Original Article

Assessment of Chemical Composition of *Spirulina Platensis* and its Effect on Fasting Blood Glucose and Lipid Profile in Diabetic Rats

Sara El-Moataaz [1], Hanaa Ismael [2], Samar Abo-Rhyem [2 ¥]

[1] Department of Nutrition, Faculty of Allied Medicine, Faros University, Alexandria, Egypt

[2] Department of Nutrition (Food Analysis), High Institute of Public Health, Alexandria University, Egypt

Abstract

Background & Objective(s): *Spirulina platensis* is an incredibly powerful source of proteins, fibers and antioxidants. The aim of the study was to assess the nutritional value of *Spirulina platensis* cultivated in Egypt and study its effect on fasting blood glucose and lipid profile in diabetic rats.

Methods: A total of one kilogram of *Spirulina platensis* was used in the study. The nutritional value of *Spirulina platensis* was characterized by measuring its proximate, vitamins, amino acids, minerals and antioxidants contents. We extended our analysis to study its effect on fasting blood glucose (FBG), low density lipoprotein cholesterol (LDL-C), high density lipoprotein cholesterol (HDL-C), serum triglycerides (TG), and total cholesterol (TC) parameters in sixty male albino rats grouped as 10 control rats and 50 diabetic rats in five groups. The latter comprised 10 diabetic control rats and 40 test rats that were orally administered 10, 20, 30 mg/kg body weight *Spirulina platensis* and 300 mg/kg of the anti-diabetic medication Metformin respectively for four weeks.

Results: *Spirulina platensis* contained high amount of high quality protein, as eight essential amino acids were present (56.79%). It was also a rich source of Ca, phenolic compounds and flavonoids (363.7, 997 and 711 mg/100g respectively). *Spirulina* significantly managed body weight of diabetic rats and reduced blood glucose level in treated groups compared to diabetic control by 67.8% and 68.3% in rats treated by 10 and 30 mg/kg body weight *Spirulina* respectively. Rats administered 30 mg/kg body weight *Spirulina* achieved decreased TG, TC, and LDL-C levels to reach 101.9, 75.43, and 6.2 mg/dl respectively, with improvement in HDL-C level by 58.7% compared to diabetic control rats.

Conclusion: Chemical analysis of *Spirulina platensis* produced in Egypt showed high nutritional value with high concentration of several functional ingredients. In addition, its effect on the measured FBG, LDL-C, HDL-C, TG, and TC parameters in experimental rats was dose dependent.

Keywords: *Spirulina platensis*; diabetes; nutritional value; blood glucose level, lipid profile.

INTRODUCTION

The International Diabetes Federation (IDF) declared Egypt as the ninth country worldwide for its number of diabetic patients with type 2 diabetes mellitus (T2DM). Over the last two decades the prevalence of T2DM in Egypt has rapidly raised to reach 15.6% of all adults aged 20 to 79. This sharp rise may be due to the increase in the risk variables such as obesity, changes in eating pattern and physical inactivity or other distinctive risk factors related to Egypt. Globally, diabetic patients accounted for 12% in 2010, which amounted to around 376 billion adults and the anticipated figure 490 billion in 2030.[1] Primary prevention of T2DM is to reduce insulin resistance and to enhance and maintain β-cell pancreatic function. Lifestyle modification is an important tool for managing weight, altering diet composition and also effective in reducing the risk of developing T2DM.[2] Hippocrates had a deep concept that, food should be the medicine and medicine should be the food. Currently the consumers are interested in that concept by claiming healthy food and always searching for healthier alternatives besides seeking unique requirements such as fat-free foods, sugar-free foods and low calorie items.[3] The selection of particular foods that provide extra advantages beyond the fundamental nutritional needs can

provide a healthier life. Foods generally have dietary importance, aroma and taste, but people are now looking for other physiological characteristics such as vitamin content, antioxidant, immune system enhancement, digestion aid, and anti-cancer properties.[4]

Medicinal plants are used since ancient times as a source of remedies and have a great deal in the therapy of various diseases.[4] Chinese, South Americans and Asian Indians stated that approximately 800 plant species have been reported to have anti-diabetic characteristics through their hypoglycemic activity mechanisms. Antioxidant activity was recorded in the majority of medicinal plants owing to active antioxidant compounds such as flavonoids, anthocyanins, isoflavones, flavones, coumarins, catechins, lignans, and isocatechins, in addition vitamins such as vitamin C and E, b-carotene and α-tocopherol which known to possess antioxidant potential.[5]

Spirulina platensis is a blue-green microalga, which has been marketed and consumed as a human food and has been approved as a food for human consumption by many governments one of them is Egyptian health agencies and associations.[6] It is one of the naturally occurring sources of protein, which, reaching to five times that of meat and can provide the majority of essential and non-essential amino acids. Chemical analysis of *Spirulina platensis* showed the plant as an outstanding source of proteins, vitamins and minerals.[7]

Spirulina platensis is frequently used in traditional medicine to treat and prevent various chronic diseases including cancer, arterial hypertension, obesity, and dyslipidemia.[8] Potential health impacts included immunomodulation, antioxidant, anticancer, antiviral and antibacterial activity, as well as positive effects against malnutrition, hyperlipidemia, diabetes, obesity, inflammatory allergic reactions, heavy metal/chemical-induced toxicity, radiation damage and anemia.[6] Drugs prescribed for diabetics patients often lead to various side effects. Common issues such as nausea, headache, weight gain, bloating, constipation, and diarrhea may arise or other serious problems such as liver damage, heart complication, pancreatitis, tumor, bone loss, erectile dysfunction, psychosis, and muscle spasm are encountered.[6]

The purpose of this study was to characterize the chemical composition of a natural product known as *Spirulina platensis* in addition to studying its hypoglycemic and lipid lowering effect on rats with type 2 diabetes mellitus compared to the widely used anti-diabetic drug.

METHODS

The dried form of *Spirulina platensis* was used in this study. *Spirulina platensis* powder was purchased from National Research Center, Cairo governorate. Chemical composition of *Spirulina platensis* was conducted at Marine Toxins Laboratory, Food Toxins and Contaminants Department, National Research Center in Dokki, and Central Laboratory of High Institute of Public Health, Alexandria University.

Part I: Chemical composition and Nutritional value
To characterize the chemical composition and the nutritional value of *Spirulina platensis*, all the following tests were done in triplicates.
Proximate analysis
1.1 Moisture percentage
Moisture is the measurements of water evaporated at or near boiling point. Moisture was measured according to Association of Official Analysis Chemists (AOAC), (2000) method 990.19.[9]
Calculation:
Moisture (%) = (W1-W2) $\times$ 100 $\div$ wt of sample in grams
Where
W_1 = weight of crucible + sample prior to drying.
W_2 = weight of crucible + sample after drying.
1.2 Ash percentage
Ashing refers to the inorganic residues remaining after oxidation or complete ignition using muffle furnace at temperature of 500 $^{\circ}$C − 600 $^{\circ}$C. Water and volatiles are vaporized and organic substances are burned in the presence of oxygen to CO_2 and oxides of N_2 according to AOAC 900.02 A, (2000) method.[9]
Calculation:

$$\text{Ash (wet) (\%)} = \frac{(\text{wt. crucible and ash} - \text{wt. crucible})}{(\text{wt. crucible and sample} - \text{wt. crucible})} \times 100$$

1.3 Fat percentage
Bligh and dyer (1959) technique, was used for crude fat determination. *Spirulina* powder was mixed with a mixture of methanol and chloroform to give a single phase miscible with water. Additional chloroform was then added to give a separation of phases. The solvents were separated by centrifugation and the chloroform layer containing the dissolved fat was removed then evaporated till dryness on a steam bath.[10]
Calculation:
% Crude fat = weight of fat / weight of sample$\times$100
1.4 Crude protein percentage
Proteins and other organic food components in the sample were digested with sulfuric acid in the presence of catalysts. The total nitrogen was then converted to ammonium sulfate. The digest was neutralized with alkali and distilled into boric acid solution. The borate anions formed were titrated with standardized acid, which was converted to nitrogen in the sample. The result of the analysis represents the nitrogen content of the food since nitrogen also comes from non-protein components and should be multiplied by factor (6.25) to be converted to crude protein using (semi-micro Kjeldahl method) which have been established by AOAC in methods 976.06, 976.05, and 960.52 (2000) method.[9]
1.5 Crude fiber percentage
Crude fiber is the insoluble and combustible organic residue which remains after the sample has been treated

under prescribed conditions. The most common conditions are consecutive treatments with light petroleum, boiling diluted sulfuric acid, boiling diluted sodium hydroxide, diluted hydrochloric acid, alcohol and ether. This empirical treatment provides a crude fiber consisting largely of the cellulose content together with a proportion of lignin and hemicelluloses content of the sample according to AOAC, (2000) method number 991.43.[9]

1.6 Carbohydrate percentage

Carbohydrate content was calculated according to AOAC, (2000) method using the following equation:[9]

Carbohydrates (%) = 100 − (Protein % + Fat % + Ash% + Moisture % + Fiber %).

2. Determination of amino acid profile

Amino acid analysis is a technique based on ion exchange liquid chromatography, used in a wide range of application areas to provide qualitative and quantitative compositional analysis. In the biochrom systems, this basic principle has been refined to produce fully automatic, high speed, sensitive analyses. This is sometimes referred to as classical amino acid analysis.

2.1 Procedure

Biochrom amino acid analysis systems were used. The sample was loaded onto a column of cation-exchange resin. Buffers of varying pH and ionic strength were then pumped through the column to separate the various amino acids. The column temperature was accurately controlled and was adjusted when necessary to produce the required separation. The column eluent was mixed with the ninhydrin reagent and the mixture was allowed to pass through the high temperature reaction coil. In the reaction coil, ninhydrin reacted with the amino acids present in the eluate to form coloured compounds.

The amount of colored compound produced is directly proportional to the quantity of amino acid present in the eluate. From the reaction coil, the eluate/ninhydrin mixture was fed to the photometer unit where the amount of each colored compound is determined by measuring the amount of light absorbed. The light absorption was measured at two wavelengths (570 nm and 440nm), because amino acids produce colored compounds which absorb light with a wavelength of 440nm, whereas other amino acid colored compounds absorb light at 570 nm.

The photometer output was connected either to a two channel chart recorder which plots the amino acid concentrations as a series of peaks or to an appropriate integration system. The retention time of the peak on the chart identifies the amino acid, the area under the peak indicating the quantity of amino acid present. As an amino acid analyzer is a comparative instrument, a calibration analysis had to be performed before starting a series of analyses to produce a standard trace for comparison purposes. After sample analysis, the column was regenerated by pumping a strong base through the column followed by buffer 1 which equilibrates the column prior to the next analysis.[11]

3. Determination of minerals

Calcium, Iron, Phosphorus, Zinc, Potassium and Sodium content of *Spirulina platensis* was determined according to AOAC, (2000) [9] at 550 − 600 °C. Digestion using wet ashing was done then the fluffy ash was diluted with 1:1 (10% HCL: Water). Finally, the filtrated solution was measured in Atomic Absorption Spectroscopy Shimadzu Model (AA-6650),where samples were aspired into a flame and atomized.[9] A light beam was directed through the flame into the detector that measured in which the amount of light absorbed by the atomized element in the flame. Wavelength absorbed in the flame is proportional to the concentration of the element in the sample.

4. Determination of Vitamins

Ten grams of *Spirulina platensis* powder were homogenized with methanol for extraction of water soluble vitamins while acetone-chloroform (30:70 v/v) was used for extraction of fat soluble vitamins. The mixtures were shaken on a vortex mixer for 5 min, centrifuged at 4000 rpm for 5 min and filtered through a Millipore™ filter (45 ìm). The filtrates were evaporated under nitrogen and the residues were re-dissolved in 1ml water for water soluble vitamins (Niacin, Riboflavin and Thiamine) and in 1 ml butanol for fat soluble vitamins (vitamin E, K), that were quantified by HPLC.[12] β- carotene level was determined by spectrophotometric method according to AOAC, (2000) method number 2005.07.[9]

5. Antioxidant activity

5.1 Determination of total antioxidant activity

Total antioxidant activity was determined using documented method by Moukette et al., 2015[13] using the following equation:

$$\% \text{ Inhibition} = \frac{Ac - As}{Ac} \times 100$$

Where Ac: Absorbance of control (methanol)
As: Absorbance of sample

5.2 Quantification of total flavonoid compounds

Total flavonoid content was determined using aluminum chloride ($AlCl_3$) according to a known method developed by Zhishen et al., 1999 using quercetin as a standard. The absorbance was measured at 510 nm by UV- visible spectrometer. The results were expressed as quercetin equivalent mg/g of dried extract (QE/g dried ext).[14]

5.3 Quantification of phenolic compounds by HPLC

High Performance Liquid Chromatography (HPLC) with UV detection was used for identification and detection of phenolic profile of the extract. The solution of *Spirulina platensis* extract was dissolved in pure water in the ratio of 30 mg extract/1 mL water and centrifuged at 4000 rpm for 10 minutes. The supernatant was filtered through a cellulose acetate membrane filter 0.20 μm - 0.45μm. The analysis of the filtrated solution was performed on an Agilent Technologies 1200 HPLC system fitted with a SUPELCOSIL LC-18 column (length 250 mm, diameter 4.6 mm, packaging size 5 mm). The column temperature was set at 20 °C. The mobile phase was made of a mixture

of solution of acetic acid 0.5% (A) by volume and acetic nitrile (B). The elution was performed by using 100% of A for the first 2 minutes of the run, 40% of A and 60% of B from 2 to 60 minutes. The flow rate was set equal to 1 mL/min and the volume 25 μL was injected in the column. Polyphenols were detected by a UV detector (280 nm). The retention times of the identified different phenolic compounds were measured using a single standard solution (100 mg/L). This method was also previously used by Moukette et al., (2015).[13]

Part II: Experimental animal model
1. Preparation of *Spirulina platensis* extract
Three different concentrations of *Spirulina platensis* were prepared 10, 20, and 30 mg/kg BW as stock solutions using distilled water. Each group of rats was orally administrated *Spirulina platensis* extract by esophageal syringe (Gavage).

2. Experimental design
A total of sixty healthy adult male albino rats of Wistar strain weighing around 120 to 150g were procured from the animal house of Faculty of pharmacology, Pharos University. The animals were housed under laboratory conditions (relative humidity 45-65 %, temperature 22 ± 1°C and 12h light and 12h dark cycle). They were fed with standard rodent pellet diet and distilled water ad-libitum. Rats were divided randomly into six equal groups of 10 rats each as follows; Group I (control group) 10 rats were left as control (fed with standard diet only). The remaining fifty rats were injected subcutaneously with double doses of Alloxan, the first dose was 120 mg/kg BW and the second was 60 mg/kg BW.[15]

After injection, animals were left for one week until stabilization of diabetes, provided free access for food and water. Animals were checked for the presence of glycosuria using glucose strips. They were considered to be diabetic when glycosuria was present for 3 consecutive days. Diabetic rats were subdivided into the four groups: i) Group II (diabetic control): 10 diabetic rats were left without treatment, ii) Group III; 10 diabetic rats were treated with 10 mg of *Spirulina platensis* water extract per kg body weight per day, iii) Group IV; 10 diabetic rats were treated with 20 mg of *Spirulina platensis* water extract per kg body weight per day, iv) Group V; 10 diabetic rats were treated with 30 mg of *Spirulina platensis* water extract per kg body weight per day, v) Group VI; 10 diabetic rats were treated with 300 mg of Metformin (a widely used anti-diabetic drug) per kg body weight per day. Doses were given daily for four weeks. At the end of the experimental period (4 weeks), rats were fasted overnight, euthanized by isoflurane inhalation > 5% and blood samples were collected. The separated serum was used for the following biochemical analysis; fasting blood glucose level and serum lipid profile [total triglycerides, total cholesterol (TC), low density lipoprotein cholesterol (LDL-C), and high density lipoprotein cholesterol (HDL-C)].

Weight measurement
Weight of each rat was measured at the base line then weekly from base line till the end of the 4 weeks using a sensitive balance.

Statistical analysis
Data were fed to the computer and analyzed using IBM SPSS software package version 20.0. (Armonk, NY: IBM Corp).[16] Qualitative data were described using numbers and percent. The Kolmogorov-Smirnov test was used to verify the normality of distribution. Quantitative data were described using range (minimum and maximum), mean, standard deviation (SD) and median. Significance of the obtained results was judged at the 5% level.[17]

ANOVA test was used for normally distributed quantitative variables, to compare between more than two groups, and Post Hoc test (Tukey) for pairwise comparisons.

Percent of change was calculated for all of the following measurements: blood glucose level, lipid profile using the following equation:

$$\% \text{ change} = \frac{X_2 - X_1}{X_1} \times 100$$

X_1: Mean of control group, X_2: Mean of dose group
It was calculated for body weight using the following equation:

$$\% \text{ change} = \frac{X_2 - X_1}{X_1} \times 100$$

X_1: weight of control group
X_2: weight of the week

Ethical considerations
Experimental protocol and procedures were approved by the institutional animal care and use committee (IACUC) AU0919022623 of High Institute of Public Health, Alexandria University.

RESULTS

Table 1 illustrates that, *Spirulina platensis* is a rich source of protein which reached 56.79%. The protein content was of high quality as it comprised 8 essential amino acids and 9 of the non-essential amino acids. Concerning mineral analysis, *Spirulina platensis* was rich in Ca, Na, K, and P which amounted for 363.7, 216.7, 170, and 123.1mg/100g respectively. Antioxidant activity of *Spirulina platensis* was 39.18%. Additionally, it was a rich source of phenolics, flavonoids, β carotene and vitamin E that reached 997, 711, 70, and 60 mg/100g respectively. Figure 1 shows that the body weight of all diabetic rat groups decreased after one week then started to increase from the second week till the end of the experiment. The increase of body weight was highest in rats treated with 20 mg *Spirulina platensis* (45.47%) followed by those treated with 10 mg *Spirulina platensis* (42.54%) followed by those treated with Metformin (40.53%) after four weeks of

treatment. The percentage increase of body weight was comparable to that achieved by the normal control group. The lowest increase in body weight was traced in rats treated with 30 mg of *Spirulina platensis* (27.39%).

Table 1: Chemical composition of *Spirulina platensis*

Chemical/Element	Content in *Spirulina platensis* (Mean ± SD)
Proximate composition (%)	
Protein	56.79 ± 1.53
Carbohydrates	13.60 ± 0.60
Ash	10.05 ± 0.31
Lipids	8.33 ± 0.16
Moisture	6.98 ± 0.05
Fibers	4.25 ± 0.25
Essential amino acids (mg/100g)	
Leucine	29.11 ± 0.46
Phenylalanine	23.78 ± 1.23
Lysine	19.10 ± 0.16
Valine	18.40 ± 0.62
Isoleucine	14.12 ± 0.76
Threonine	13.59 ± 0.14
Histidine	13.46 ± 0.50
Methionine	5.31 ± 0.82
Non-Essential amino acids (mg/100g)	
Glutamic acid	47.03 ± 1.12
Arginine	44.91 ± 1.02
Aspartic	36.69 ± 1.09
Alanine	33.8 ± 1.21
Cysteine	3.30 ± 0.41
Tyrosine	19.74 ± 1.03
Serine	18.43 ± 1.14
Glycine	15.0 ± 1.04
Proline	14.88 ± 0.34
Minerals (mg/100g)	
Calcium	363.7 ± 0.73
Sodium	216.7 ± 4.41
Potassium	170.0 ± 2.89
Phosphorus	123.1 ± 1.46
Iron	12.4 ± 0.16
Zinc	2.6 ± 0.21
Vitamins (mg/100g)	
B-Carotene	70.0
Vitamin E	60.0
Niacin	12.2
Riboflavin	3.7
Thiamin B1	3.0
Phenolic compounds	997.1
Flavonoids	711.1
Total antioxidant activity	39.2

There was a highly significant difference in body weight of all treated groups except in rats treated with Metformin.

Table 2 and figure 2 reveal that the highest mean fasting blood glucose (FBG) level was detected in diabetic rats treated with 300 mg/kg metformin (221.8 mg/dl), followed by diabetic rats treated with 20 mg/kg *Spirulina platensis* (160.4 mg/dl), and diabetic rats treated with 10 mg/kg *Spirulina platensis* (158.4 mg/dl), while the lowest FBG was detected in rat group treated with 30 mg/kg *Spirulina platensis* (155.8 mg/dl). There was a highly significant difference between each treated rat group and positive control group regarding the mean FBG level ($p < 0.001$).

Likewise, Table 3 and figure 3 illustrated that the highest mean TC level was detected in diabetic rats treated with Metformin (97.57 mg/dl), followed by diabetic rats treated with 10 mg/kg *Spirulina platensis* (81.36 mg/dl), and diabetic rats treated with 20 mg/kg *Spirulina platensis* (79.43mg/dl). The lowest TC level was detected in rats treated with 30 mg/kg *Spirulina platensis* (75.43 mg/dl). There was highly significant difference between treated rat groups in relation to diabetic control ($p < 0.001$).

Table 4 and figure 4 revealed the highest mean triglycerides level was detected in diabetic rats treated with Metformin (103.7 mg/dl), followed by diabetic rats treated with 10 mg/kg *Spirulina platensis* (102.6 mg/dl), and diabetic rats treated with 30 mg/kg *Spirulina platensis* (101.9 mg/d).

The lowest triglycerides level was detected in rats treated with 20 mg/kg *Spirulina platensis* (101.1 mg/dl). There was a high significant difference between each treated rat groups in relation to diabetic control ($p < 0.001$).

Table 5 and figure 5 showed that the highest percentage change of HDL-C level was detected in diabetic rats treated with Metformin (87%), followed by diabetic rats treated with 30 mg/kg *Spirulina platensis* (58.7%).This level was reduced to 46.7% in diabetic rats treated with 20 mg/kg *Spirulina platensis*. The lowest change of HDL-C level (43.6%) was detected in diabetic rats treated with 10 mg/kg *Spirulina platensis* compared to the diabetic control group.

Table 6 and figure 6 showed that the highest mean value of LDL-C was detected in diabetic rat group which did not receive any treatment (29.4 mg/dl). LDL-C decreased to 12.8 mg/dl in diabetic rat group treated with 10 mg/kg *Spirulina platensis*, followed by 8.8 mg/dl in diabetic rat group treated with 20 mg/kg *Spirulina platensis*, and 7.0 mg/dl in diabetic rat group treated with Metformin. The lowest mean level of LDL-C was detected in diabetic rat group treated with 30 mg/kg *Spirulina platensis* (6.2 mg/dl). There was highly significant difference between different groups in relation to diabetic control ($p < 0.001$).
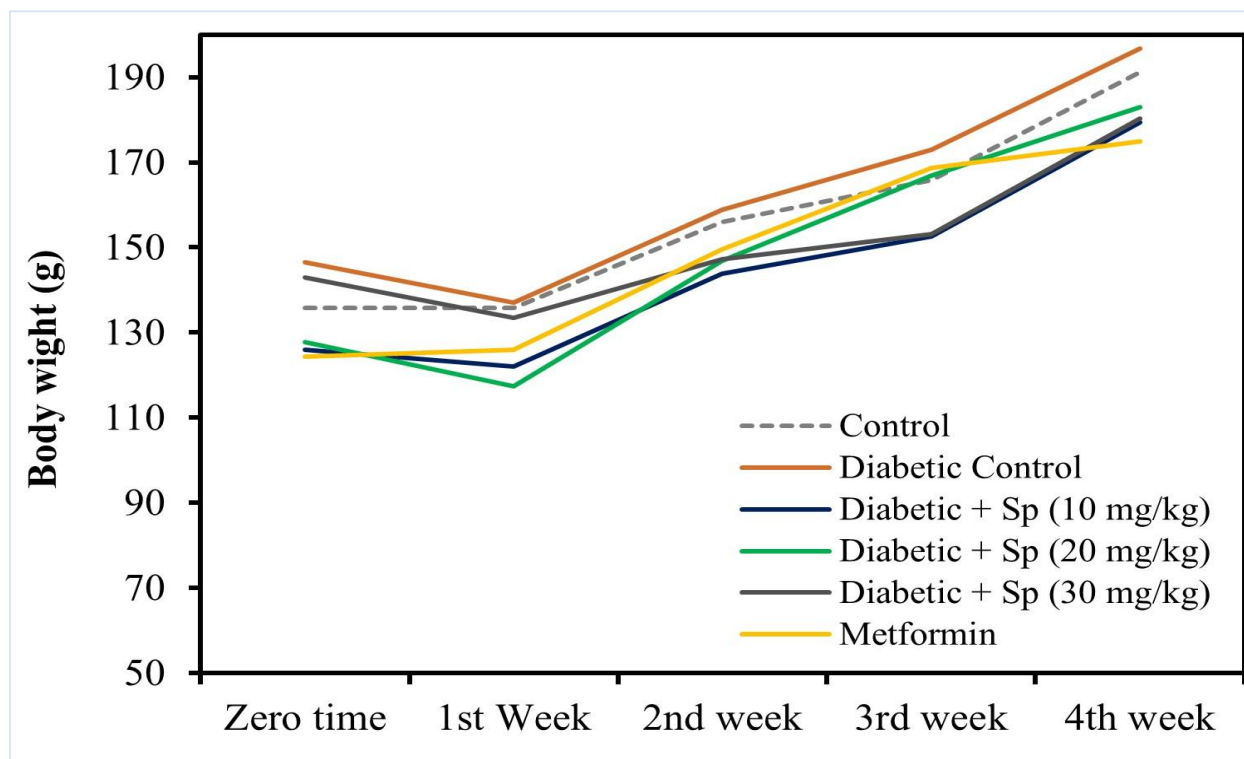
Figure 1: Effect of *Spirulina platensis* on body weight of rat groups treated with different doses

Sp: *Spirulina platensis*

Table 2: Glucose level of rat groups treated with different doses of *Spirulina platensis*

Blood Glucose (mg/dl)	Group						p
	Control (negative)	Diabetic Control (positive)	Diabetic+*Sp* 10 mg/kg	Diabetic+*Sp* 20 mg/kg	Diabetic+*Sp* 30 mg/kg	Metformin 300 mg/kg bw	
Min.	74.0	287.0	109.0	138.0	148.0	171.0	
Max.	152.0	648.0	189.0	196.0	166.0	337.0	<0.001[*]
Mean ± SD.	124.2 ± 31.31	491.2 ± 142.7	158.4 ± 31.09	160.4 ± 22.94	155.8 ± 8.56	221.8 ± 67.89	
p with Negative		<0.001[*]	0.965	0.956	0.975	0.241	
p with Positive			<0.001[*]	<0.001[*]	<0.001[*]	<0.001[*]	

p: *p* for ANOVA test, Pairwise comparison between each 2 groups was done using Post Hoc Test (Tukey)

p with Negative: *p* value for comparing between Control Negative with each other group

p with positive: *p* value for comparing between Control Positive with each other group*: statistically significant at $p \leq 0.05$
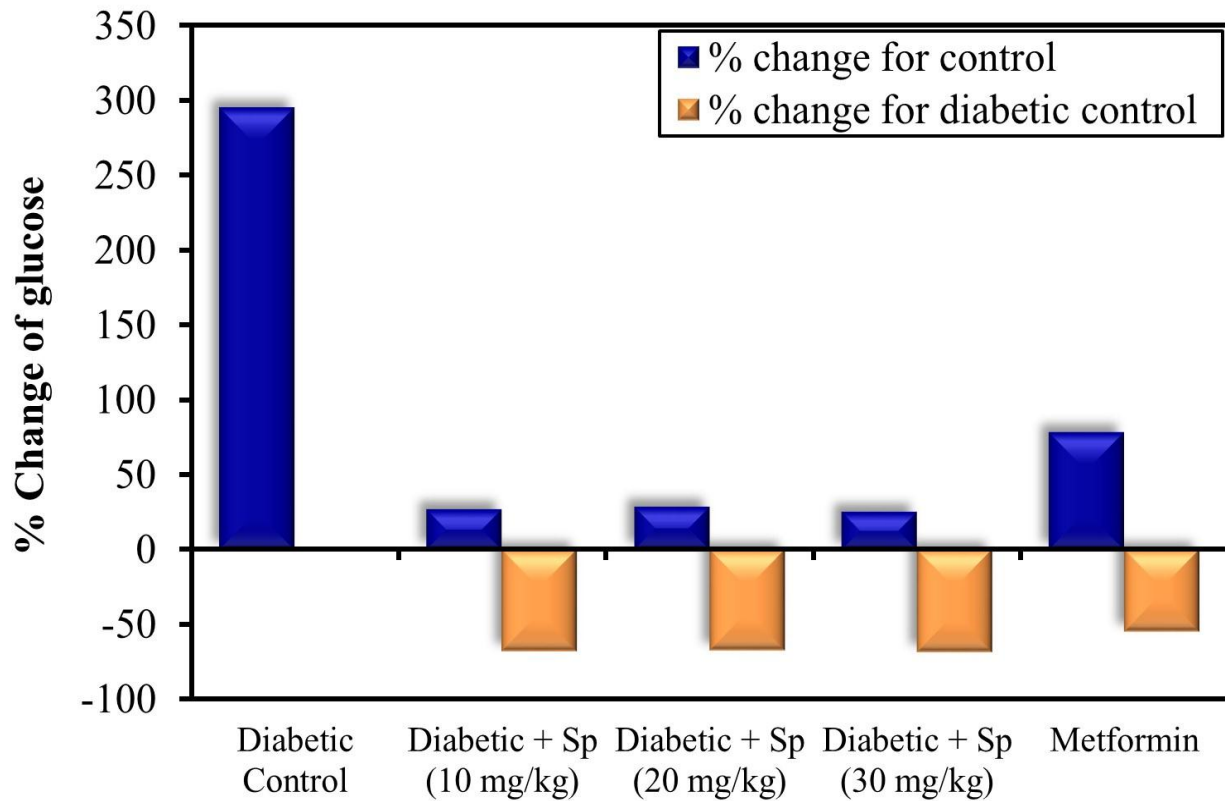
BW: body weight

Sp: *Spirulina platensis*

Figure 2: Percent of change of fasting blood glucose level in different treated rat groups

Sp: *Spirulina platensis*

Table 3: Total cholesterol level of rat groups treated with different doses of *Spirulina platensis*

Total cholesterol (mg/dl)	Groups						*p*
	Control (negative)	Diabetic Control (positive)	Diabetic+*Sp* 10 mg/kg	Diabetic+*Sp* 20 mg/kg	Diabetic+*Sp* 30 mg/kg	Metformin 300 mg/kg bw	
Min.	59.0	225.0	61.0	58.0	61.0	51.0	
Max.	120.0	270.0	97.50	99.0	95.0	145.0	<0.001[*]
Mean ± SD.	86.0 ± 23.44	247.3 ± 15.34	81.36 ± 14.49	79.43 ± 16.56	75.43 ± 10.74	97.57±29.26	
p with Negative		<0.001[*]	0.907	0.997	0.987	0.870	
p with Positive			<0.001[*]	<0.001[*]	<0.001[*]	<0.001[*]	

p: p for ANOVA test, Pairwise comparison between each 2 groups was done using Post Hoc Test (Tukey)
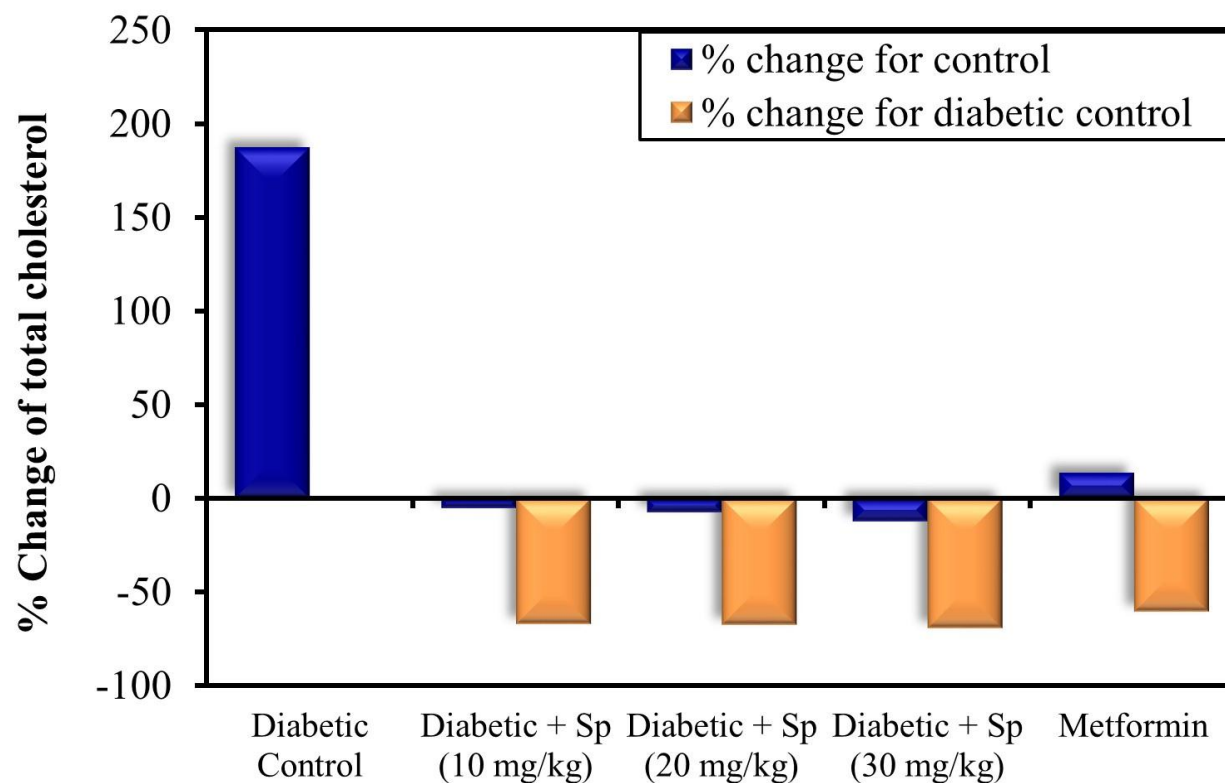
p with Negative: *p* value for comparing between Control Negative with each other group

p with positive: *p* value for comparing between Control Positive with each other group

*: statistically significant at *p*≤ 0.05

BW: body weight

Sp: *Spirulina platensis*

Figure 3: Percent change of total cholesterol in rat groups treated with different doses of *Spirulina platensis*

Sp: *Spirulina platensis*

Table 4: Serum triglyceride level of rat groups treated with different doses of *Spirulina platensis*

Serum triglyceride (mg/dl)	Groups						p
	Control (negative)	Diabetic Control (positive)	Diabetic+*Sp* 10 mg/kg	Diabetic+*Sp* 20 mg/kg	Diabetic+*Sp* 30 mg/kg	Metformin 300 mg/kg bw	
Min.	48.0	120.0	52.0	48.0	49.0	43.0	
Max.	101.0	591.0	166.0	181.0	155.0	168.0	0.006^*
Mean ± SD.	73.86 ± 18.86	231.7 ± 161.0	102.6 ± 40.13	101.1 ± 44.87	101.9 ± 36.33	103.7 ± 38.06	
p with Negative		0.004^*	0.978	0.983	0.981	0.974	
P with Positive			0.029^*	0.026^*	0.028^*	0.031^*	

p: *p* for ANOVA test, Pairwise comparison between each 2 groups was done using Post Hoc Test (Tukey)

p with Negative: p value for comparing between Control Negative with each other group
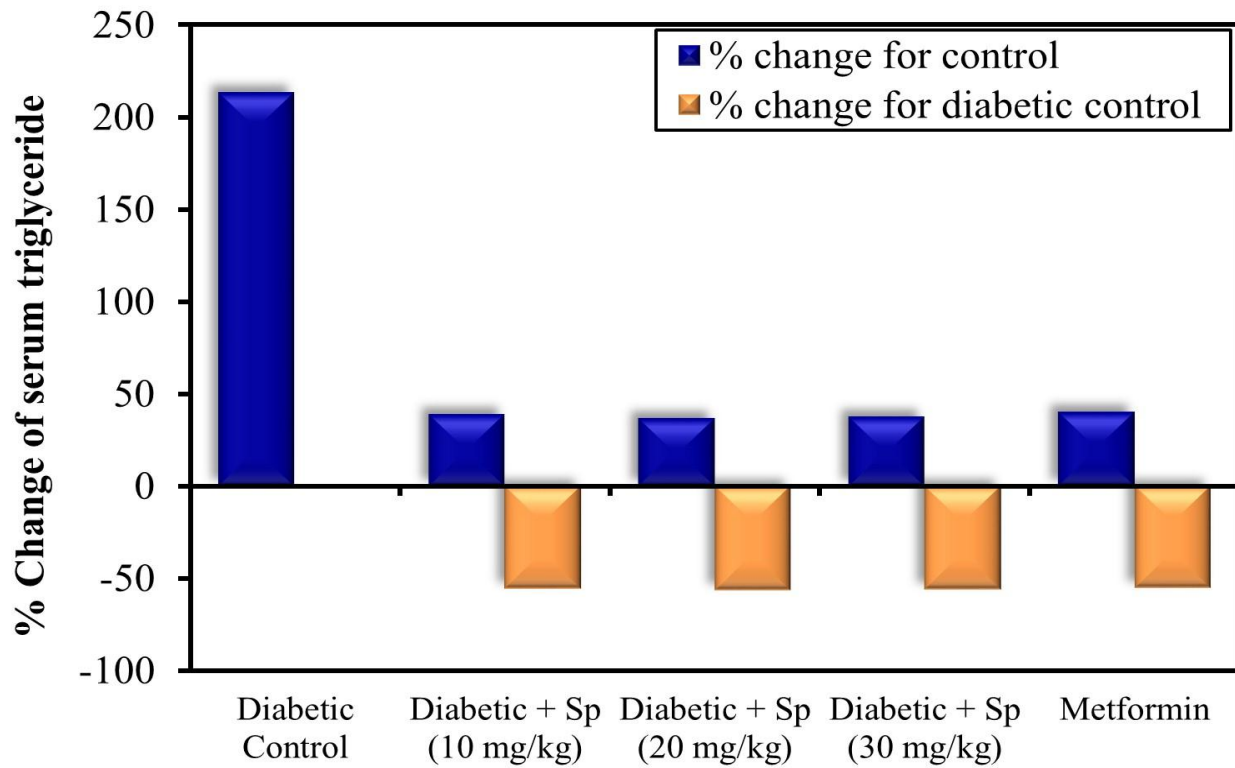
p with positive: p value for comparing between Control Positive with each other group

*: statistically significant at $p \leq 0.05$

BW: body weight

Sp: *Spirulina platensis*

Figure 4: Percent change of serum triglyceride in rat groups treated with different doses of *Spirulina platensis*

Sp: *Spirulina platensis*

Table 5: High Density Lipoprotein Cholesterol (HDL-C) levels (mg/dl) of rat groups treated with different doses

HDL-C (mg/dl)	Groups						*p*
	Control (negative)	Diabetic Control (positive)	Diabetic+*Sp* 10 mg/kg	Diabetic+*Sp* 20 mg/kg	Diabetic+*Sp* 30 mg/kg	Metformin 300 mg/kg bw	
Min.	36.0	35.0	52.0	55.0	55.0	45.0	
Max.	88.0	51.0	90.0	86.0	87.50	129.0	0.001[*]
Mean ± SD.	65.71 ± 16.75	47.14 ± 5.58	67.71 ± 12.51	69.14 ± 10.42	74.79 ± 11.19	88.14 ± 26.49	
p with Negative		0.232	1.000	0.874	0.998	0.091	
p with Positive			0.146	0.020[*]	0.102	<0.001[*]	

p: *p* for ANOVA test, Pairwise comparison between each 2 groups was done using Post Hoc Test (Tukey)
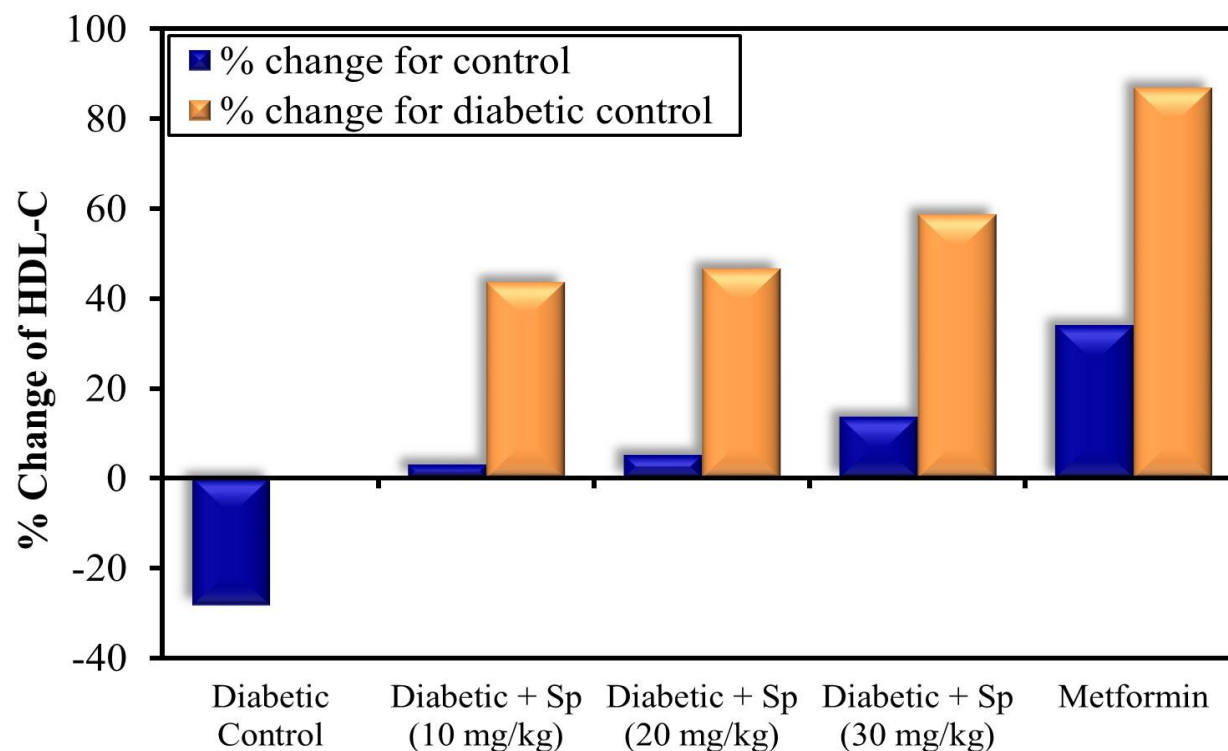
p with Negative: *p* value for comparing between Control Negative with each other group

p with positive: *p* value for comparing between Control Positive with each other group

*: statistically significant at $p \leq 0.05$

BW: body weight

Sp: *Spirulina platensis*

Figure 5: Percent change of High Density Lipoprotein Cholesterol (HDL-C) in diabetic rats

Sp: *Spirulina platensis*

Table 6: Low Density Lipoprotein Cholesterol (LDL-C) levels of rat groups treated with different doses of *Spirulina platensis*

LDL-C (mg/dl)	Groups						p
	Control (negative)	Diabetic Control (positive)	Diabetic+*Sp* 10 mg/kg	Diabetic+*Sp* 20 mg/kg	Diabetic+*Sp* 30 mg/kg	Metformin 300 mg/kg bw	
Min.	8.0	21.0	7.0	5.0	2.0	4.0	
Max.	31.0	42.0	29.0	13.0	13.0	9.0	<0.001*
Mean ± SD.	15.20 ± 9.31	29.40 ± 8.76	12.80 ± 9.18	8.80 ± 3.03	6.20 ± 4.66	7.0 ± 2.0	
p with Negative		0.034*	0.993	0.334	0.683	0.432	
p with Positive			0.009*	<0.001*	0.001*	<0.001*	

p: *p* for ANOVA test, Pairwise comparison between each 2 groups was done using Post Hoc Test (Tukey)
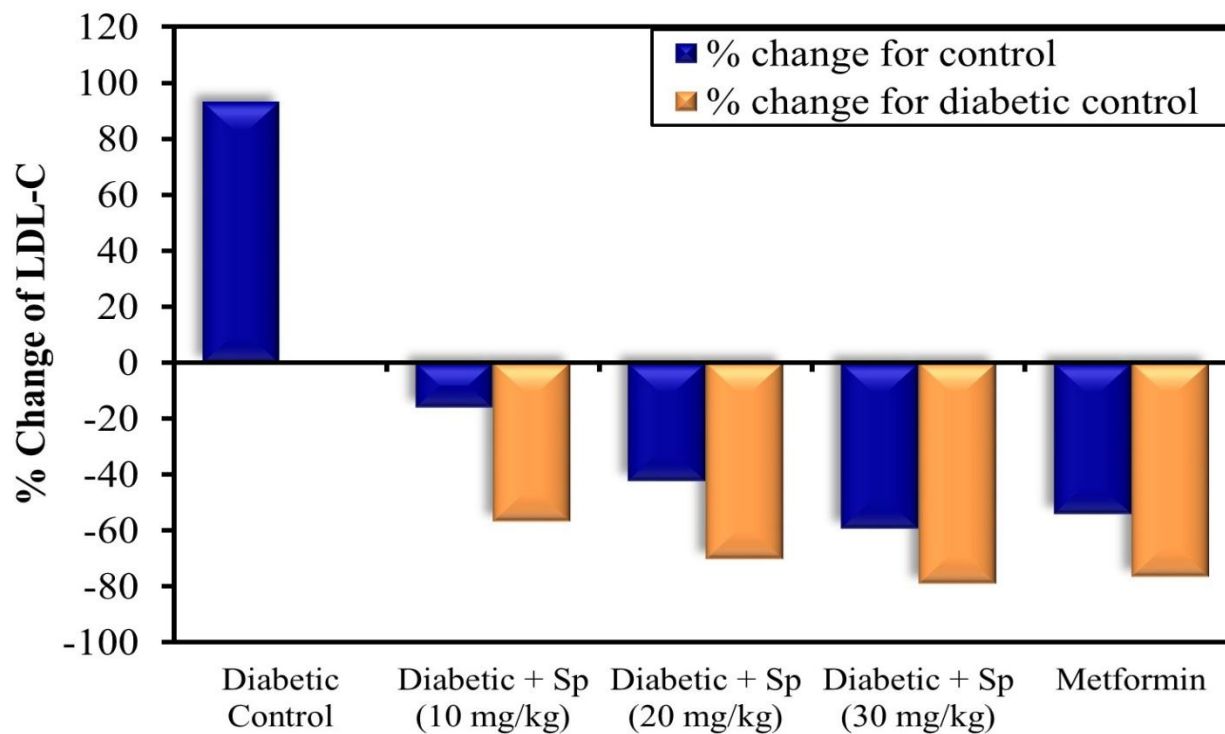
p with Negative: *p* value for comparing between Control Negative with each other group

p with positive: *p* value for comparing between Control Positive with each other group

*: statistically significant at $p \leq 0.05$

BW: body weight

Sp: *Spirulina platensis*

Figure 6: Percent change of Low Density Lipoprotein Cholesterol (LDL-C) in rat groups treated with different doses of *Spirulina platensis*

Sp: *Spirulina platensis*

DISCUSSION

Chemical composition of *Spirulina platensis*
A study conducted by Ali et al., (2017)[18] stated that lipids and ash content of *Spirulina platensis* was 5.3 % and 6.9% respectively which were lower than our results, while protein and fibers content were 61.8 % and 9.5% respectively which were higher than our results. *Spirulina platensis* of our study demonstrated a higher concentration of protein compared to that harvested in Nomayos and Chad which accounted for 37.55% and 50.24% respectively. Our analyses revealed a lower concentration of protein compared to that harvested in Switzerland and Burkina Faso which reached 65% and 61.3% respectively.[19] This variation may suggest that the protein content in *Spirulina platensis* could vary with cultivation period in relation to the photons (sunlight) period.[19, 20] In addition to the quantity, it is also important to assess the protein's quality, which is determined by the contents, proportion and availability of the protein's amino acids. The present study showed that *Spirulina platensis* was enriched with 8 essential amino acids including isoleucine, leucine, lysine, methionine, histidine, threonine, phenylalanine and valine as well as a number of nonessential amino acids including alanine, arginine, aspartic acid, cysteine, glutamic acid, glycine, proline, serine and tyrosine. Such findings were in agreement with Ravi et al., (2010)[21] who specified that *Spirulina platensis* contains 8 essential amino acids.

The current work revealed that cysteine and methionine constituted the lowest concentration of amino acids. This agreed with some authors[22] who stated that *Spirulina platensis* is somewhat low in these amino acids. although this might appear true, we must put into consideration that such a conclusion was reached by comparing amino acid concentrations versus a standard or reference protein usually, egg albumin or milk casein.[22] Mineral analyses in our results were lower than values reported by Nasirian et al., (2018)[23] where Iron, Zinc and Copper contents reached 90, 15 and 20 mg/100g respectively. Another study conducted by Ali et al., (2017)[18] who obtained promising data about Ca, P and Fe, that reached 27%, 84% and 17% respectively. These differences may be due to difference in environmental conditions and climate. In addition, it depends on the soil enrichment by fertilizers and the techniques used for cultivation of *Spirulina platensis*.

Data concerning vitamins were higher than that reported by Cuellar-Bermudez et al.,(2015)[24] who stated that vitamin B1, B2, B3 and E concentrations were 3.5,

0.4, 1.3 and 10 mg/100g respectively. On the other hand, our study showed that the mean concentration of β Carotene was lower than that reported by Cuellar-Bermudez et al., (2015)[24] who found that β Carotene content was 212 mg/100g. This discrepancy may be attributed to the differences in the cultivation area and also depends on the drying techniques.

Effect on Body weight

Diabetic rats treated with Metformin showed improvement in body weight as compared with diabetic rats. This result was in line with that reported Lee et al., (2008)[25], although it disagreed with Lee and Morley, (1998)[26] who stated that Metformin exhibited no change in the body weight with respect to control group. *Spirulina* increased body weight in diabetic rats, which may be explained by increased insulin secretion or increased food consumption, since it helps diabetic patients to maintain their body weight.

Effect on FBG level

The current study demonstrated that a significant does dependent reduction in FBG level in diabetic rat groups treated with increasing doses *Spirulina platensis* compared to diabetic control group. This was in accordance with the study conducted by Metwally (2015)[27] who found that oral administration of *Spirulina* to diabetic rats significantly reduced the FBG level. In fact, *Spirulina platensis* as a blue-green algae (cyanobacteria) can act like insulin or stimulate the β cells of islets of Langerhans to increase the output of insulin, thereby lowering blood sugar level.[28] Furthermore, the hypoglycemic outcome could be due to the effect of fiber content of *Spirulina platensis* that interfered with the absorption of glucose. In the current study, Metformin was used as a reference to evaluate hypoglycemic activity since it has proved to be an effective anti-diabetic agent. Such a strong effect of *Spirulina* is in good agreement with previous studies.[28]

Effect on lipid profile

Diabetic rats exhibited a significant increase in serum TC, triglyceride and LDL-C when compared to normal control rats. This comes in agreement with Hussaini et al., (2018)[29] who reported that Alloxan causes hyperlipidemia in animals. In the current study, there was a significant reduction in serum TC, triglyceride and LDL-C and improvement in HDL-C of diabetic rats treated with different tested doses of *Spirulina platensis* as compared with diabetic control. These results are in agreement with Deng and Chow (2010)[30], who demonstrated that *Spirulina platensis* concentrate could bind cholesterol metabolites "bile acids" and decrease cholesterol solubility. Also, this effect might be due to the presence of γ-linoleic acid in *Spirulina*, which prevents accumulation of fats and cholesterol in human body.

CONCLUSION AND RECOMMENDATIONS

Chemical analysis of *Spirulina platensis* cultivated in Egypt showed high nutritional value with high concentration of functional ingredients including vitamins, minerals, antioxidants, and high quality protein. Tested

doses of *Spirulina platensis* significantly decreased blood glucose levels compared to diabetic control. The effect of *Spirulina* on blood glucose level was dose dependent. *Spirulina platensis* significantly decrease blood cholesterol level in hyperglycemic rats compared to diabetic control in addition it was found to be effective in normalizing the triglyceride levels in hyperglycemic rats. *Spirulina* has a significant potential in improving HDL-C levels in hyperglycemic rats.

Conflict of Interest: None to declare.

REFERENCES

1. Hegazi R, El-Gamal M, Abdel-Hady N, Hamdy O. Epidemiology of and risk factors for type 2 diabetes in Egypt. Ann glob health. 2015;81(6):814-20.

2. Ahmad LA, Crandall JP. Type 2 diabetes prevention: A review. Clin Diab. 2010;28(2):53–9.

3. Laila Kiani. (2007). Natural Miracles: what Functional foods Can Do for You. ProQuest Discovery Guides. http://www.csa.com/discoveryguides/discoveryguides-main.php.

4. Rao MU, Sreenivasulu M, Chengaiah B, Reddy KJ, Chetty CM. Herbal medicines for diabetes mellitus: A review. Int J Pharm Tech Research. 2010;2:1883-92.

5. Prior RL. Fruits and vegetables in the prevention of cellular oxidative damage. Amr J Clin Nutr. 2003;78(3 Suppl):570s-8s.

6. Henrikson R. Earth Food *Spirulina*. Hana, Maui, Hawaii: Ronore Enterprises, Inc. 2012.

7. Azabji-Kenfack M, Dikosso SE, Loni EG, Onana EA, Sobngwi E, Gbaguidi E, et al. Potential of *Spirulina platensis* as a Nutritional supplement in malnourished HIV-Infected adults in Sub-Saharan Africa: A randomised, single-blind study. Nutr Metab insights. 2011;4:29-37.

8. Araldi RP, Rechiutti BM, Mendes TB, Ito ET, Souza EB. Mutagenic potential of Cordia ecalyculata alone and in association with *Spirulina maxima* for their evaluation as candidate anti-obesity drugs. Gen Mole Res. 2014;13(3):5207-20.

9. Association of Official Analysis Chemists (AOAC). International, official methods of analysis. 17th ed. New York, USA: AOAC; 2000. 816-51.

10. Bligh EG, Dyer WJ. A rapid method of total lipid extraction and purification. Can J Biochem Physiol. 1959;37(8):911-7.

11. Pearson D. The Chemical Analysis of Food. 8th ed. London: Churchill JA; 1981.

12. Manz U, Vuilleumier JP. Determination of added canthaxanthin in complete feeds and premixes with HPLC. In: Keller HE, Hoffmann F, editors. Analytical methods for vitamins and carotenoids in feed. Basel, Switherland: La Roch Ltd; 1988. p. 68-71.

13. Moukette Moukette B, Constant Anatole P, Nya Biapa CP, Njimou JR, Ngogang JY. Free radicals quenching potential, protective properties against oxidative mediated ion toxicity and HPLC phenolic profile of a Cameroonian spice: Piper guineensis. Toxicology reports. 2015;2:792-805.

14. Zhishen J, Mengcheng T, Jianming W. The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. Food Chem. 1999;64(4):555-9.

15. Radenkovic M, Stojanovic M, Prostran M. Experimental diabetes induced by alloxan and streptozotocin: The current state of the art. J Pharm Toxicol methods. 2016;78:13-31.

16. Kirkpatrick LA, Feeney BC. A simple guide to IBM SPSS statistics for version 20.0. Student ed. Belmont, Calif.: Wadsworth, Cengage Learning. 2013.

17. Kotz S, Balakrishnan N, Read CB, Vidakovic B. Encyclopedia of statistical sciences. 2nd ed. Hoboken, N.J.: Wiley-Interscience. 2006.

18. Ali O. Effects of dietary supplementing of *spirulina platensis* and chlorella vulgaris microalgae on hematologic parameters. J Pediatri Hematol Oncol. 2017;7(3):163-9.

19. Branger B, Cadudal JL, Delobel M, Ouoba H, Yameogo P, Ouedraogo D, et al. *Spiruline* as a food supplement in case of infant malnutrition in Burkina-Faso. Archives de pediatrie: organe officiel de la Societe francaise de pediatrie. 2003;10(5):424-31.

20. Association française pour l'a1gologie appliquée (AFAA). Actes du premier symposium sur la spiruline *Spirulina platensis* (Gom.) Geitler de l'AFAA. 1982.

21. Ravi M, De SL, Azharuddin S, Paul S. The beneficial effects of *Spirulina* focusing on its immunomodulatory and antioxidant properties. Nutr Diet Suppl. 2010;2:73-83.

22. Morales de Leon J, Camacho ME, Bourges H. Amino acid composition of some Mexican foods. Archivos latinoamericanos de nutricion. 2005;55(2):172-86.

23. Nasirian F, Dadkhah M, Moradi-kor N, Obeidavi Z. Effects of *Spirulina platensis* microalgae on antioxidant and anti-inflammatory factors in diabetic rats. Dovepress J. 2018;11:375-80.

24. Cuellar-Bermudez SP, Garcia-Perez JS, Rittmann BE, Parra-Saldivar R. Photosynthetic bioenergy utilizing CO2: an approach on flue gases utilization for third generation biofuels. J Cleaner Product. 2015;98:53-65.

25. Lee MG, Choi YH, Lee I. Effects of diabetes mellitus induced by alloxan on the pharmacokinetics of metformin in rats: restoration of pharmacokinetic parameters to the control state by insulin treatment. J Pharm Pharm Sci. 2008;11(1):88-103.

26. Lee A, Morley JE. Metformin decreases food consumption induces weight loss in subjects with obesity with type II non-insulin-dependent diabetes. Obes Res. 1998 Jan;6(1):47-53.

27. Metwally NS, Maghraby AS, Farrag EK, El Baky HHA, Farrag ARH, Foda DS, et al. Efficiency of the algae *spirulina platensis* as antidiabetic agent. W J Pharm Res. 2015;4(11).

28. Senthil N, Balu P.M, Murugesan K. Antihyperglycemic effect of *spirulina*, insulin and Morinda citrifolia against streptozotocin induced diabetic rats. Int J Curr Microbiol App Sci. 2013;2:537–59.

29. Hussaini S, Hossain M, Islam M, Rafiq K. Effects of *Spirulina platensis* on alloxan induced diabetic rats. Prog Agricult. 2018;29(2):139-46.

30. Deng R, Chow TJ. Hypolipidemic, antioxidant, and antiinflammatory activities of microalgae *Spirulina*. Cardiovasc Ther. 2010;28(4):e33-45.