EFFICIENCY OF THE ALGAE *SPIRULINA PLATENSIS* AS ANTIDIABETIC AGENT

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ABSTRACT

This study was conducted to define the effect of the green algae *Spirulina platensis* on rats as antidiabetic agent. A comparison between amaryl, a currently available antidiabetic drug and the algae was studied. Some biochemical and immunological parameters were investigated in this study. Also histological and immunohistochemical investigations were performed. Rats were divided into 6 groups. Group1 is considered as naïve animals. Group 2 The diabetic non treated group injected with a single dose (45 mg/kg body weight) of streptozotocin (STZ) for three days. Group 3 The pre-treated group orally administrated algae (15 mg/kg body weight) daily for three weeks. Group 4 orally administered with 15 mg/kg body weight for three weeks then injected with STZ. Group 5 The post –treated group intraperitoneally injected with STZ then treated with algae for 4 weeks. Group 6 injected intrraperitoneally with STZ then treated with the drug amaryl at a dose of 0.15 mg/ kg body weight for 4 weeks. Results showed that oral administration of the present algae modulated the diabetic increase in blood glucose level revealing the antihyperglycemic effect of the used algae. It effectively increased insulin and albumin concentrations and decreased the total cholesterol and triglycerides levels with consequence decrease in lactate dehydrogenase (LDH) activity. Furthermore, it decreased lipid peroxidation product MDA and increased the

activity of the antioxidant enzyme glutathione reductase. Treatment of diabetic animals with amaryl drug improved diabetic induced altertions in most of the above studied markers. The immunological data showed that spirulina had no effect on the mean number of thymocytes. Diabetes affected the mean number of thymocytes as it decreased non significantly compared to the naïve group. this decrease was restored by spirulina either in the pre or post- treated groups. Spirulina showed a neutral effect on the mean number of splenocytes. Small improvement was observed in the splenocytes number in the pre- and post-treated algae groups compared to diabetic non treated group. The IgG level in sera from spirulina pre-treated group showed more improvement levels as compared to control. Post alga-treated group showed decrease in IgG level compared to diabetic non treated group. Microscopic investigations of the pancreas of rats given spirulina before diabetes induction showed the normal structure of the exocrine and endocrine component. The pre-treated spirulina diabetic groups showed an unchanged immunoreactivity for insulin as compared to the control group. On the other hand there was a reduction in the immunoreactivity for insulin in spirulina post-treated groups. A reduction in the immunoreactivety for glucagon was observed in the pre- and post-treated spirulina diabetic group. It could be concluded that the current algae has multi-beneficial actions in controlling diabetes and consequence complications induced in pancreas and liver and may be candidate as natural antidiabetic drug.

KEYWORDS: Diabetes- Streptozotocin- Spirulina- Glucose – Amaryl- Thymocytes-splenocytes- IgG.

INTRODUCTION

Diabetes mellitus (DM) is a prevalent chronic disease in many countries. Changing lifestyles, reduced physical activity and increased obesity contribute to increasing the number of patients with DM.[1] DM is a metabolic disorder characterized by increased fasting and postprandial blood sugar levels.[2] It was resulting from a defect in insulin secretion, insulin action or both. Insulin deficiency in turn leads to chronic hyperglycemia with disturbances of carbohydrates, fat and protein metabolism.[3] Globally, the estimated incidence of diabetes and projection for year 2030, as given by International Diabetes Federation is 350 million.[4] Moreover, Arafa and Amin[5] reported that 8.6 million people in Egypt will be diabetic by year 2030. Type 2 diabetes mellitus is responsible for 90 to 95% of diabetes worldwide.[6]

Although the etiology of this disease is not well defined, viral infection, autoimmune disorder, and environmental factors have been implicated.[7] Increased oxidative stress,

impaired antioxidant defense systems and consequently lipid peroxidation are major participants in the development and progression of DM and its complications.[8] The therapeutic management of DM with minimal side effects remains a clinical challenge and the currently available pharmotherapies for the treatment of DM including oral hypoglycemic agents and insulin injection have a number of limitations, such as adverse effects and high rates of secondary failure of β-cells.[9] Also, they do not restore normal glucose homeostasis and they are accompanied with side effects.[10]

This situation has led to the search for alternative therapies for diabetes from natural products and medicinal plants as these are commonly cheaper, less toxic, with fewer side effects and multi-target actions.[11,12] Even the WHO expert committee on diabetes has recommended that this area warrants further attention.[13]

Spirulina is a microscopic and filamentous cyanobacterium (blue-green alga) that has a long history of use as a food for humans. Spirulina microalgae including, *Spirulina platensis*, *Spirulina maxima*, and *Spirulina fusiformis* are considered as valuable additional food source of some macro and micronutrients including high quality protein, iron, gamma linolenic fatty acid, vitamins, especially vitamin B12, minerals, carotenoides, and phycocyanins. Its safety as food has been established through Toxicological studies.[14,15] Several studies have reported that spirulina can prevent or inhibit cancers in animals [16, 17] *In vitro* and animal studies have suggested that *Spirulina* possesses antiviral effects.[18,19] Spirulina is a powerful stimulant for the immune system by increasing the phagocytic and natural killer activities.[20] spirulina and many other cyanobacteria had been found to exhibit many immune-stimulating and antiviral activities not only *in-vitro* but also in animals and human volunteers. It had been found to activate macrophages, NK cells, T cells, B cells, and to stimulate the production of antibodies and cytokines.[21,22] Moreover, hypocholesterolemic effects have been reported in some animal studies.[23] Anuradha and Vidhya[24] reported that spirulina has antihyperglycemic effect in clinical trails which could represent a protective mechanisms against the development of atherosclerosis and maintain euglycemia. Also, Layam and Reddy [25] reported that spirulina has antidiabetic effect on STZ (45mg/kg body wieght) induced diabetes in male albino Wistar rats. Recently, Jarouliya *et al* estimated[26] the anti-hyperglycemic, anti-hyperlipidaemic and hepatoprotective effects of *Spirulina maxima* in rats fed with excessive fructose diet to develop hyperglycemia and hypertriglyceridaemia as well as oxidative damage.

The present work was undertaken to explore the effect of *Spirulina platensis* on some biochemical, immunological and immunohistochemical parameters in diabetic rats. Amaryl was used as a reference drug.

MATERIALS AND METHODS

Chemicals: All chemicals used were of analytical grade. Kits used for the quantitative determination of different parameters were purchased from Stanbio laboratory, Texas USA and Quinica Clinica Aplicada S.A., Spain.

Streptozotocin was purchased from Sigma Aldrich. Amaryl drug (glimepiride as active ingredient) is a product purchased by Sanofi – Aventis, Egypt.

Immunological reagents were obtained from, Biochrome KG; Berlin, Germany and KPL, Gaithersburg, MD, USA. Immunohistochemical detection kit of insulin and glucagon was purchased from FLEX Denmark.

Spirulina platensis algae were obtained from the culture collection of Texas University, Austin, USA. The algae strain was maintained in Zarrouk's medium [27], a standard synthetic medium prepared by the Plant Biochemistry Department, National Research Center, Cairo, Egypt.

Animals: Adult female Wistar albino rats weighing 150-200g supplied from the animal house of National Research Center, Dokki, Giza, Egypt. Rats were fed a standard diet and free access of tap water. They were kept for two weeks to acclimatize to the enviromental conditions. All the studies were conducted in accordance with the Animal Ethical Committee of the National Research Center under the ethics number (09085).

Induction of diabetes: Diabetes was induced by STZ, each rat was injected intraperitoneally with a single dose of STZ (45 mg/kg b.w) dissolved in 0.01 M citrate buffer immediately before use.[28] The animals were fasted for 16 hrs before STZ injection, after injection, they had free access of food and water and were given 5% glucose solution to drink overnight to counter hypoglycemic shock.[29] After 3 days of STZ injection, blood glucose level was checked using the glucometer. The animals with a blood glucose level of more than 180 mg/dL were considered diabetic and included in the study.

Experimental Design: The rats were divided into 6 groups each of eight rats. Group 1: normal healthy control rats (not received any supplementation). Group2: diabetic group. Group3: normal healthy rats supplemented with spirulina (15 mg/kg body weight according to Layam and Reddy, 2006). Group 4: rats given spirulina (15 mg/kg body weight) for 3 weeks then injected with STZ (prophylactic group). Group 5: rats injected with a single dose of STZ then supplemented with spirulina (15 mg/kg body weight) for 4 weeks (therapeutic group). Group 6: rats treated with amaryl drug (0.15 mg/ kg body weight). At the end of experimental period, the animals were fasted overnight then subjected to mild ether anesthesia and blood samples were collected. The blood was allowed to coagulate and centrifuged at 3000 rpm for 15 minutes at 4°C to separate the serum for biochemical analysis. After blood collection, rats of each group were sacrificed under ether anesthesia and the liver samples were collected, minced and homogenized in ice cold bidistilled water to yield 10% homogenates using a glass homogenizer. The homogenates were centrifuged for 15 minutes at 3000 rpm at 4°C and the supernatants were used for different biochemical tissue analysis. Also, spleen and thymus were used for cellular immunological studies. Also sections of pancreas were fixed in formaldehyde and stained for determination of the destruction in β-cells number after induction of diabetes or restoration of β-cells number after treatment with algae. Immunohistochemical assay of insulin and glucagon in the pancreas islets was performed.

Biochemical assay

Serum analysis: Fasting blood glucose was measured using the glucometer. Insulin was determined using ELISA kit for the quantitative measurement of insulin according to Finlay and Dillard.[30] Total cholesterol was determined according to the method of Stein [31], triglycerides was determined in serum according to Wahlefeld.[32] LDH was measured according to Kachmar and Moss[33], albumin was determined according to Dumas and Biggs[34], ALP was measured According to Babson *et al*,[35] ALT and AST activities were determined according to Reitman and Frankel.[36] The levels IgG of all the experimental animals were detected by ELISA according to Maghraby and Bahgat.[37]

Liver analysis

Preparation of liver homogenates for biochemical assays: One gram liver from each rat was homogenized in 10 ml of distilled water using an electrical homogenizer with a teflon rod and then centrifuged at 3000. Glutathione reductase was assayed kinetically in liver tissue

homogenate according to Erden and Bor.[38] Lipid peroxidation was determined in liver tissue homogenates according to Ruiz-Larrrea.[39] Protein was determined colorimetrically in liver tissue homogenates According to Gornal *et al*.[40]

Detection of the total number of splenocytes and thymocytes using trypan blue

According to Maghraby [41], the spleen and thymus were excised, gently teased in Petri dishes containing Phosphate buffer saline tween-fetal calf serum (PBST-FCS) using glass slides. Splenocytes and thymocytes from individual rat were washed three times with (PBST-FCS) by centrifugation at 1500 g at 4°C for 10 min. The cell pellet was re-suspended in PBST-FCS. Red blood cells were lysed with lysis buffer for 3 min at room temperature. The lymphocytes were washed twice by centrifugation in PBST-FCS. Equal volume (0.1 ml) of whole suspension and trypan blue were mixed and examined under LEITZ microscope using Neubaur haemocytometer. Viable lymphocytes exclude the dye while dead cells appear blue. Viable lymphocytes were counted according to the equation: N X Y 2 X 10^4. Where N: number of viable cell per 16 large squares. Y: the volume of cell suspension.

The number of both thymocytes and splenocytes from individual rat were determined according to Maghraby.[41]

Histopathological examination of pancreas tissues.

Sections of 6 μm thickness were prepared and stained with hematoxylin and eosin as described by Afifi.[42] The stained sections were observed under the light microscope, cytoplasm stained in shades of pink to red and the nuclei gave blue colour.

Immunohistochemical analysis of insulin and glucagon in sections of pancreas

Immunohistochemical analysis of insulin and glucagon in sections of pancreas were detected by using the anti-insulin and anti-glucagon monoclonal antibodies as described by Ha *et al*.[43]

Statistical analysis: Data were analyzed by comparing values for different treatment groups with the values for individual control. All values were expressed as the mean±SE. Significant differences between the groups were statistically analyzed using a one- way analysis of variance (ANOVA). *P* value of 0.05 or less was considered statistically significant. Immunological data were estimated using the Graph pad Instat statistics program.

RESULTS

1- Biochemical results

Table (1) represented that amaryl group and spirulina as post-and pre-treated groups produced a significant decrease in glucose level by (-76.64%, -72.76% and -38.26%) as compared with diabetic non treated group. This decrease was confirmed by a significant increase in the insulin level by (114.74%, 106.50% and 50.29%) for amaryl, spirulina post- and- pre-treated algae groups respectively as compared to diabetic non treated group.

Table (2) represented the different biochemical parameters, (cholesterol, triglycerides, LDH and albumin) measured in normal rat sera supplemented with spirulina indicated that it is so far safe to be used in this study. The levels of these parameters did not show a significant difference when compared to normal negative control group.

The pre-treatment of diabetic rats with spirulina did not induce a significant change in the above mentioned parameters when compared to diabetic non-treated group. Spirulina post treatment was ranked as the best one in improving the four parameters when compared with diabetic non treated group.

The three liver enzymes AST, ALT and ALP activities showed a marked increase in sera of diabetic rats (160.75%, 150.19% and 168.75% respectively) when compared to control animals as shown in table (3).

Table (3) represents a significant decrease in the liver enzymes activities after post-treatment with spirulina (-40.24%, -41.38% and -41.88%) for AST, ALT and ALP activities respectively compared to diabetic non treated group. It is observed that the post-treated group with spirulina decreased the elevation in the enzyme activities more than the pre-treated group.

Glutathione reductase activity showed a marked decrease in diabetic rats (-66.36%) when compared to control group. This decrease was elevated in the post-treated spirulina and amaryl groups by (89.4% and 79.07%) respectively as represented in table (4).

Also the elevated level of MDA due to diabetes was corrected by the pre-and post-treatment with spirulina (-27.11 % and -40.25% respectively) and by amaryl treatment (-23.39%) as shown in table (4).

Cellular and Humoral Immune response

Mean number of thymocytes: Spirulina had no effect on the mean number of thymocytes as the control group (table, 5). Diabetes affected the mean number of thymocytes as it was non significantly decreased by (-21.62%) compared to the control group. This decrease was restored by spirulina either in the pre- (21.45%) or post- (23.77%) treated groups. Amaryl-treated diabetic group showed a significant increase in thymocytes number (101.04%) compared to diabetic non treated group as shown in table (5).

Mean number of splenocytes: Spirulina showed a neutral effect on the mean number of splenocytes as shown in table (5). In the normal supplemented group, splenocyte mean number was non significantly decreased (-14.94%) than those found in the control group.

A non significant decrease in mean number of splenocytes was observed (-29.96%) in diabetic non treated group compared to the control group as shown in table (5).

Small improvment was observed in the splenocytes number in the pre- (12.81%) and post- (17.15%) treated diabetic groups compared to diabetic non treated group. On the other hand, there was an obvious significant increase (185.37%) in the amaryl treated group compared to diabetic non treated group as shown in table (5).

Serum IgG level: The IgG level in sera from normal spirulina showed a non significant change (18.26%)   compared to control as shown in table (5). Spirulina pre-treated group showed greater improvement levels (-27.04%) and was followed by amaryl treated group (-24.91%). On the other hand, the post-treated group showed decrease by (-8.54%) compared to diabetic non treated group.

HISTOPATHOLOGICAL RESULTS

(i)- Hematoxylin & Eosin dye technique: Histological examination in pancreas sections of control rats showed that the cells of the pancreas were all present in their normal proportions. The acinar cells that were stained strongly were arranged in lobules with prominent nuclei. The islet cells were seen embedded within the acinar cells and surrounded by a fine capsule (figure1).

The microscopic investigations of pancreas of non diabetic rats supplemented with spirulina showed that the structure of both the endocrine pancreas and the exocrine pancreas appeared more or less as the control animals (figures, 2).

In sections of pancreas of diabetic rats, the acinar cells around the islets though seem to be in normal proportion did not look classical. The islets were large and occupied by a uniform eosinophilic material and few atrophic cells. Eosinophilic materials also surrounded the blood vessels. On the other hand, an interlobular haemorrhage was noticed (figure 3).

In case of diabetic rats treated with amaryl drug, sections showed the normal shape of the islets of Langerhans surrounded by normal exocrine part (figure 4).

Microscopic investigations of the pancreas of rats given spirulina before diabetes induction showed the normal structure of the exocrine and endocrine component. The islets were present with a large proportion of islet cells but with a smaller volume as compared with control. There are very scanty inflammatory cell infiltration and no eosinophilic deposits were not seen (figure 5).

Sections of pancreas of diabetic rat post- treated with spirulina showed the normal structure of the exocrine and endocrine components. The islets are present with a large proportion of islet cells though with a smaller volume as compared with control and inflammatory cells infiltration were not seen (figure 6).

(ii)-Immunohistochemical assay of insulin and glucagon

Insulin: In control and normal spirulina supplemented groups, beta cells in the islets of Langerhans appeared normal and the insulin immunoreactivity was shown in a classical pattern in the center of the islets which was stained with deep brown color. The normal distribution of the immunoreactivity of insulin in the normal algae treated groups proved the non toxic and safe dose used in this work as shown in figures (7, 8).

Diabetic non treated group showed weak immunoreactivity for insulin which reflected the uncompleted destruction of the beta cells secreting insulin while the amaryl treated group showed normal distribution of the immunoreactivity of insulin as shown in figures (9, 10 respectively).The pre-treated spirulina diabetic group showed an unchanged immunoreactivity for insulin as compared to the control group as shown in figure (11). On the other hand there was a reduction in the immunoreactivity for insulin in spirulina post-treated group as shown in figure (12).

Glucagon: In the control and normal spirulina supplemented groups, normal distribution of alpha cells secreting glucagon appeared in the marginal zone of the islets of Langerhans

which was stained in brown color. Spirulina treated group showed a remarkable reduction in the immunoreactivety for glucagon as shown in figures (13,14).

Diabetic non treated group showed much more abundant existance of immunoreactivety for glucagon which revealed absence of the insulin controlling effect on alpha cells due to the destruction of most of the beta cells, on the other hand there was pale immunoreactivety for glucagon in case of amaryl treated group as shown in figures (15,16).

A reduction in the immunoreactivety for glucagon was observed in the pre and post-treated spirulina diabetic groups, as shown in figures (17, 18).

Table (1): Effect of *Spirulina platensis* supplementation on glucose and insulin levels in sera of diabetic and non diabetic groups.

Groups / Parameters	Normal control	Diabetic non treated	Normal spirulina	Spirulina pre-treated	Spirulina post-treated	Amaryl drug
Glucose (mg/dl)	96.24±4.31	495.7±13.98[a]	102.0±6.75[n]	306.0±16.04[a,*]	135.0±7.22[a,*]	115.76±2.16[n,*]
% change compared to control group	-	415.06	5.98	217.95	40.27	20.28
% change compared to diabetic group	-	-	-	-38.26	-72.76	-76.64
Insulin (ng/ml)	5.31±0.07	1.69±0.07[a]	4.87±0.36[n]	2.54±0.26[a,*]	3.49±0.44[a,*]	3.63±0.29[a,*]
% change compared to control group	-	-68.17	-8.28	-52.16	-34.27	-31.63
% change compared to diabetic group	-	-	-	50.29	106.50	114.79

Data are represented as Mean ±SE of 8 rats.
P^a Significant at $P \leq 0.05$ compared to control group.
P^n Non significant compared to control group.
P^* Significant at $P \leq 0.05$ compared to diabetic non treated group.
$P^\#$ Non significant compared to diabetic non treated group.

Table (2): Effect of *Spirulina platensis* supplementation on some biochemical parameters in sera of diabetic and non diabetic groups.

Groups / Parameters	Normal control	Diabetic non treated	Normal Spirulina	Spirulina pre-treated	Spirulina post-treated	Amaryl drug
Total cholesterol (mg/dl)	81.87±2.19	214.34±37.28[a]	84.41±5.88[n]	190.08±12.71[a,#]	144.94±7.73[a,*]	151.70±21.95[a,*]
% change compared to control group		161.80	3.10	132.17	77.03	85.29
% change compared to diabetic group				-11.31	-32.37	-29.22
Triglycerides (mg/dl)	80.45±1.74	208.04±19.38[a]	83.29±5.29[n]	193.06±12.16[a,#]	150.3±9.0[a,*]	152.21±15.20[a,*]

	Normal control	Diabetic non treated	Normal Spirulina	Spirulina pre-treated	Spirulina post-treated	Amaryl drug
% change compared to control group		158.59	3.53	139.97	86.82	89.19
% change compared to diabetic group				-7.20	-27.75	-26.92
LDH (U/L)	350.22±3.03	749.64±11.24[a]	343.96±20.8[n]	522.32±31.04[a,*]	413.02±24.05[a,*]	557.19±26..35 [a,*]
% change compared to control group		113.99	-1.78	49.14	17.9.	59.09
% change compared to diabetic group				-30.30	-44.89	-25.65
Albumin (g/dl)	5.13±0.02	2.76±0.18[a]	5.15±0.42[n]	3.17±0.44[a,#]	4.25±0.41[n,*]	4.12±0.21[a,*]
% change compared to control group		-46.19	0.38	-38.20	-17.15	-19.68
% change compared to diabetic group				14.85	53.98	49.27

Data are represented as Mean ±SE of 8 rats.

P^a Significant at $P \le 0.05$ compared to control group.

P^n Non significant compared to control group.

P^* Significant at $P \le 0.05$ compared to diabetic non treated group.

$P^\#$ Non significant compared to diabetic non treated group.

Table (3): Effect of *Spirulina platensis* supplementation on liver function enzymes in sera of diabetic and non diabetic groups.

Groups / Parameters	Normal control	Diabetic non treated	Normal Spirulina	Spirulina pre-treated	Spirulina post-treated	Amaryl drug
AST (U/ml)	37.89±1.28	98.8±2.12[a]	40.24±3.82[n]	74.50±8.19[a,*]	59.04±7.19[a,*]	78.96±0.90[a,*]
% change compared to control group		160.75	6.20	96.62	55.81	108.39
% change compared to diabetic group				-24.59	-40.24	-20.08
ALT (U/ml)	38.93±0.42	97.4±1.29[a]	36.77±3.41[n]	69.02±5.60[a,*]	57.09±5.23[a,*]	77.26±3.43[a,*]
% change compared to control group		150.19	-5.54	77.29	46.64	98.45
% change compared to diabetic group				-29.13	-41.38	-20.67
ALP(U/L)	36.49±0.62	98.07±2.50[a]	36.98±3.35[n]	69.36±5.44[a,*]	56.99±4.71[a,*]	80.56±2.37[a,*]
% change compared to control group		168.75	1.34	90.07	56.17	120.77
% change compared to diabetic group				-29.27	-41.88	-17.85

Data are represented as Mean ±SE of 8 rats.

P^a Significant at $P \le 0.05$ compared to control group.

P^n Non significant compared to control group.

P^* Significant at $P \le 0.05$ compared to diabetic non treated group.

$P^\#$ Non significant compared to diabetic non treated group.

Table (4): Effect of *Spirulina platensis* supplementation on glutathione reductase enzyme and lipid peroxidation in liver tissue of diabetic and non diabetic groups.

Groups / Parameters	Normal control	Diabetic non treated	Normal Spirulina	Spirulina pre-treated	Spirulina post-treated	Amaryl drug
GR (nmol/min./ mgprotein)	10.94±0.24	3.68±0.25[a]	10.31±1.07[n]	5.44±0.83 [a,*]	6.97±1.02 [a,*]	6.59±0.60 [a,*]
% change compared to control group		-66.36	-5.75	-50.27	-36.28	-39.76
% change compared to diabetic group				47.82	89.40	79.07
MDA(ng/gmtissue)	10.41±0.58	21.24±0.68[a]	9.68±0.99[n]	15.48±1.29 [a,*]	12.69±1.09 [n,*]	16.27±1.37 [a,*]
% change compared to control group		104.03	-7.01	48.70	21.90	56.29
% change compared to diabetic group				-27.11	-40.25	-23.39

Data are represented as Mean ±SE of 8 rats.

P^a Significant at $P \le 0.05$ compared to control group.

P^n Non significant compared to control group.

P^* Significant at $P \le 0.05$ compared to diabetic non treated group.

$P^{\#}$ Non significant compared to diabetic non treated group.

Table (5): Effect of *Spirulina platensis* supplementation on the mean number of thymocytes and splenocytes and serum IgG levels in diabetic and non diabetic groups.

Groups / Parameters	Control	Diabetic non treated	Normal spirulina	Spirulina Pre-treated	Spirulina post-treated	Amaryl drug
Thymocytes	111.43±11.23	87.33±8.30 [n]	112.00±8 [n]	106.07±5.03 [n,#]	108.09±1.41 [n,#]	175.57±30.07 [n,*]
% change compared to control group		-21.62	0.51	-4.81	-2.99	57.56
% change compared to diabetic group				21.45	23.77	101.04
Splenocytes	316.85±20.33	221.92±43.73 [n]	269.50±18.25 [n]	250.35±16.65 [n,#]	260.00±13.60 [n,#]	633.3±57.71 [a,*]
% change compared to control group		-29.96	-14.94	-20.98	-17.94	99.87
% change compared to diabetic group				12.81	17.15	185.37
IgG level	0.33±0.01	0.56±0.10 [n]	0.39±0.03 [n]	0.41±0.02 [n,#]	0.51±0.04 [n,#]	0.42±0.08 [n,#]
% change compared to control group		68.26	18.26	24.24	54.54	27.27
% change compared to diabetic group				-27.04	-8.54	-24.91

Data are represented as Mean ±SE of 8 rats.

P^a Significant at $P \le 0.05$ compared to control group.

P^n Non significant compared to control group.

P^* Significant at $P \le 0.05$ compared to diabetic non treated group.

$P^{\#}$ Non significant compared to diabetic non treated group.

Histopathological Results

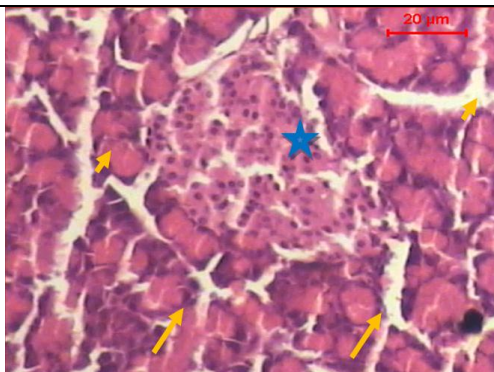**(i)- Hematoxylin & Eosin dye technique**

Figure (1): A section of pancreas of control rat shows the exocrine component consisting of closely packed acini (arrows). The interlobular ducts (arrowhead) appear surrounded with the supporting tissue. The endocrine tissue of the pancreas, islet of Langerhan (asterisk), is scattered throughout the exocrine tissue (H & E stain X 200, Scale bar: 20µm).
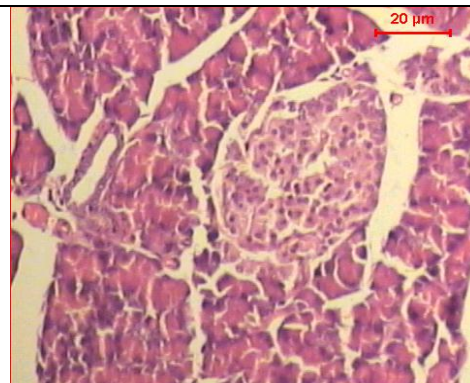

Figure (2): A section of pancreas of rat supplemented with *Spirulina Platensis* shows the normal structure of the exocrine and endocrine component (H & E stain X 200, Scale bar: 20µm).
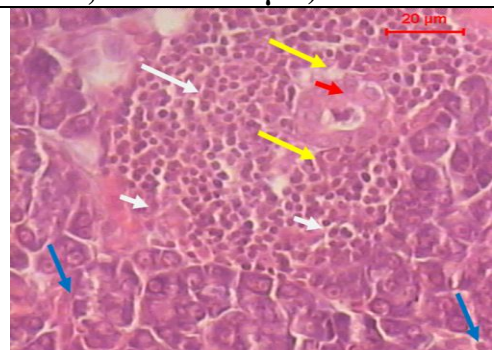
Figure (3): A section of pancreas of diabetic non treated rat showing the acinar cells around the islets though seem to be in normal proportion does not look classical. The islets are largely (white arrow) occupied by a uniform eosinophilic material (white arrowhead) and few atrophic cells (yellow arrows). Eosinophilic materials also surround the blood vessel (red arrowhead). Notice the interlobular haemorrhage (blue arrows) (H & E stain X 200, Scale bar: 20 µm).
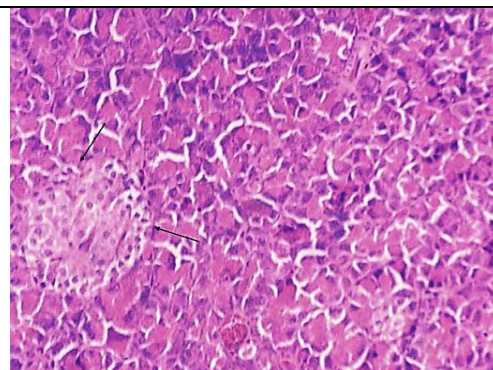

Figure (4): A section of pancreas of diabetic rat treated with amaryl drug showing the islets of Langerhans with distinct border from surrounding exocrine part(↖). (H & E stain X 200, Scale bar: 20 µm).
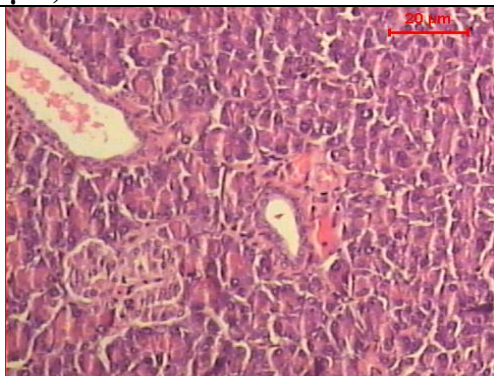

Figure (5): A section of pancreas of diabetic rat pre-treated with *Spirulina Platensis* shows the normal structure of the exocrine and endocrine component. The islets are present with a large
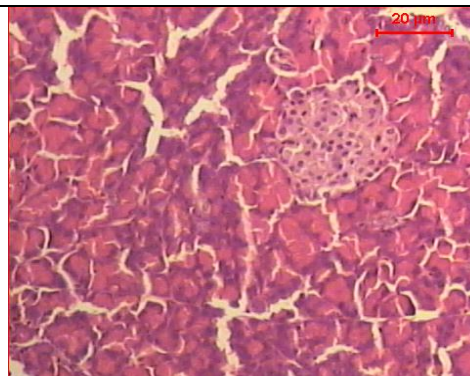

Figure (6): A section of pancreas of diabetic rat post-treated with *Spirulina Platensis* shows the normal structure of the exocrine and endocrine

proportion of islet cells though with a smaller volume as compared with control. There is very scanty inflammatory cell infiltration and no eosinophilic deposits were seen (H & E stain X 200, Scale bar: 20µm).	component. The islets are present with a large proportion of islet cells though with a smaller volume as compared with control and inflammatory cell infiltration were not seen (H & E stain X 200, Scale bar: 20µm).

(ii)-Immunohistochemical assay of insulin and glucagon

1- Insulin
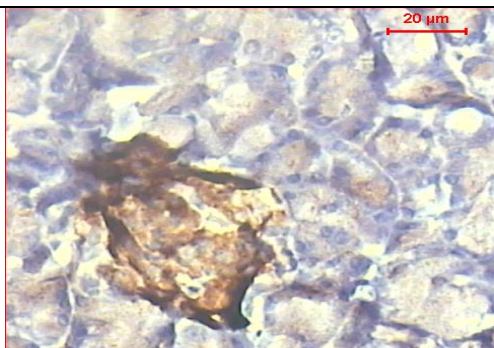
Figure (7): Immunohistochemical staining for insulin antibody to identify the beta cells in the islets of Langerhans of pancreas in control rat. Note that insulin immunoreactivity is distributed in the centre of the islets (X200, Scale bar = 20 mm).
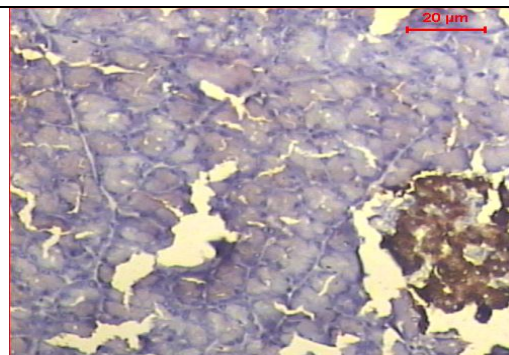

Figure (8): Immunohistochemical staining for insulin antibody of pancreas in rat supplemented with *Spirulina Platensis* shows normal distribution of immunoreactivity (X200, Scale bar = 20 mm).

Figure (9): Immunohistochemical staining for insulin in STZ diabetic non treated pancreas shows weak immunostaining for insulin compared with normal one (X200, Scale bar = 20 mm).
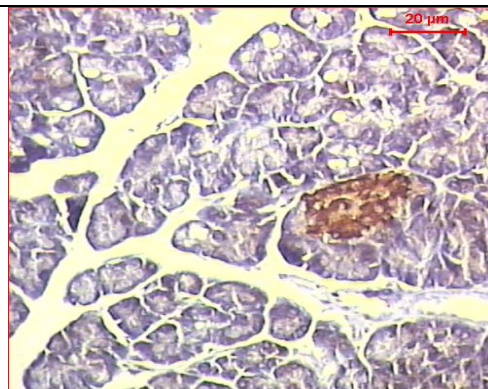

Figure (10): Immunohistochemical staining for insulin antibody of pancreas in diabetic rat treated with amaryl drug shows distribution of immunoreactivity that appear more or less like control (X200, Scale bar = 20 mm).
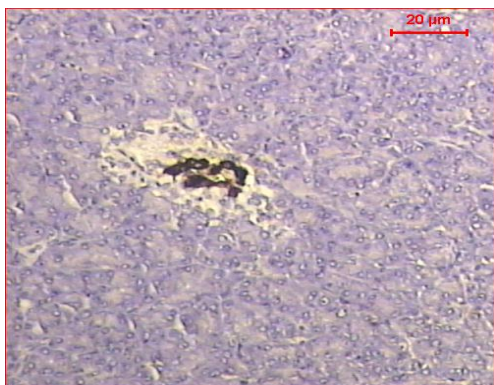

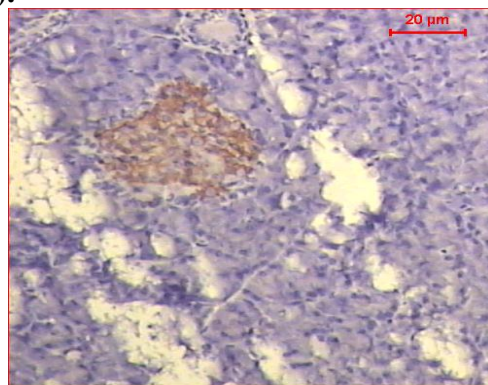**Figure (11):** Immunohistochemical staining for

Figure (12): Immunohistochemical staining for insulin

insulin antibody of pancreas in diabetic rat pre-treated with *Spirulina Platensis* shows the normal distribution of insulin immunoreactivity as compared with control (X200, Scale bar = 20 mm).

antibody of pancreas in diabetic rat post- treated with *Spirulina Platensis* shows the reduction in the normal distribution of insulin immunoreactivity as compared with control (X200, Scale bar = 20 mm).
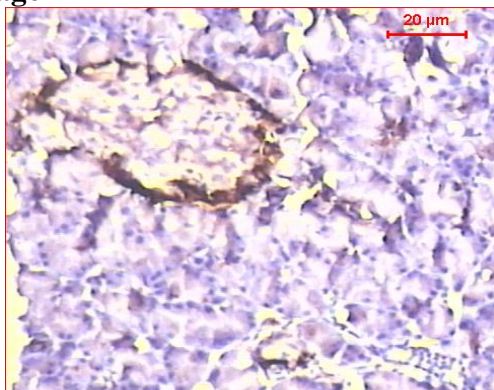
2- Glucagon

Figure (13): Immunohistochemical staining for glucagon antibody to identify the alpha cells in the islets of Langerhans of pancreas in control rat. Note that glucagon immunoreactivity is distributed in the marginal zone of the islets (X200, Scale bar = 20 mm).
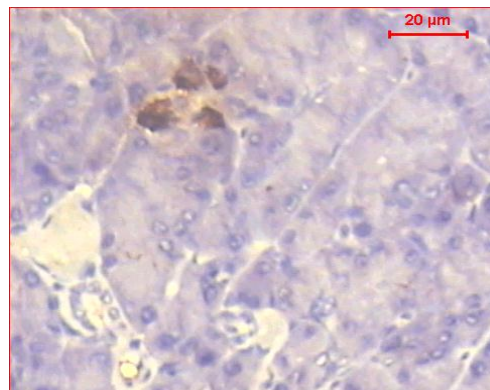

Figure (14): Immunohistochemical staining for glucagon antibody of pancreas in rat supplemented with *Spirulina Platensis* shows reduction in the distribution of glucagon immunoreactivity (X200, Scale bar = 20 mm).
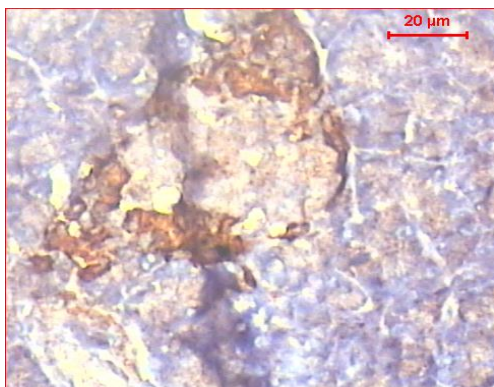

Figure (15): Immunohistochemical staining for glucagon in STZ diabetic non treated pancreas shows much more abundant and ubiquitous immunostaining for glucagon as compared with normal one (X200, Scale bar = 20 mm).

Figure(16): Immunohistochemical staining for glucagon antibody of pancreas in diabetic rat treated with amaryl drug shows pale immunoreactivity of glucagon as compared with control (X200, Scale bar = 20 mm).
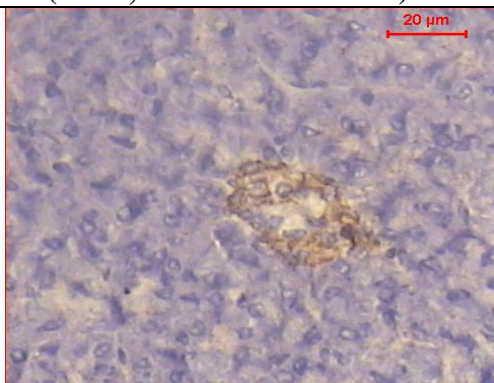

Figure (17): Immunohistochemical staining for glucagon antibody of pancreas in diabetic rat pre-treated with *Spirulina Platensis* shows reduction in
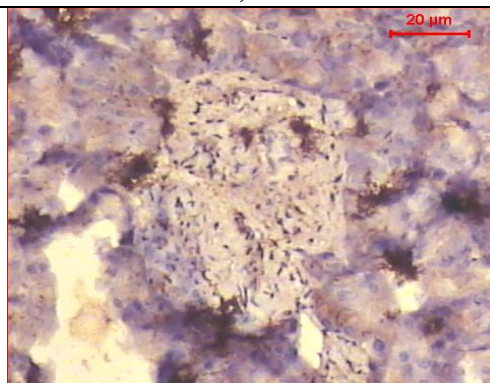

Figure (18): Immunohistochemical staining for glucagon antibody of pancreas in diabetic rat post-treated with *Spirulina Platensis* shows the reduction in

the normal distribution of glucagon immunoreactivity as compared with control (X200, Scale bar = 20 mm).	the normal distribution of glucagon immunoreactivityas compared with control (X 200, Scale bar = 20 mm).

DISCUSSION

The pathogenesis and pathophysiological processes of T2DM are extreme complex and remain to be controversial. However, a growing number of evidences showed that oxidative stress and inflammation might play important role in the development of T2DM (Evans *et al*., 2005; Donath & Shoelson, 2011). Several studies showed that diabetes is accompanied with metabolic disorders (Mc Gill & Felton, 2007) in which inflammation and oxidative stress closely relates each other (Munoz & Costa 2013; Gratas- Delamarche *et al*., 2014; Lugrin *et al*., 2014). In diabetes mellitus, oxidative stress is enhanced through various sources such as hyperglycemia, dyslipidemia, hyperinsulinemia, insulin resistance, and impaired antioxidant defense (Wiernsprger, 2003). Accordingly a great attention was directed for the use of antioxidants to normalize the harm caused by diabetes.

Natural products and micronutrients, as prophylactic or treating agents in various diseases are becoming promising in last decades. In this study, an attempt was done to investigate the changes which occurred in sera, liver and pancreas of STZ - induced diabetic rats after administration of the alga *Spirulina platensis*. The effect of a reference drug, amaryl, was compared with that of the supplemented antioxidant.

In the present study we used STZ for diabetes induction in rats. STZ is a nitrosourea compound produced by *Streptomyces achromogenes*, which is specifically induces DNA strand breakage in β- cells of pancreas causing diabetes mellitus (Kumar et al., 2012). The present study has demonstrated that injection of rats with STZ resulted in a significant elevation of blood glucose level in diabetic group as compared with normal animals indicating establishment of diabetic state.

Our study showed that the administration of *S. platensis* suspension for 3 weeks to hyperglycemic rats produced a marked decrease in the blood glucose level. This data was in accordance with Layam et al. (2006) who found that oral administration of spirulina to diabetic rats significantly reduced the blood glucose level and the authors attributed this effect to high fiber content of *S. platensis* that interfered with the absorption of glucose. Another theory was based on the possible action of peptides and polypeptides generated by the digestion of spirulina proteins (Mani et al., 2000). In addition, Takai (1991) reported that

several therapeutically important compounds/ molecules like β- carotene, phycocyanin,γ- linolenic acid, etc.identified in *S.platensis* are shown to possess immnuomodulatory and biomodulatory functions.

STZ is well known for its selective pancreatic islet β- cell cytotoxicity. It interferes with cellular metabolic oxidative mechanisms (Papaccio et al., 2000). Intraperitoneal administration of STZ (45 mg/kg) effectively induced diabetes in normal rats, as reflected by glycosuria, hyperglycemia, polyphagia, polydipsia and body weight loss when compared with normal rats (Calabresi and Chabner, 1990). In addition, Wuarin et al. (1996) demonstrated that insulin levels are reduced in type 1 diabetes, while resistance to insulin is coupled with a partial reduction in insulin production in type 2 diabetes. The authors reported that insulin growth factor (IGF) gene expression is reduced in STZ − diabetic rat brain and liver, moreover, Lee and Park (2000) found that most β- cells were destroyed by treatment with STZ, only few of them shows a weak secretory activity of insulin. Recently, Serbedzija et al. (2009) reported that substantial evidence showed that patients have diminished brain insulin and IGF.

In the present study, it was observed that oral administration of spirulina could reverse the above mentioned diabetic effects. The possible mechanism by which spirulina brings about its antihyperglycemic action may be through potentiation of the pancreatic secretion of insulin from islet β- cell or due to enhanced transport of blood glucose to the peripheral tissue. This was clearly demonstrated by the increased levels of insulin in diabetic rats treated with spirulina (Layam and Reddy, 2006).

The abnormal high concentration of serum lipids of diabetic rats is mainly due to increase in the mobilization of free fatty acids from the peripheral fat deposits, because insulin inhibits the hormone sensitive lipase production (Udayakumar et al., 2009).

The current work showed a significant increase in serum lipid cholesterol level in STZ- treated rats compared to control animals. Administration of *S. platensis* suspension to rats after hyperglycemia produced a marked decrease in blood cholesterol level. Similar observations were obtained by Rodriguez-Hernandez et al. (2001), who found that the dietary administration of 5% *S. maxima* for 4 weeks to diabetic mice reverted the LDL and VLDL levels to normal level. Also, the present data are in harmony with Moura et al. (2012) who found that cholesterol, LDL and VLDL showed a significant reduction with an increase in

HDL- cholesterol following administration of algae for 30 days. This effect might be due to the presence of γ-linoleic acid in spirulina, which prevents accumulation of fats and cholesterol in human body (Mani et al., 2002). In addition, Ramamoorthy and Premakumari (1996) attributed this hypocholesterolemic effect to the presence of c- phycocyanin in spirulina. Phycocyanin is a water soluble protein and enriched in spirulina and it was suggested that phycocyanin might be the active ingredient in spirulina responsible for the hypolipidemic activity (Nagaoka et al., 2005).

Our present study showed a marked increase in blood triglycerides concentration in STZ-treated rats. This data is in agreement with Krishnakumar et al.(2000) who reported that diabetes-induced hyperlipidemia is attributable to excess mobilization of fat from the adipose due to the under utilization of glucose. The observed regression of the diabetic state due to the administration of our algae suspension may have increased the utilization of glucose, thereby depressing the mobilization of fat (Akpan et al., 2012).

Spirulina was found effective in normalizing the triglyceride levels, the reduction in triglyceride level could be through lipoprotein lipase, a key enzyme in the metabolism of triglyceride rich lipoprotein (Nassir et al., 1993). In addition, our results are in accordance with Lee et al. (2008) who found a significant lowering effect of spirulina on plasma triglyceride concentration of type 2 diabetes mellitus patients. Recntly, Jarouliya et al. (2012) found that *S. maxima* was effective in normalizing the triglyceride levels and the authors attributed this effect to decreased VLDL triglyceride production or increased VLDL clearance in the peripheral tissues.

The present study was extended to assess   the biochemical mechanism (s) of the action of glycemic control of alga under investigation compared to the antidiabetic drug, amaryl. Liver glycolytic and gluconeogenic enzymes are the key enzymes responsible for maintaining the homeostasis of the blood glucose **(Maiti et al. 2004).** A significant reduction of glycolytic enzyme LDH in serum of diabetic animals was documented in the current work. This data is in consistent with previous reports stated that diabetes is developed due to obstruction of glucose utilization by the tissues through glycolysis and its over-production through excessive hepatic gluconeogenesis **(Klover and Mooney, 2004)** and this may attribute to the relative deficiency of insulin which responsible for increasing the expression of the glycolytic enzymes in the liver (**Sun et al., 2002).** Administration of the studied alga normalized the

alteration in LDH enzyme in serum of diabetic rats   which enlightened its possible way of antidiabetogenic activity.

Albumin is the most abundant plasma protein synthesized exclusively in the liver, albumin constitutes over half of the total plasma proteins. It has diverse physiological functions ranging from maintenance of colloid pressure to transfer/transport of various metabolites and is a powerful extracellular antioxidant **(Peters, 1996, Bourdon et al.1999).** It contains 17 disulphide bridges and has a single remaining cysteine residue which is responsible for the capacity of albumin to react with and neutralize peroxyl radicals **(Young and Woodside, 2001).** Albumin is also widely considered as an important nonenzymatic plasma antioxidant. This is primarily due to the single cysteine sulfhydryl residue. The sulfhydryl groups of albumin can react with oxidants and free radicals causing oxidation of the albumin molecule in order to ''spare'' other important regulatory or long-lived proteins **(Wratten et al., 2001)**. Decreased in albumin due to its glycation during diabetes may consider one of the important factors responsible for oxidative stress related to diabetes (**Jin et al. 2008**). Recently, Zheng et al., (2013) found that Oral administration of phycocyanin (300 mg/kg), derived from *Spirulina platensis*, for 10 wk protected against albuminuria in db/db mice.

Serum transaminases and alkaline phosphatase activities were generally increased in streptozotocin-induced diabetic rats. Similar data were recorded (El-Agouza et al., 2000; Gawronska-Szlarz et al., 2003; Yanardag et al., 2005). Increase in the activities of liver enzymes may be due to STZ toxicity, extensive tissue destruction, disturbances in the transphosphorylation and in the general metabolism of the different cells and tissues of diabetic rats (Tanaka et al., 1998). It is known that elevation of transaminases could be a common sign of impairment in liver function. Acute cellular necrosis librates alkaline phosphatase in the circulation and serum enzyme level is elevated (Elyazi, 2000).

Our results decleared that oral administration of Spirulina ameliorates the increase in lipid peroxidation, this effect is attributed to the presence of phycocyanin and phycocyanobilin which have antioxidant effects against oxidative stress as demonstrated by **Zheng *et al.* (2012)**.

Oxidative stress is produced under diabetic condition and it is likely to be involved in progression of pancreatic β-cell dysfunction (Kajimoto and. Kaneto, 2004). High levels of free radicals, due to insufficiency of the antioxidant defense system, may lead to disruption of

cellular function, oxidative damages to membranes, and enhance their susceptibility to lipoperoxidation (Baynes, 1991).

Several studies have documented that oxidative stress is accelerated in diabetes mellitus owing to an increase in the production of oxygen free radicals, lipid peroxidation and low-density lipoprotein (Reddy et al., 2005) Free radicals can diffuse intracelluarly and result in mitochondrial enzyme damage and DNA breaks, all of which impair cellular function and contribute to the pathophysiology of diabetes (Bonnefont-Rousselot et al., 2000). Oxygen free radicals exert their cytotoxic effects on membrane phospholipids, resulting in the formation of MDA. As a product of lipid peroxidation, the levels of MDA reflect the degree of oxidation in the body. Increased levels of TBARS, an end-product of lipoperoxidation, were found previously in the liver of streptozotocin- induced diabetic rats (Dias et al., 2005; Di Naso et al., 2010, 2011). In this study, the TBARS increase confirms this finding, which indicates an overall oxidative stress increase in diabetic rats.

Our results decleared that oral administration of Spirulina ameliorates this increase in lipid peroxidation, this effect attributed to the presence of phycocyanin and phycocyanobilin which have antioxidant effects against oxidative stress. These data are in accordance with Zheng et al., (2013).

Our immunological data show that the mean of total count of thymocytes and splenocytes are affected by diabetes. Decreasing in lymphocytes count is recorded in both the thymocytes and splenocytes in diabetic non treated rats when compared to control rats due to the defects in insulin secretion. On the other hand, there is a significant increase in IgG level in sera from diabetic non treated rats. These results are in accordance with **Kim *et al*. (2014)** who reported an immunological injury associated with diabetes which happened due to the toxic effect of hyperglycemia. They detected a reduction in splenic lymphocyte subpoulations helper T cell and cytotoxic T cell in addition to an increase in IgG1 level in diabetic mice compared to non diabetic mice.

The immunological studies by many authors demonstrated that C-phycocyanin found in *S. platensis* possess anti-oxidant, anti-inflammatory and radical scavenging properties that protect rats from chemical induced thymic atrophy and it serve as an effective natural antioxidant for chemical oxidative stress and enhances biological defense activity and

reduces allergic inflammation in addition to enhancement of the immune response **(Nemoto-Kawamura *et al*. 2004; Grzanna *et al*., 2006)**.

In our study, the mean number of thymocytes in *S. platensis* supplemented non diabetic rats showed no change w.r.t. control. However, the pre-and post-spirulina treated groups showed an improvement in thymocytes number w.r.t. the diabetic non treated group.

Our data showed that the decrease in the mean number of splenocytes due to diabetes was restored but not to the normal level in case of treated groups with spirulina. The mean number of splenocytes was increased from rats administrated spirulina before or after diabetes compared with cells from diabetic non treated rats. This is in agreement with **Hayashi *et al*. (1994)** who showed that a fed of *Spirulina platensis* diet increased numbers of splenic antibody-producing cells in the primary immune response to sheep red blood cells (SRBC).

In our study, spirulina supplementation to diabetic rats showed non significant improvement in IgG levels in the pre-treated spirulina diabetic group w.r.t. diabetic non treated group. Various Igs are increased in diabetes specifically,IgG1 and IgM (**Zhang *et al.*,2008; Cui *et al*., 2010**).This observation is in accordance with our data as the IgG level increased by 68.26% in the diabetic group in comparison to control group. **Qureshi *et al*. (1996)** reported that dietary *Spirulina platensis* enhanced humoral and cell mediated immune functions in chickens.

Hayashi *et al*. (1998) investigated the antibody productions of IgA, IgE and IgG1 in mice as an evidence of the protective effects of Spirulina toward food allergy and microbial infection. Histological examinations on pancreas show inflammatory cell infiltration in both the pre-and post-spirulina treated groups in spite of the normal appearance of the exocrine and the endocrine component of the pancreas. These results demonstrated the moderate antioxidant activity of spirulina in protecting the pancreas cells.

In addition, the immunohistochemical examinations revealed a reduction in insulin and glucagon in the post-treated group and a reduction in glucogon only in the pre-treated one. This observation can be discussed as an incomplete recovery of the pancreas or may be due to the elevated levels of ROS that cannot be overcomed by spirulina.

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Discussion: Natural products and micronutrients, as prophylactic or treating agents in various diseases are becoming promising in last decades. In this study, an attempt was done to investigate the changes which occurred in sera, liver and pancreas of STZ - induced diabetic rats after administration of the alga *Spirulina platensis*. The effect of a reference drug, amaryl, was compared with that of the supplemented antioxidant spirulina.

In the present study we used STZ for diabetes induction in rats. STZ is a nitrosourea compound produced by *Streptomyces achromogenes*, which is specifically induces DNA strand breakage in β- cells of pancreas causing diabetes mellitus.[44] The current work has demonstrated that injection of rats with STZ resulted in a significant elevation of blood glucose level in diabetic group as compared with normal animals indicating establishment of diabetic state.

The present study revealed that almost all the parameters showed a greater improvement due to post treatment with spirulina than the pre treatment which can be attributed to its mode of action. We will try to explain the effect of spirulina supplementation on each parameter separately.

Our study showed that the administration of *S. platensis* suspension for 4 weeks to hyperglycemic rats produced a marked decrease in the blood glucose level when compared with diabetic non treated group. This data was in accordance with Layam and Reddy [25] who found that oral administration of spirulina to diabetic rats significantly reduced the blood glucose level. The authors attributed this effect to high fiber content of *S. platensis* that interfered with the absorption of glucose. Another theory was based on the possible action of peptides and polypeptides generated by the digestion of spirulina proteins.[45] In addition,

Takai [46] reported that several therapeutically important compounds/ molecules like β-carotene, phycocyanin,γ- linolenic acid, etc. identified in *S.platensis* are shown to possess immnuomodulatory and biomodulatory functions. Recently, Qu *et al* reported [47] reported that administration of phycocyanin of *spirulina platensis* showed a significant decreased effect on fasting blood glucose levels in alloxan-induced diabetic mice. This result suggests that phycocyanin protected cells of pancreatic islets from alloxan induced injury and/or promotes pancreatic -cell regeneration.[48] This was confirmed by morphological analysis of pancreatic islets.

STZ is well known for its selective pancreatic islet β- cell cytotoxicity, it interferes with cellular metabolic oxidative mechanisms.[49]

Intraperitoneal administration of STZ (45 mg/kg) effectively induced diabetes in normal rats, as reflected by glycosuria, hyperglycemia, polyphagia, polydipsia and body weight loss when compared with normal rats.[50] In addition, Wuarin [51] demonstrated that insulin levels are reduced in type 1 diabetes, while resistance to insulin is coupled with a partial reduction in insulin production in type 2 diabetes. The authors reported that insulin growth factor (IGF) gene expression is reduced in STZ – diabetic rat brain and liver, moreover, Lee and Park [52] found that most β- cells were destroyed by treatment with STZ, only few of them shows a weak secretory activity of insulin.

In the present study, it was observed that oral administration of spirulina could reverse the above mentioned diabetic effects. The possible mechanism by which spirulina brings about its antihyperglycemic action may be through potentiation of the pancreatic secretion of insulin from islet β- cell or due to enhanced transport of blood glucose to the peripheral tissue. This was clearly demonstrated by the increased levels of insulin in diabetic rats treated with spirulina.[25]

The abnormal high concentration of serum lipids of diabetic rats is mainly due to increase in the mobilization of free fatty acids from the peripheral fat deposits, because insulin inhibits the hormone sensitive lipase production. [53]

The current work showed a significant increase in serum lipid cholesterol level in STZ-treated rats compared to control animals. Administration of *S. platensis* suspension to rats after hyperglycemia produced a marked decrease in blood cholesterol level. Similar

observations were obtained by Rodriguez-hernandez *et al* [54], who found that the dietary administration of 5% *S. maxima* for 4 weeks to diabetic mice reverted the LDL and VLDL levels to normal level. Also, the present data are in harmony with Moura *et al* who found that cholesterol, LDL and VLDL showed a significant reduction with an increase in HDL-cholesterol following administration of algae for 30 days. This effect might be due to the presence of γ-linoleic acid in spirulina, which prevents accumulation of fats and cholesterol in human body.[56] In addition, Ramamoorthy and Premakumari [57] attributed this hypocholesterolemic effect to the presence of c- phycocyanin in spirulina. Phycocyanin is a water soluble protein and enriched in spirulina and it was suggested that phycocyanin might be the active ingredient in spirulina responsible for the hypolipidemic activity.[23]

Our present study showed a marked increase in blood triglycerides concentration in STZ-induction rats. This data is in agreement with Krishnakumar *et al* who [58] who reported that diabetes-induced hyperlipidemia which is attributable to excess mobilization of fat from the adipose tissue due to the under utilization of glucose.

In our study, administration of spirulina to rats resulted in a significant reduction in triglycerides concentration. These data are in accordance with Iwata *et al* and Nassir *et al* who postulated that the hypotriglyceridemic effect of spirulina might be due to its effect on the metabolism of lipoproteins. They reported that rats fed a diet containing spirulina showed a significant increase in the activity of lipoprotein lipase, a key enzyme in the metabolism of triglyceride rich lipoprotein, compared to rats fed a high fructose diet. In addition, our results are in accordance with Lee *et al* who found a significant lowering effect of spirulina on plasma triglyceride concentration in type 2 diabetes mellitus patients. Also, Jarouliya *et al* found that *S. maxima* was effective in normalizing the triglyceride levels. The observed regression of the diabetic state due to the administration of our algae suspension may have increased the utilization of glucose, thereby depressing the mobilization of fat.

Liver glycolytic and gluconeogenic enzymes are the key enzymes responsible for maintaining the homeostasis of the blood glucose.[62] A significant increase of the glycolytic enzyme LDH in serum of diabetic animals was documented in the current work. This data is in consistent with previous reports stated that diabetes is developed due to obstruction of glucose utilization by the tissues through glycolysis and its over-production through excessive hepatic gluconeogenesis [63] which may be attributed to the relative deficiency of insulin responsible for increasing the expression of the glycolytic enzymes in the liver.[64] Administration of the

studied alga normalized the alteration in LDH enzyme in serum of diabetic rats which enlightened its possible way of antidiabetogenic activity.

Albumin is the most abundant plasma protein synthesized exclusively in the liver, it constitutes over half of the total plasma proteins. It has diverse physiological functions ranging from maintenance of colloid pressure to transfer/transport of various metabolites and is a powerful extracellular antioxidant.[65, 66] It contains 17 disulphide bridges and has a single remaining cysteine residue which is responsible for the capacity of albumin to react with and neutralize peroxyl radicals.[67] Albumin is also widely considered as an important nonenzymatic plasma antioxidant, this is primarily due to the single cysteine sulfhydryl residue. The sulfhydryl groups of albumin can react with oxidants and free radicals causing oxidation of the albumin molecule in order to ''spare'' other important regulatory or long-lived proteins.[68]

Results in our work showed a significant decrease in albumin concentration of diabetic rats compared with control healthy group. This data is in harmony with Jin *et al* who reported that decreased in albumin due to its glycation during diabetes may consider one of the important factors responsible for oxidative stress related to diabetes.

Treatment of rats with our algae modulated this decrease of albumin concentration due to STZ induction. These results are in accordance with Zheng *et al* who found that oral administration of phycocyanin (300 mg/kg), extracted from *Spirulina platensis*, for 10 wk protected against albuminuria in db/db mice.

Serum transaminases and alkaline phosphatase activities were generally increased in streptozotocin-induced diabetic rats. Similar data were recorded by several authors.[71,72,73] Increase in the activities of liver enzymes may be due to STZ toxicity, extensive tissue destruction, disturbances in the transphosphorylation and in the general metabolism of the different cells and tissues of diabetic rats.[74] It is known that elevation of transaminases could be a common sign of impairment in liver function.

The present study showed a significant decrease in the liver functions enzymes AST, ALT and ALP due to spirulina treatment. The results are in accordance with Jarouliya *et al* who found that administration of spirulina for 30 days resulted in significant decrease in plasma AST and ALT activities in fructose fed Wistar rats. The authors reported that restoration of the enzymes activities to their respective normal levels after administration of spirulina

showed hepatoprotective effect of this alga. In addition Becker *et al* mentioned that the presence of β-carotene in spirulina, a fat soluble pigment, the precursor of vitamin A, known for its antioxidant activity helps in the hepatoprotective activity.

Oxidative stress is produced under diabetic condition and it is likely to be involved in progression of pancreatic *β*-cell dysfunction.[76] High levels of free radicals, due to insufficiency of the antioxidant defense system, may lead to disruption of cellular function, oxidative damages to membranes, and enhance their susceptibility to lipoperoxidation.[77]

Several studies have documented that oxidative stress is accelerated in diabetes mellitus owing to an increase in the production of oxygen free radicals, lipid peroxidation and low-density lipoprotein.[78] Free radicals can diffuse intracelluarly and result in mitochondrial enzyme damage and DNA breaks, all of which impair cellular function and contribute to the pathophysiology of diabetes.[79] Oxygen free radicals exert their cytotoxic effects on membrane phospholipids, resulting in the formation of malondialdehyde (MDA). As a product of lipid peroxidation, the levels of MDA reflect the degree of oxidation in the body. Increased levels of Thiobarbituric Acid Reactive Substance (TBARS), an end-product of lipoperoxidation, were found previously in the liver of streptozotocin- induced diabetic rats.[80,81,82] In this study, the TBARS increase confirms this finding, which indicates an overall oxidative stress increase in diabetic rats.

Our results decleared that oral administration of Spirulina ameliorates this increase in lipid peroxidation, this effect attributed to the presence of phycocyanin and phycocyanobilin which have antioxidant effects against oxidative stress. These data are in accordance with   Zheng *et al.*

Lipid peroxidation data revealed a moderate improvement in decreasing the levels of MDA in drug treated diabetic rats which was in accordance with Krauss [83] who denoted that glimepiride administration caused a decreasing level in peroxides, MDA and increase in activity of superoxide dismutase and glutathione peroxidase in STZ diabetic rats.

Our immunological data show that the mean of total count of thymocytes and splenocytes are affected by diabetes. Decreasing in lymphocytes count is recorded in both the thymocytes and splenocytes in diabetic non treated rats when compared to control rats due to the defects in insulin secretion. On the other hand, there is a significant increase in IgG level in sera from

diabetic non treated rats. These results are in accordance with Kim *et al* who reported an immunological injury associated with diabetes which happened due to the toxic effect of hyperglycemia. They detected a reduction in splenic lymphocyte subpoulations helper T cell and cytotoxic T cell in addition to an increase in IgG1 level in diabetic mice compared to non diabetic mice.

The immunological studies by many authors demonstrated that C-phycocyanin found in *S. platensis* possess anti-oxidant, anti-inflammatory and radical scavenging properties that protect rats from chemical induced thymic atrophy and it serve as an effective natural antioxidant for chemical oxidative stress and enhances biological defense activity and reduces allergic inflammation in addition to enhancement of the immune response.[85,86]

In our study, the mean number of thymocytes in *S. platensis* supplemented non diabetic rats showed no change w.r.t. control. However, the pre-and post-spirulina treated groups showed an improvement in thymocytes number w.r.t. the diabetic non treated group.

Our data showed that the decrease in the mean number of splenocytes due to diabetes was restored but not to the normal level in case of treated groups with spirulina. The mean number of splenocytes was increased from rats administrated spirulina before or after diabetes compared with cells from diabetic non treated rats. This is in agreement with Hayashi *et al* who showed that a fed of *Spirulina platensis* diet increased numbers of splenic antibody-producing cells in the primary immune response to sheep red blood cells (SRBC).

In our study, spirulina supplementation to diabetic rats showed non significant improvement in IgG levels in the pre-treated spirulina diabetic group w.r.t. diabetic non treated group. Various Igs are increased in diabetes specifically,IgG1 and IgM.[88,89]This observation is in accordance with our data as the IgG level increased by 68.26% in the diabetic group in comparison to control group. Qureshi *et al* reported that dietary *Spirulina platensis* enhanced humoral and cell mediated immune functions in chickens.

Hayashi *et al* investigated the antibody productions of IgA, IgE and IgG1 in mice as an evidence of the protective effects of Spirulina toward food allergy and microbial infection. Undesirable cell number count was noticed in the drug amaryl-treated-group in which a significant increase was detected in thymocytes and splenocytes, an improvment in serum levels of IgG were detected w.r.t the diabetic non treated rats.

The histological data of the pancreatic sections of diabetic rats show that although the acinar cells around the islets of Langerhans seem to be in normal proportion but it does not look classical. The islets are largely occupied by a uniform eosinophilic material and few atrophic cells. Eosiniphilic materials also surround the blood vessels and interlobular haemorrhage is noticed. The histological changes in diabetic animals are a sign for the oxidant and inflammatory state caused by diabetes.

The data are in accordance with several authors who found that diabetic rats showed marked pancreatic structural alterations, possibly due to hyperglycemia-induced oxidative stress. Pancreatic islets of Langerhans represent a main structure to regulate glucose metabolism and homeostasis. Compared with liver, the islets contain much less antioxidant activities. Thus, β-cells are considered to be low in antioxidant defense and more susceptible to oxidative stress.[92] Also, Bonnevie-Nielsen [93] observed the atrophy and vacuolation of pancreatic islets in diabetic rats. They attributed their observation to the inflammatory changes detected in pancreatic islets result from selective destruction of insulin producing *β*-cells. Masjedi [94] observed a decrease in the number and diameter of pancreatic islets in diabetes. Recently, El-Desouki et al [95] demonstrated that diabetes caused changes in rat pancreatic tissue as islet vacuolization, degeneration and necrosis of b-cells, dilation of intercalated duct and infiltration of inflammatory cells. Moreover, the expression of insulin secreting cells (b-cells) by using monoclonal anti insulin markedly demonstrated the destruction and reduction of b-cells immunoreaction in diabetic rats. Also it was found that spirulina -treatment minimized and improved the changes associated with pancreatic rat diabetes and enhanced the expression of b-cells in the islets of Langerhans.

All these findings are in accordance with our histological and immunohistochemical examinations results on pancreas. Our results showed inflammatory cell infiltration in both the pre-and post-spirulina treated groups in spite of the normal appearance of the exocrine and the endocrine component of the pancreas. These results demonstrated the moderate antioxidant activity of spirulina in protecting the pancreas cells.

The immunohistochemical studies revealed parallel results in which the diabetic non treated group showed weak immunoreactivity for insulin which reflected the uncompleted destruction of the beta cells secreting insulin in addition to much more abundant existence of the immuneoreactivity for glucagon which revealed the absence of insulin controlling effect on alpha cells due to the destruction of most of the beta cells.

In addition, the immunohistochemical examinations revealed a reduction in insulin and glucagon in the post-treated group and a reduction in glucagon only in the pre-treated one. This observation can be discussed as an incomplete recovery of the pancreas or may be due to the elevated levels of ROS that cannot be overcomed by spirulina.

The histological and the immunohistochemical investigations in the amaryl treated diabetic group showed the normal appearance of the pancreas cells and the normal existence of insulin and some reduction in glucagon immunoreactivites.

CONCLUSION

- Spirulina showed no toxicity on the rats which encourage us to use it as hypoglycemic agent.

- The antioxidant activity of spirulina was pronounced in ameliorating the antioxidant parameters to the control non-diabetic group.

- Although there is no great differences found in some parameters between the use of the reference drug and the algae, but the side effects of the drug directed us to make more trust in using the algae.

- Our recommendation is to use spirulina parallel with the drug in a trial to reduce the dose recommended for the drug.

REFERENCES

1. Shaw JE, Sicree RA, Zimmet PZ. Global Estimates of the Prevalence of Diabetes for 2010 and 2030. Diabetes Res. Clin. Pract. J., 2010; 87: 4–14.

2. Okigbo RN, Anuagasi CL, Amadi JE. Advances in Selected Medicinal and Aromatic Plants Indigenous to Africa. Journal of Medicinal Plant Research, 2009; 3(2): 86-95.

3. Kumar S, Kumar V, Prakash O. Antidiabetic and Hypolipidemic Activities of Dillenia indica Extract in Diabetic Rats. Zhong Xi Yi Jie He Xue Bao, 2011; 9(5): 570-574.

4. Ananda Prabu K, Kumarappan CT, Christudas S, Kalaichelvan VK. Effect of Biophytum sensitivum on Streptozotocin and Nicotinamide Induced Diabetic Rats. Asian Pac. J. Trop. Biomed., 2012; 2(1): 31-35.

5. Arafa NA, Amin GE. The epidemiology of Diabetes Mellitus in Egypt: Results of a National Survey. Egypt J. Commun. Med., 2010; 28: 29–41.

6. Achenbach P, Bonifacio E, Koczwara K, Ziegler AG. Natural History of Type 1 Diabetes. Diabetes, 2005; 54 (2), 25-31.

7. Shewade Y, Tirth S, Bhonde RR. Pancreatic Islet-Cell Viability, Functionality and Oxidative Status Remain Unaffected at Pharmacological Concentrations of Commonly Used Antibiotics in vitro. J. Biosci., 2001; 26: 349–55.

8. Rudge MV, Damasceno DC, Volpato GT, Almeida FC, Calderon IM, Lemonica IP. Effect of Ginkgo biloba on the Reproductive Outcome and Oxidative Stress Biomarkers of Streptozotocin-Induced Diabetic Rats. Braz. J. Med. Biol. Res., 2007; 40: 1095–9.

9. Ajjan RA, Grant PJ. Cardiovascular Disease Prevention in Patients with Type 2 Diabetes: The Role of Oral Anti-Diabetic Agents. Diab. Vasc. Dis. Res., 2006; 3(3): 147-58.

10. Bandawane D, Juvekar A, Juvekar M. Antidiabetic and Antihyperlipidemic Effect of Alstonia scholaris Linn Bark in Streptozotocin Induced Diabetic Rats. Indian J. Pharm. Educ. Res., 2011; 45(2): 114-120.

11. Ozsoy-Sacan O, Karabulut-Bulan O, Bolkent S, Yanardag R, Ozgey Y. Effects of Chard (Beta vulgaris L. var cicla) on the Liver of the Diabetic Rats: A Morphological and Biochemical Study. Biosci. Biotechnol. Biochem., 2004; 68(8): 1640-8.

12. Nissen SE, Wolski K. Effect of Rosiglitazone on the Risk of Myocardial Infarction and Death from Cardiovascular Causes. N. Engl. Med., 2007; 356:2457–71.

13. WHO Expert Committee on Diabetes Mellitus: Second Report. Geneva, Switzerland: World Health Organization, Technical report series, 1980; 646. 18.

14. Salazar M, Chamorro GA, Salazar S, Steele CE. Effect of Spirulina maxima Consumption on Reproduction and Peri- and Postnatal Development in Rats. Food Chem. Toxicol., 1996; 34(4): 353-9.

15. Mazo VK, Gmoshinshii IV and Zilova IS . Microalgae spirulina in human nutrition. V. Opr. Pitan., 2004; 73(1): 45 -53.

16. Mohan IK, Khan M, Shobha JC, Naidu MU, Prayag A, Kuppusamy P, Kutala VK. Protection against Ciplatin-Induced Nephrotoxicity by Spirulina in Rats. Cancer Chemother. Pharmacol., 2006; 58: 802-808.

17. Roy KR, Arunasree KM, Reddy NP, Dheeraj B, Reddy GV, Reddanna P.. Alteration of Mitochondrial Membrane Potential by Spirulina platensis C-Phycocyanin Induces Apoptosis in the Doxorubicin-Resistant Human Hepatocellular-Carcinoma Cell Line HepG2. Biotechnol. Appl. Biochem., 2007; 47:159–167.

18. Gorobets OB, Blinkova LP, Baturo AP. Action of Spirulina platensis on Bacterial Viruses. Z. Immunitatsforsch Immunobiol., 2002; 6:18–21.

19. Shih SR, Tsai KN, Li YS, Chueh CC, Chan EC. Inhibition of Enterovirus 71–Induced Apoptosis by Allophycocyanin Isolated from a Blue-Green Alga Spirulina platensis. J. Med. Virol., 2003; 70:119–25.

20. Qureshi MA, Ali RA. Spirulina platensis Exposure Enhances Macrophage Phagocytic Function in Cats. Immunopharmacol. Immunotoxicol., 1996; 18: 457–463.

21. Pugh N, Ross SA, ElSohly HN, ElSohly MA, Pasco DS. Isolation of Three High Molecular Weight Polysaccharide Preparations with Potent Immunostimulatory Activity from Spirulina platensis, Aphanizomenon flos-aquae and Chlorella pyrenoidosa. Planta Med., 2001; 67(8): 737-42.

22. Hernández-Corona A, Nieves I, Meckes M, Chamorro G, Barron BL. Antiviral Activity of Spirulina maxima against Herpes Simplex Virus Type 2. Antiviral Res., 2002; 56(3):279-85.

23. Nagaoka S, Shimizu K, Kaneko H, Shibayama F, Morikawa K, Kanamaru Y. 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.

24. Anuradha V, Vidhya D. Impact of Administration of Spirulina on the Blood Glucose Levels of Selected Diabetic Patients. Indian J Nutr Diet, 2001; 38: 40-44.

25. Layam A, Reddy CLK. Antidiabetic Property of Spirulina. Diabetologia Croatica, 2006; 29-33.

26. Jarouliya U, Anish ZJ, Kumar P, Bisen PS and Prasad GBKS. Alleviation of Metabolic Abnormalities Induced by Excessive Fructose Administration in Wistar Rats by Spirulina maxima. Indian J. Med. Res., 2012; 135(3): 422-428.

27. Zarrouk C. Contribution a l ʾetude d; une cyanophycee. Influence de Divers facteurs Physiques et Chimiques sur la Croissance et la Photosynthese de Spirulina maxima (Setch.et gardner) Geitler Ph.D. Thesis, University of Paris, France, 1966.

28. Milani E, Nikfar, S, Khorasani R, Zamani MJ, Abdollahi, M. Reduction of Diabetes-Induced Oxidative Stress by Phosphodiestrase Inhibitors in Rats. Comp. Biochem. Physiol., 2005; 140 C: 251-255.

29. Bhandari U, Kanojia R, Pillai KK. Effect of Ethanolic Extract of Zingiber officinale on Dyslipidaemia in Diabetic Rats. J. Ethnopharmacol., 2005; 97: 227-230.

30. Finlay JWA, Dillard RF. Appropriate Calibration Curve Fitting in Ligand Binding Assays. AAPS Journal, 2007; 9(2):260-267.

31. Stein EA. In Textbook of Clinical Chemistry, NW Tietz, ed. WB Saunders, Philadelphia, 1986; 879-886.

32. Wahlefeld AW. In Methods of Enzymatic Analysis, Hu Bergmeyer Ed. Academic Press, New York, 1974; 1831-1835.

33. Kachmar JF, Moss DW. In Fundamentals of Clinical Chemistry, 2nd ed .NW Tietz, Editor. WB Saunders, Philadelphia, 1976; 652.

34. Dumas BT and Biggs HG. In Standard Methods of Clinical Chemistry, Vol 7, Academic Press, New York, 1972; 175.

35. Babson LA, Greeley SJ, Coleman CM, Phillips GD. Phenolphthalein Monophosphate as a Substrate for Serum Alkaline Phosphatase. Clin. Chem., 1966; 12, 482-490.

36. Reitman, S and Frankel, S. A Colorimeteric Method for the Determination of Serum Glutamic Oxaloacetic, Glutamic Pyruvic Transaminases. A.J. Clinical. Pathol., 1957; 28: 56-63.

37. Maghraby AS and Bahgat M. Immunostimulatory Effect of Coumarin Derivatives before and after Infection of Mice with the Parasite Schistosoma mansoni. Arzneimittelforschung, 2004; C 54: 445-450.

38. Erden M and Bor NM. Changes in Reduced Glutathione, Glutathione Reductase and Glutathione Peroxidase after Radiation in Guinea Pigs. Biochem. Med., 1984; 31: 217–227.

39. Ruiz- Larrea MB, Leal AM, Liza MH, Lacort M and De Groot H. Antioxidant Effects of Estradiol and 2- Hydroxyestradiol on Iron – Induced Lipid Peroxidation of Rat Liver Microsomes. Steroids, 1994; 59 (6): 383- 388.

40. Gornal AC, Bardawill CJ, David MM, Determination of Serum Proteins by Means of the Biuret Reaction. J. Biol. Chem., 1949; 177: 751- 766.

41. Maghraby A. Effect of Bilharcid on the Immune System of Healthy and Schistosoma mansoni Infected Mice, Thesis submitted to the Faculty of Science, Cairo University, Egypt, 1989; 43-46.

42. Afifi A. Contrbution to the Chemical Carcinogensis in Xenopus. Ph. D.Thesis, UCL, Louvain La Neuve, Belgium, 1986; 90- 91.

43. Ha BG, Shin EJ, Park J, Shon YH . Anti-Diabetic Effect of Balanced Deep-Sea Water and its Mode of Action in High-Fat Diet Induced Diabetic Mice. Mar.Drugs, 2013; 11: 4193-4212.

44. Kumar S, Kumar V and Prakash. Antidiabetic and Hypolipidemic Activities of Kigelia pinnata Flowers Extract in Streptozotocin Induced Diabetic Rats. A. Sian. J. Trop. Biomed., 2012; 2(7): 543-546.

45. Mani UV, Desai S, Iyer UM. Studies on Long Term Effect of Spirulina Supplementation on Serum Lipid Profile and Glycated Proteins in NIDDM Patients. J. Nutraceut., 2000; 2: 25-32.

46. Takai Y. Effect of Water Soluble and Insoluble Fractions of Spirulina over Serum Lipids and Glucose Resistance of Rats. J. Jpn. Soc. Nutr. Food Sci., 1991; 44: 273-7.

47. Qu Y, Lina L, Pana Q, Yanga X and Cheng X. Preventive Effect of Phycocyanin from Spirulina platensis on Alloxan-Injured Mice. Environmental Toxicology and Pharmacology, 2012; 34: 721–726.

48. Jothivel N, Ponnusamy SP, Appachi M, Singaravel S, Rasilingam D, Deivasigamani K and Thangavel S. Anti-Diabetic Activity of Methanol Leaf Extract of Costus pictus D. DON in Alloxan-Induced Diabetic Rats. J. Health Sci., 2007; 53 (6): 655–663.

49. Papaccio G, Pisanti FA, Latronico MV, Ammendola E, Galdieri M. Multiple low-dose and single high-dose treatments with streptozotocin do not generate nitric oxide. J. Cell Biochem., 2000; 77(1):82-91.

50. Calabresi P, Chabner BA. Antineoplastic Agents. In : Goodman A, Rall JW, eds. The Pharmacological Basis of Therapeutics, 8 th Edition. New York: Pergamon press, 1990; 1209-1263.

51. Wuarin L, Namdev R, Burns JG, Fei ZJ, Ishii DN. Brain Insulin-Like Growth Factor-II mRNA Content is Reduced in Insulin-Dependent and Non-Insulin-Dependent Diabetes Mellitus. J. Neurochem.,1996; 67(2):742-51.

52. Lee SH, Park IS. Effects of Soybean Diet on the Beta Cells in the Streptozotocin Treated Rats for Induction of Diabetes. Diabetes Res. Clin. Pract., 2000; 47(1):1-13.

53. Udayakumar R, Kasthurirengan S, Mariashibu TS, Rajesh M, Anbazhagan VR, Kim SC, Ganapathi A, Choi CW. Hypoglycaemic and Hypolipidaemic Effects of Withania somnifera Root and Leaf Extracts on Alloxan-Induced Diabetic Rats. Int. J. Mol. Sci., 2009; 10(5):2367-82.

54. Rodríguez-Hernández A, Blé-Castillo JL, Juárez-Oropeza MA, Díaz-Zagoya JC.. Spirulina maxima Prevents Fatty Liver Formation in CD-1 Male and Female Mice with Experimental Diabetes. Life Sci., 2001; 69(9):1029-37.

55. Moura LP, Gurjao ALD, Filho JCJ,Mizuno J, Suemi C, deMello MAR..Spirulina Exercise and Serum Glucose Control in Diabetic Rats. Arq. Bras. Endocrinol. Metab., 2012; 56 (1):25-32.

56. Mani UV, Lyer UM, Nayak US. Hypocholesterolemic Effect of Spirulina in Patients with Hyperlipidemic Nephritic Syndrome J. Med. Food, 2002; 5: 91-96.

57. Ramamoorthy A, Premakumari S. Effect of Supplementation of Spirulina on Hypercholesterolemic Patients. J. Food Sci. Technol., 1996; 33:124-128.

58. Krishnakumar K, Augustti KT, Vijayammal PL. Hypolipidaemic Effect of Salacia oblonga Wall. Root bark in Streptozotocin Diabetic Rats. Med. Sci., 2000; 28: 65-67.

59. Iwata K, Inayama T and Kato T. Effect of Spirulina platensis on Plasma Lipoprotein Lipase Activity in Fructose – Induced Hyperlipidemic Rats. Journal of Nutritional Science and Vitaminology, 1990; 36: 165 -171.

60. Nassir F, Mazur A, Felgines C, Rayssiguier Y. Age Related Response to Dietary Fructose in the Rat: Discrepancies in Triglyceride and Apolipoprotein B Synthesis as a Possible Mechanism for Fatty Liver Induction in Adult Rat. Proc. Soc. Exp. Biol. Med., 1993; 204: 180-183.

61. Lee EA, Park JE, Choi YJ, Huh KB, KimWY. A Randomized Study to Establish the Effects of Spirulina in Type 2 Diabetes Mellitus Patients. Nutr. Res. Pract., 2008; 2(4):295-300.

62. Maiti R, Jana D, Das UK, Ghosh D. Antidiabetic Effect of Aqueous Extract of Seed of Tamarindus indica in Streptozotocin-Induced Diabetic Rats. J. Ethnopharmacol., 2004; 92(1): 85-91.

63. Klover PJ, Mooney RA. Hepatocytes: Critical for Glucose Homeostasis. Int. J. Biochem. Cell Biol., 2004; 36(5):753-8.

64. Sun Y, Liu S, Ferguson S, Wang L, Klepcyk P, Yun JS, Friedman JE. Phosphoenolpyruvate Carboxykinase Overexpression Selectively Attenuates Insulin Signaling and Hepatic Insulin Sensitivity in Transgenic Mice. J. Biological Chem., 2002; 277: 23301–23307.

65. Peters, T. All about Albumin. San Diego: Academic Press, 1996.

66. Bourdon E, Loreau N, Blache D. Glucose and Free Radicals Impair the Antioxidant Properties of Serum Albumin. FASEB J., 1999; 13(2): 233-44.

67. Young IS, Woodside JV. Antioxidants in health and disease. J. Clin. Pathol., 2001; 54:176–86.

68. Wratten ML, Sereni L and Tetta C. Oxidation of Albumin is Enhanced in the Presence of Uremic Toxins. Ren. Fail., 2001; 23 (3-4): 563-571.

69. Jin L, Xue HY, Jin LJ, Li SY, Xu YP. Antioxidant and Pancreas-Protective Effect of Aucubin on Rats with Streptozotocin-Induced Diabetes. Eur. J. Pharmacol., 2008; 582 : 162–167.

70. Zheng J, Inoguchi T, Sasaki S, Maeda Y, McCarty MF, 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; 15; 304(2): 110-20.

71. El-Agouza I, Rawy A, Saad A, Lashin A and El-Sisi S. Effect of Sulfur Containing Amino Acids and Insulin Injection on Streptozotocin Diabetic Rats. J. Drug Res. Egypt, 2000; 23: 213- 224.

72. Yanardag R, Ozsoy-Sacan O, Orak H and Ozgey Y. Protective Effects of Glurenorm (gliquidone) Treatment on the Liver Injury of Experimental Diabetes. Drug Chem. Toxicol., 2005; 28(4):483-497.

73. Mwafy SN, Yassin MM . Antidiabetic Activity Evaluation of Glimepiride and Nerium oleander Extract on Insulin, Glucose Levels and Some Liver Enzymes Activities in Experimental Diabetic Rat Model. Pakistan Journal of Biological Sciences, 2011; 1-7.

74. Tanaka T, Tanaka M and Mitsui Y.The Cloning and Sequencing of Ribosomal Protein S18 of Parasitic Protozoa, Entamoeba histolytica. DNA Seq., 1998; 8(4): 267-270.

75. Becker E, jakober R, Luft D and Schmiilling RM. Clinical and Biochemical Evaluation of the Algae Spirulina with regard to its Application in the Treatment of Obesity. A double blind cross over study. Nutrition Reports International, 1986; 33: 565 -572.

76. Kajimoto Y, Kaneto H. Role of Oxidative Stress in Pancreatic β-cell Dysfunction, Annals of the New York Academy of Sciences, 2004; 1011: 168–176.

77. Baynes JW. Role of Oxidative Stress in Development of Complications in Diabetes. Diabetes, 1991; 40 (4): 405–412.

78. Reddy SV, Tiwari AK, Kumar US, Rao RJ, Rao JM. Free Radical Scavenging, Enzyme Inhibitory Constituents from Antidiabetic Ayurvedic Medicinal Plant Hydnocarpus Wightiana Blume. Phytother. Res., 2005; 19: 277-81.

79. Bonnefont-Rousselot D, Bastard JP, Jaudon MC, Delattre J. Consequences of the diabetic status on the oxidant/antioxidant balance. Diabetes Metab., 2000; 26:163-76.

80. Dias AS, Porawski M, Alonso M, Marroni N, Collado P S, Gonz´alez-Gallego J. Quercetin Decreases Oxidative Stress, NF-κB Activation, and iNOS Overexpression in Liver of Streptozotocin-Induced Diabetic Rats. Journal of Nutrition, 2005; 135(10): 2299–2304.

81. Di Naso FC, Simoes Dias A, Porawski M, Marroni NAP. Exogenous Superoxide Dismutase: Action on Liver Oxidative Stress in Animals with Streptozotocin-Induced Diabetes. Experimental Diabetes Research, 2011; 2011: 6.

82. Di Naso FC, de Mello R N, Bona S. Effect of Agaricus Blazei Murill on the Pulmonary Tissue of Animals with Streptozotocin-Induced Diabetes. Experimental Diabetes Research, 2010; 2010: 8.

83. Krauss H, Koźlik J, Grzymisławski M, Sosnowski P, Mikrut K, Piątek J and Paluszak J. The Influence of Glimepiride on the Oxidative State of Rats with Streptozotocin-Iinduced Hyperglycemia. Med. Sci. Momni., 2003; 9(11): 389-393.

84. Kim JJ, Choi j, Lee MK, Kang KY, Paik MJ, Jo SK, Jung U, Park HR and Yee ST. Immunomodulatory and Antidiabetic Effects of a New Herbal Preparation (Hemo HIM) on Streptozotocin-Induced Diabetic Mice. Evidence-Based Complementary and Alternative Medicine, 2014; (2014): Article ID 461685.

85. Nemoto-Kawamura C, Hirahashi T, Nagai T, Yamada H, Katoh T and Hayashi O. Phycocyanin Enhances Secretary IgA Antibody Response and Suppresses Allergic IgE Antibody Response in Mice Immunized with Antigen-Entrapped Biodegradable Microparticles. J. Nutr. Sci. Vitaminol., 2004; 50(2):129-136.

86. Grzanna R, Polotsky A, Phan PV, Pugh N, Pasco D and Frondoza CG. Immolina, a High-Molecular-Weight Polysaccharide Fraction of Spirulina, Enhances Chemokine Expression in Human Monocytic THP-1 cells. J. Altern. Complement. Med., 2006; 12(5): 429-435.

87. Hayashi O, Katoh T and Okuwaki Y. Enhancement of Antibody Production in Mice by Dietary Spirulina platensis. 1994; 40(5): 431-441.

88. Zhang X, Cui J, Qiu MC, Li DQ, Zhang P and Zhang JS. The Effects of Cyclosporine A on Immunoglobulins Deposition in Retina of Streptozotocin-Induced Diabetic Rats. Zhonghua Nei Ke Za Zhi, 2008; 47 (2): 125-128.

89. Cui J, Qiu MC, Li DQ, Zhang X, Zhang JS and Zhang P. The Protective Effects of Cyclosporine A on Aortic Immunological Injuries in STZ- Induced Diabetic Rats. Zhonghua Nei Ke Za Zhi, 2010; 38 (5): 440-444.

90. Qureshi MA, Garlich JD and Kidd MT. Dietary Spirulina platensis Enhance Shumoraland Cell-Mediated Immune Functions in Chickens. Immunopharmacol. Immunotoxicol., 1996; 18(3): 465-476.

91. Hayashi O, Hirahashi T, Katoh T, Miyajima H, Hirano T and Okuwaki Y. Class Specific Influence of Dietary Spirulina platensis on Antibody Production in Mice. J. Nutr. Sci. Vitaminol., 1998; 44(6): 841-851.

92. Lei XG and Vatamaniuk MZ. Two Tales of Antioxidant Enzymes on B Cells and Diabetes. Antioxidants and Redox Signaling, 2011; 14 (3): 489-503.

93. Bonnevie-Nielsen V, Steffes MW and Lernmark AA. Major Loss in Islet and B Cell Function Preceds Hyperglycemia in Mice Given Multiple Low Doses of Streptozotocin. Diabetes, 1981; 30: 424-429.

94. Masjedi F, Gol A and Dabiri S. Preventive Effect of Garlic (Allium Sativum L.) on Serum Biochemical Factors and Histopathology of Pancreas and Liver in Streptozotocin – Induced Diabetic Rats. Iran J. Pharm. Res., 2013; 12(3): 325 -338.

95. El-Desouki NI, Tabl GA, Abdel-Aziz KK, Salim EI, Nazeeh N. Improvement in Beta-islets of Langerhans in Alloxan-Induced Diabetic Rats by Erythropoietin and Spirulina. The Journal of Basic & Applied Zoology, 2015; 71: 20 –31.