation, polyol pathway, protein glycation) are initiated and augmented under oxidative stress. Furthermore, exposure of endothelial cells to high glucose (as indicated in the present results), leads to augmented production of superoxide anions, which may quench nitric oxide, a potent endothelium-derived vasodilator that participates in the general homeostasis of the vasculature (16). In further support of the consequential injurious role of oxidative stress, is the finding that many of the adverse effects of high glucose on endothelial functions are reversed by antioxidants (16). Moreover, antioxidant therapy may be a suitable approach for halting the intrinsic changes within liver and retinal capillary bed that lead to the development of diabetic liver fibrosis and retinopathy.

The present histological examination at the cellular level reveal foci of inflammatory cells in between hepatocytes and surrounding a central vein, necrosis and degenerative changes in hepatocytes of rats indicating establishment of diabetic state (Fig. 3). Holemans et al. (32) stated that, as a result of streptozotocin action, beta cells undergo destruction by necrosis. STZ is widely used for inducing type 2 diabetes in a variety of animals. It selectively induces degenerative alterations and necrosis of pancreatic beta-cells resulting in, insulin deficiency and impairment in glucose oxidation. The use of lower dose of streptozotocin (40 mg/kg b.wt.) produced an incomplete destruction of pancreatic beta cells even though rats became permanently diabetic (31). In accordance to the present study, Mitra et al. (33), earlier reported that, the diabetic liver showed degeneration and congestion after injection of STZ. Hyperglycemia is observed with a concomitant drop in blood insulin followed by hypoglycemia about six hours due to decrease in insulin levels. Akbarzadeh et al. (34), confirmed the destruction of islet cells in pancreatic biopsy of diabetic rats due to the effect of streptozotocin and added that 60 mg/kg dose of STZ

ensured induction of diabetes in rats and hyperglycemia. The present results demonstrate significant elevation in liver function markers associated with significant reduction in total protein content as compared to the normal control group. The high serum levels of these enzymes post STZ treatment are associated with inflammation and/or injury to liver cells, a condition known as hepatocellular liver injury and apoptosis. In parallel with the present work, previous reports revealed significant increased activities of serum enzymes relative to their normal levels (35). Supporting our findings, it has been found that hyperglycemia resulted in hepatolysis reflected by increased blood serum aminotransferases as one of the consequences of diabetic complication. The increment of such serum markers may be due to the leakage of these enzymes from the liver cytosol into the blood stream as a result of hepatomegaly (fatty liver) (35). The significant reduction in total protein content in diabetic rats is in concomitant with the results of Otsuki and Williams (36), who found significant reduction in serum total protein concentrations in diabetic rats and this may be due to reduction in the three major phases in protein secretion, intracellular transport and discharge. Alderson et al. (37) explained the reduction in total protein due to significant increase in protein excretion. Mendez et al. (38), reported that non-enzymatic glycation of albumin was the potential to alter its biological structure and function. It is mainly due to the formation of a Schiff base between amino-group of lysine (and sometimes arginine) residues and excess glucose molecules in blood to form glycoalbumin. Hypoalbuminemia is one of the factors responsible for the onset of ascites related to liver fibrosis (39).

In addition to, abnormal glucose metabolism, Diabetes often involves abnormal lipid metabolism which is considered as additional metabolic disorder, in diabetic complications. The same results were achieved by Sethi et al. (40), who found significant elevation in lipid profile in serum of diabetic rats. In a good agreement with the present data, Jurgonski et al. (41), revealed that hyperglycemia produced marked increase in the serum level of triglycerides, total cholesterol, LDL-cholesterol, while HDL-cholesterol showed reduced concentration in diabetic rats. It was reported that, hepatic fat accumulation is a well-recognized complication of DM. The most common clinical presentation in DM is hepatomegaly. This hyperlipidemia associated with DM may be attributed to insulin deficiency and elevated cortisol level, which has an important role in the process of fat accumulation (42). Under normal circumstances insulin activates lipoprotein lipase which hydrolyzes triglycerides. Insulin deficiency results in failure to activate the enzyme, thereby causing hypertriglyceridemia (43). On the other hand, in insulin deficiency, the plasma free fatty acids concentration is elevated as a result of increased free fatty acids outflow from fat depots, where the balance of the free fatty acids esterification, triglycerides lipolysis is displaced in favor of lipolysis (43). Also elevated cortisol promotes the liberation of free fatty acids from adipose tissue into blood stream by inducing and maintaining the synthesis of the hormone sensitive lipase, thus increasing free fatty acids level which contributes to cardiovascular risk (44).

The reduction in cardioprotective HDL-C means decrease of cholesterol efflux from the tissues, the first step in reverse cholesterol transport from the peripheral tissues to the liver. The antioxidant and antiatherogenic activities of HDL-C are enhanced when its circulating level is increased. LDL-C particles become small and dense which undergo oxidative modification, thus leading to a diabetic complication (45). In addition, Mir et al. (46), reported hypercholesterolemia, hypertriglyceridemia associated with DM and explained these increments at the basis of streptozotocin induced diabetes. There is excess of fatty acids in the serum, which promotes conversion of excess fatty acids into phospholipids and cholesterol in liver. These two substances along with excess triglycerides formed at the same time in liver may be discharged into blood in the form of lipoproteins. In parallel results, Mir et al. (46) found high concentration of total lipid in serum of diabetic rabbits and attributed this elevation mainly to increase mobilization of free fatty acids from the peripheral fat depots, since insulin inhibits the hormone sensitive lipase.

The present results indicate also, significant elevation in oxidative stress markers, NO and lipid peroxidation products (MDA) in liver of diabetic rats. These elevated levels may be due to oxidative stress which is considered as one of the necessary causative factors that link diabetes with the pathogenic complications of several tissues (47). It was reported that NO over production has been linked to a variety of clinical inflammatory diseases (48). Experimental studies suggested that NO may be responsible for the increased liver injury. The direct toxicity of NO is enhanced by reacting with superoxide radicals to give powerful secondary toxic oxidizing species, such as peroxynitrite (ONOO) which is capable of oxidizing cellular structure and causes lipid peroxidation (49), a process leading to membrane damage and considered the proximal cause of cell death. Lipid peroxidation can damage protein, lipid, carbohydrates and nucleic acids, and is one of the risk factor of protein glycation. Oxygen free radicals are implicated as mediators of tissue injury in cardiovascular pathology. Cytotoxic effect of ROS is related to lipid peroxidation and subsequent membrane destruction (50). Oxygen free radicals, in addition to the myocardial damaging effect, may also be responsible for the release of lysosomal or hydrolytic enzymes such as elastase (51). On other hand, the study of Moustafa (52), reported elevated rates of liver lipid peroxidation accompanied with the deterioration in glucose tolerance in GSH-depleted rats. It has been suggested that, in free radicals initiating systems, the deterioration in glucose tolerance is attributed to impaired insulin action (53). Initiating lipid peroxidation by free radicals, in the lipid moiety of the cell membrane was supposed to result in distortion of the structural and functional integrity of the cell membrane or internal cellular components. This would interfere with the ability of insulin to initiate and propagate its normal sequence of actions which may account, at least in part, for STZ-induced hyperglycemia (54). Moreover, the current data show also that, STZ caused a reduction in GSH and SOD levels in the liver of diabetic rats. The decline in the activity of free radical scavenging

enzyme SOD may be due to its inactivation caused by excess ROS production. SOD neutralizes superoxide as it cannot cross lipid membrane producing hydrogen peroxide. Hydrogen peroxide can cross biological membranes. Catalase detoxifies hydrogen peroxide which has the principle role in tissue damage. So, the reduction in SOD may damage the first line of enzymatic defence against superoxide anion and hydrogen peroxide (54). The significant depletion of GSH in liver of diabetic rats indicates damage to the second line of antioxidant defence. This probably further exacerbates oxidative damage by adversely affecting critical GSH related processes such as free-radical scavenging, detoxification of electrophilic compounds, modulation of cellular redox status and thiol-disulphide status of proteins and regulation of cell signalling and repair pathways (56).

Concerning glucoytic (LDH, PK and HK) and gluconeogenic (PEPCK) enzymes, significant decrease in glucoytic enzymes was noticed, while significant increase was recorded in gluconeogenic enzyme in diabetic group as compared to the normal control. In accordance to the present results Sherlock and Dooley (57), found that in diabetic state, degradation of liver glycogen and gluconeogenesis are increased and glycolysis is decreased while glucose utilization is inhibited. Glucose-6-phosphatase increases in the liver, facilitating glucose release into the blood. The opposing enzyme which phosphorylates glucose, i.e hexokinase, is unaffected by insulin and decreases in diabetes. As a result, the liver continues to produce glucose even with severe hyperglycemia. Under this circumstance the normal liver would shut off and deposit glycogen. As the liver plays a central and crucial role in the regulation of carbohydrate metabolism, its normal function is essential for the maintenance of blood glucose levels and of a continued supply to organs that require a glucose energy source. This central role for the liver in glucose homeostasis offers a clue to the pathogenesis of glucose intolerance in liver diseases which is attributed mainly to an impaired insulin action (57).

It was found that, during renal dysfunction or renal damage, the concentration of the metabolites increased in blood that may be due to high activities of xanthine oxidase, lipid peroxidation, and increased triacylglycerol and cholesterol levels (48). Urea is the major nitrogen-containing metabolic product of protein catabolism. Urea is the main product of protein catabolism and creatinine is another product of protein metabolism. The increase in serum urea and creatinine levels in STZ diabetic group indicates impairment in the normal kidney function of the animal, as the mechanism of removing there from the blood might have been affected. It may also be an indication of dysfunction at the glomerular and tubular levels of the kidney, it is well known that, many biochemical and histopathological findings confirmed renal damage in diabetic conditions (58).

In the present study, oral administration of Spirulina could reverse the above mentioned diabetic effects. This may be through potentiating the pancreatic secretion of insulin from islet  $\beta$ -cell or due to elicit the transport of blood glucose to the peripheral tissue. In addition, Spirulina might be increased the

levels of insulin and C-peptide in diabetic rats (5).

The antihyperglycemic effect of Spirulina may be due to the down-regulation of NADPH and NADH, a cofactor in the fat metabolism. The higher activity of glucose-6-phosphatase provides H<sup>+</sup>, which binds with NADP<sup>+</sup> in the form of NADPH and is helpful in the synthesis of fats from carbohydrates. When glycolysis slows down because of cellular activity, the pentose phosphate pathway still remains active in the liver to break down glucose that continuously provides NADPH, which converts acetyl radicals into long chain fatty acid chains (16). Spirulina may be capable of oxidizing NADPH (16). The enhanced hexokinase and other glycolytic enzyme activities (LDH, PK and HK), while suppression in gluconeogenic enzyme PEPCK in Spirulina treated rats suggested a greater uptake of glucose from blood by liver cells. The activities of these enzymes suggested that enhanced lipid metabolism during diabetes is shifted towards carbohydrate metabolism and it enhances the utilization of glucose at peripheral sites. One of the possible actions of Spirulina may be due to its inhibition of endogenous synthesis of lipids. The decreased activity of glucose-6-phosphatase through pentose phosphate shunt results in a high reduced glutathione to oxidized glutathione ratio (GSH/GSSG), which is coupled with conversion of NADPH to NADP (59). Spirulina may produce high NADP<sup>+</sup>, which results in down regulation of lipogenesis and lower risk of the tissues for oxidation stress and high resistance for diabetes (59). Furthermore, the microalga Spirulina, was found to have therapeutic potential in several areas, including the capacity of preventing and decreasing the damages caused by hyperlipidemia and the antioxidant activity because it has proteins (55-70%), sugars (12-25%), essential fatty acids (18%), vitamins, and minerals in its chemical constitution (60).

The mechanism by which Spirulina exerts its action due to it contains carotenoid pigments, especially betacarotene, zeaxanthine, besides phycocyanine (7), phenolic compounds (61) and substances with antioxidant activity. The absence of phycotoxins is an advantage of Spirulina when compared to other cyanobacteria and studies on chronic and subchronic toxicity did not reveal any toxic effect related to its intake (62). Experiments conducted in different animal models demonstrated that a supplementary diet with Spirulina could promote a decrease in plasma and hepatic total cholesterol, LDL, triglycerides, and phospholipids, besides increasing HDL (high-density lipoprotein). In humans, studies have indicated a significant reduction in the total cholesterol, LDL, VLDL (very low-density lipoprotein), and triglycerides, elevation in HDL cholesterol and reduction of atherogenic effect (63). Nagaoka et al. (64), identified the mechanism by which how *Spirulina platensis* reduced hypercholesterolemia and concluded that, phycocyanine protein derived from the phycobiline pigment developed an essential role on such microalga's capacity. Bertolin et al. (60), attributed the antioxidant effect of Spirulina extract to beta carotene, tocopherol and phenolic compounds present in the composition of the microalga. According to Es-

trada et al. (7), the phycocyanine protein extracted from *Spirulina platensis* has the capacity to interact with the reactive species to oxygen generated during the oxidative process through the sequestration of free radicals.

Hepato-renal histopathological examination of diabetic rats showed vacuolar degeneration of hepatocytes (steatosis), proliferation of mesangial cells and matrix of glomeruli, thickened arteriole and Tubular dilatation (65, 66). Treatment of diabetic rats with Spirulina extract revealed hepato-renal normalization characterized by normal hepatic lobular architecture, with small numbers of scattered inflammatory cells, normal mesangial cells and matrix of glomeruli, focal thickened arteriole and mild tubular dilatation. Accordingly, it could be concluded that Spirulina has a beneficial effect on blood glucose, antioxidant markers, liver and kidney function parameters and carbohydrates metabolizing enzyme activities. Also, preserve hepato-renal architectures. Moreover, its hypoglycemic effect in clinical trials could represent a protective mechanism against the development of atherosclerosis and to maintain euglycemia. The results from several studies have indicated that the utilization of Spirulina as a diet supplement constituted a strategy for the prevention of health problems due to the injuries produced by the free radicals (67).

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