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Antioxidant and Antihyperglycemic Activity of *Arthrospira platensis* (*Spirulina platensis*) Methanolic Extract: *In vitro* and *in vivo* Study

Saly F. Gheda#, Atef M. Abo-Shady, Omnia H. Abdel-Karim, Gehan A. Ismail
Botany Department, Faculty of Science, Tanta University, Tanta, Egypt.

THE STUDY evaluated different extracts of *Spirulina platensis* using acetone, methanol, ethanol as well as water solvents for their antioxidant and antidiabetic potential. The results showed that all *S. platensis* extracts exhibited antioxidant activity by using DPPH (2, 2-Diphenyl-1-picrylhydrazyl), reducing power and total antioxidant capacity assays; in addition to their antidiabetic activity which was dependent on the used solvent. Among the tested extracts, *S. platensis* methanolic extract exhibited the highest antioxidant activity for all the tested assays and the maximum inhibitory effects for α-amylase (96.46 %) and α-glucosidase (97.42 %) diabetic enzymes. *Spirulina* methanolic extract showed no toxicity on normal cell lines using MTT assay. Gas chromatography–mass spectrometry (GC-MS) analysis of the methanolic extract revealed the presence of different bioactive compounds mainly phytol, 1-monolinoleoylglycerol trimethylsilyl ether, cholestan-3-ol, 2-methylene- (3β,5α) and fatty acids. These compounds might be acted synergistically to exert their obtained antioxidant and antidiabetic activity. For the *in vivo* study, administration of *S. platensis* methanolic extract (at 15 and 10 mg/Kg body weight (BW) caused antihyperglycemic activity by reducing the elevated blood glucose level. A remarkable decrease in different liver, kidney functions and hyperlipidemia related to diabetes were also detected. Furthermore, the methanolic extract treatment increased the body weight, total protein, albumin and hemoglobin levels as compared with the alloxan induced diabetic rats. Furthermore, treatment of diabetic rats with the extract improved liver and pancreas histopathological disorders related to diabetes. These results recommended using *S. platensis* methanolic extract in developing medicinal preparations for treatment of diabetes and its related symptoms.

Keywords: Antihyperlipidemia, α-glucosidase, α-amylase, Gas chromatography-mass spectrometry (GC-MS), Histopathological disorders.

Introduction

Diabetes mellitus (DM) is happened due to metabolic imbalance characterized by uncontrolled increase in blood glucose level, which is usually due to the lack of insulin, impaired effectiveness of insulin action or tissue insensitivity to insulin (Dastjerdi et al., 2015). DM is classified into two main categories: Type 1 diabetes mellitus, caused due to complete absence of insulin production and Type 2 diabetes mellitus, occurred due to the relative deficiency of insulin secretion and tissue resistance to insulin action (Xu et al., 2018).

Although diabetes mainly comprises chronic levels of hyperglycemia, many diabetic patients, specifically those with type-2, also found to have raised up in the blood pressure (hypertension), chronic high levels of insulin (hyperinsulinemia) (Yoseph & Demo, 2015) and unnatural levels of cholesterol, triglycerides and/or other blood fats (hyperlipidemia) (Mir et al., 2008). In addition, lipoprotein and hematological abnormalities are among the most common complications related to type 2 diabetes. These complications are closely linked to the disease disorders and strictly linked to its diagnoses and treatment procedures. (American Diabetes Association, 2009). Enzymes, such as α-amylase and α-glucosidase in the human body act to hydrolyze starch by pancreatic

#Corresponding author emails: salygheda@yahoo.com; sally.gheda@science.tanta.edu.eg Tel:01000925359
Fax: 3350804 https:// orcid.org-0000-0002-4458-7945.
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α-amylase and absorb glucose by intestinal α-glucosidase, which may swift postprandial hyperglycemia (Ismail et al., 2019). At this time, the offered medication for treating type 2 diabetes performances through the inhibition of these diabetic related enzymes (Rang et al., 2003). Continuous research are being proceeded to explore and identify potential inhibitors of α-amylase and α-glucosidase from natural sources. This could enable for the development of compounds that show no side effects for *in vitro* and *in vivo* antidiabetic medications (Kalita et al., 2018). In this concern, *Spirulina* is gaining attention as a source of potential pharmaceutical, natural medicinal antioxidants and antidiabetic agents (Scaglioni et al., 2018).

In a related context, antioxidants are constituents that delay or avoid the oxidation process by neutralizing or scavenging the free radicals in body cells (Kokabi et al., 2013). Nowadays, antioxidant compounds play a major role in the control of oxidative stress-related diseases, including diabetes, cancer and cardiovascular diseases (Wu & Hansen, 2008). There are many commercial synthetic antioxidants, which are used to reduce the harmful effects of free radicals. But, these synthetic antioxidants may cause side harmful effects (Wijesekara et al., 2011). The search to replace these synthetic antioxidants with natural ones has become a critical exploit in immune pharmacy discovery (Goiris et al., 2012; Abdel-Karim et al., 2020).

Spirulina platensis (*Arthrospira platensis*) is a free-floating microscopic, filamentous, and an oxygenic photosynthetic cyanobacterium occurring in fresh and marine waters. *Spirulina platensis* has been considered as a nutritional super food for humans and animals (El-Sheekh et al., 2014a) because of its richness in proteins, carbohydrates, polyunsaturated fatty acids, sterols and minerals. It is also a natural source for provitamin A and vitamins B12, E and C, as well as phenolic acids, linoleic acid, and phytopigments (El-Baz et al., 2013). These algal compounds act as antioxidant (Rady et al., 1994; Scaglioni et al., 2018; Saeed et al., 2020), antimicrobial (Gheda et al., 2013; El-Sheekh et al., 2014b; Metwally et al., 2020), antidiabetic (Hussaini et al., 2018; Okechukwu et al., 2019), anti-inflammatory (Nasirian et al., 2018), anticancer (Zaid et al., 2015), antihyperlipidemic (Salem et al., 2014), and antiviral (Yakoot & Salem, 2012). In the literature, many studies reported the role of *Spirulina* as powder (Salem et al., 2014; Hussaini et al., 2018; Okechukwu et al., 2019) as antidiabetic agent *in vivo*. Few studies informed the same role for *Spirulina* - derived extracts. In this study we investigated, *in vitro,* the effects of *Spirulina platensis* as antioxidant and antidiabetic using different solvent extracts. The work also extends to examine, *in vivo,* the effect of *Spirulina* methanolic extract treatment on the biochemical parameters and the histological features of the liver and pancreas in the alloxan-induced diabetic rats.

Materials and Methods

Axenic culture of *Spirulina platensis* cyanobacterium was obtained from Phycology Laboratory, Botany department, Faculty of Science, Tanta University, Egypt. Identification of species was confirmed using morphological and taxonomical approaches according to Desikachary (1959) and Prescott (1962). Further confirmation was made using Algae Base (http://www.algaebase.org) as *Spirulina platensis* (Gomont). Thereafter, *S. platensis* was grown on Zarrouk's medium (Zarrouk, 1966).

Cultivation of Spirulina platensis

Each culture vessel (2L) was inoculated with 200mL of *S. platensis* culture and then incubated at 28±2°C and illuminated using continuous fluorescent light tubes at 45μEm^{-2}s^{-1}. The cultures were aerated with an air pump (97% O_2 and 3% CO_2) to accelerate *Spirulina* growth and avoid its settling. The pumped air was sterilized by passing through bacterial filter of 0.45μ*m* pore diameter. The growth was detected by measuring the optical density (OD) at 750nm of the culture day after day to estimate the growth curve. The biomass was harvested at the end of exponential growth phase by centrifugation at 5000rpm for 15 min. The cell pellets were washed three times and resuspended in sterilized distilled water to remove traces of growth medium followed by centrifugation at 5000rpm for 15min afterward each wash. The collected biomass of *S. platensis* was then dried in oven at 50°C for 3 days, powdered by manual mortar and then stored in air-tight containers at -20°C until used in the further experiments of the study (Stein, 1973).

Preparation of S. platensis extracts

Known weight (2.5g) of *S. platensis* powder

was extracted in 100mL each of (80%) acetone, ethanol and methanol solvents in addition to water for 72hrs. in a shaking incubator. Each supernatant was concentrated to dryness under reduced pressure using a rotary evaporator. The extracted materials were dried at 40ºC for 48hrs by using the oven, weighed and stored in a sealed glass vials at 2 – 8ºC in a refrigerator for subsequent experiments.

Antioxidant assays

DPPH free radical scavenging assay

DPPH free radical scavenging was determined according to the procedures described by Ul-Haq et al. (2012). In a glass tube, 2800 µl of 0.1 mM methanolic DPPH solution was mixed with 200µL of *S. platensis* different (acetone, methanol, ethanol and water) extract samples. Each sample was assayed in triplicate. The ampoules were overlaid, shaken well and kept away from the light at 37ºC for 60min. The tubes were centrifuged at 3000 rpm for 5min after incubation. Then, the change in color (from deep violet to light-yellow) of DPPH free radical was measured by taking the absorbance of the reaction mixtures at 517nm on a UV/Visible spectrophotometer. Ascorbic acid was utilized as a standard. The DPPH free radical scavenging percentage for each test sample was calculated by using the following equation:

$$\% \text{ Inhibition} = \frac{Abs\ control - Abs\ extract}{Abs\ control} \times 100$$

IC_{50} value was calculated by GraphPad prism 6 software. The IC_{50} value for ascorbic acid was also calculated.

Reducing power assay

The method of Mohapatra et al. (2016) was used to assess the reducing power of different cyanobacterial extracts. Aliquot (1mL) of *S. platensis* different extracts (acetone, methanol, ethanol and water) was mixed with 2.5mL of 0.2M sodium phosphate buffer (pH 6.6, prepared from 62.5mL of 0.2M Na_2HPO_4 and 37.5mL of 0.2M $NaH_2PO_4H_2O$). After that, 2.5mL of 1% potassium ferricyanide [$K_3Fe(CN)_6$] was added and then incubated in a water bath at 50ºC for 20min. Then, 2.5mL of the supernatant was then diluted with 2.5mL distilled water and 0.5mL of 0.1% ferric chloride solution. The degree of the formed blue-green color was measured on a UV/Visible spectrophotometer at 700nm. Ascorbic acid was used as standard.

Total antioxidant capacity (TAC) assay

The total antioxidant capacity (TAC) was determined according to the method of Ahmed et al. (2013). 0.3mL of *S. platensis* different extracts (acetone, methanol, ethanol and water) were mixed with 3mL of phosphomolybdenum reagent (28mM sodium phosphate and 4mM ammonium molybdate in 0.6M sulphuric acid) in a capped vials. Incubation was then executed for 90min in a boiling water bath at 95°C. After that, the samples were cooled at room temperature and the absorbance was measured at 695nm against a blank (0.3mL solvents without extract). Ascorbic acid was utilized as reference.

Total phenolic content (TPC)

The total phenolic concentration was measured by means of Folin-Ciocalteau method (Cox et al., 2010). 100µL of cyanobacterial extract was mixed with 2.0mL of 2% Na_2CO_3 and allowed to stand for 2min at room temperature. Then, 100µL of 50% Folin-Ciocalteau's phenol reagent was added. Incubation takes place for 30min at room temperature away from the light, then the absorbance was measured at 720nm. Gallic acid was utilized as a standard. The total phenolic contents of the samples were expressed as mg Gallic acid/ g dry weight (mg GAE/g DW).

In vitro antidiabetic enzyme assays

α- amylase inhibition activity

The inhibitory activity of alpha-amylase was determined with minor modifications according to Schomburg & Salzmann (1991). Aliquot of different *S. platensis* (acetone, methanol, ethanol and water) extracts (250µL) and 250µL of 1mM phosphate buffer (pH 7.3 with 30mM $CaCl_2$) containing 0.5mg/mL of **α** -amylase (porcine pancreatic alpha amylase) were mixed; then the solution was incubated for 10 min at 25°C. After incubation, 250µL of 1% soluble starch solution in 1 mM phosphate buffer (pH 7.3 with 30mM $CaCl_2$) were added to each tube at 5sec intervals. The solutions were then incubated for 10min at 25°C. The reaction was shut-off by adding 500µL of dinitrosalicylic acid color reagent. Then, in a boiling water bubble bath the capped vials were incubated for 5min; and subsequently cooled to room temperature. Five ml of distilled water were added to the mixture for dilution then the absorbance was measured spectrophotometrically at 540nm. The inhibitory activity of **α** - amylase was expressed as:

$$\% \text{ Inhibition} = \frac{Abs\ control - Abs\ extract}{Abs\ control} \times 100$$

Concentrations of the most promising extracts that resulted in 50% inhibition of the enzyme activity (IC_{50}) were determined by Graph Pad prism 6 software.

α-glucosidase inhibition activity

The effect of the cyanobacterial extracts on the activity of α-glucosidase enzyme was determined as described by Kazeem et al. (2013) using α-glucosidase derived from *Saccharomyces cerevisiae*. 100µL of 1.0 U/mL α-glucosidase were preincubated with 50µL of different *S. platensis* (acetone, methanol, ethanol and water) extracts for 10min. Then, 50µL of 3.0mM *p*-nitrophenyl glucopyranoside (pNPG) as a substrate dissolved in 20mM phosphate buffer (pH 6.9) were added to begin the reaction. The mixture of the reaction was left at 37°C for 20min then stopped by adding 2mL of 0.1M Na_2CO_3. The α-glucosidase activity was monitored by measuring the releasing yellow colored p-nitrophenol from pNPG at 405nm on a UV/Visible spectrophotometer. The results were expressed as inhibition percentage of the blank control which calculated as:

$$\% \text{ Inhibition} = \frac{Abs\ control - Abs\ extract}{Abs\ control} \times 100$$

Concentrations of the most promising extract resulting in 50% inhibition of enzyme activity (IC50) were determined by GraphPad prism 6 software.

Cytotoxicity (MTT) assay

In order to estimate the safety of the most promising extract for further applications, the cytotoxicity (MTT) assay was established according to Kohler et al. (2005). Human prostatic stromal myofibroblast normal cell line (WPMY-1) was maintained in a standard medium consisting of DEMEM with 10% (v/v) fetal bovine serum and 1% (v/v) penicillin/streptomycin and incubated at 37°C in a 5% CO_2 prior to use. The medium was then replaced with fresh DEMEM – 10% FBS and cells were maintained by sub-culturing until arriving at an acceptable confluence. Then, cells were sowed into 96-well cell culture plates at a concentration of 1×10^4cells/mL and incubated 24hrs at standard condition. After that, cells were treated with different concentrations (250 to 1000µg/mL) of *S. platensis* methanol extract. After 48 hrs incubation period, the medium was removed and 5mg/mL of MTT reagent were suspended to each well and re-incubated for 3-4hrs. The developed formazan crystals were dissolved in 100µL acidified isopropanol and the sample absorbance was measured at 630nm using ELISA microplate reader (Bio-Rad Technology, Japan). MTT assay was made in triplicate for each extract concentration and the viability of the cells was calculated by the following equation:

$$\text{Cell viability \%} = \left(1-\frac{\text{Abs Control} - \text{Abs Sample}}{\text{Abs Control}}\right) \times 100$$

where, Abs sample and Abs control, were absorbance of the cells incubated with and without sample, respectively.

Gas chromatography-mass spectrometry analysis

Methanolic extract of *S. platensis* was subjected to Perkin Elmer model: Clarus 580/560 S Gas Chromatography-Mass Spectrometer for identification of its different secondary phytochemical compounds according to the following acquisition parameters. GC analysis was performed by injecting 1µL of sample on a Column (Rxi- 5 Sil MS column 30m, 0.25mm ID, 0.25 df) of a GC-MS instrument with helium as Carrier Gas. Oven temperature was programed as variable initial temp.: 50°C for 2.50min, 8°C/min to 250°C, hold 5min, 5°C/min to 280°C, hold 2min, injector temp.: 280°C, with Solvent Delay time: 4.00min, transfer temp.: 280°C, source temp.: 200°C, Split ratio: 20:1, Scan: 50 to 600 Da. The documentation of compounds was based on comparison of their mass spectra with the database of the National Institute Standard and Technology (NIST) installed with the GC-MS computer system.

In vivo antidiabetic assays

Animals and housing conditions

Forty-nine male Wister albino rats (200± 50g) were obtained from animal house of the Egyptian Organization for Vaccine and Biological Preparation, Cairo, Egypt. Rats were fed *ad libitum* with a standard pellet diet and left free accessed to tap water. They were housed in individual cages in a well-ventilated animal room at 23°C to 27°C with 12hrs. light/dark cycle. They were kept for two weeks to be acclimatized to the prescribed environmental conditions. All protocols and procedures adopted for the present investigation agreed with the approval of the Institutional Animal Ethics Committee of National Research Center and in accordance with the recommendation of the appropriate care and usage of laboratory animals, regulated by Faculty of Science, Tanta University (IACUC-SCI-

TU-0115 30/3/2019).

Induction of diabetes mellitus in rats

Diabetes mellitus was induced in the animals according to Bromme et al. (2000) by three intraperitoneal (I.P.) injections of alloxan monohydrate freshly dissolved in acetate buffered saline (Merck). The first dose was 150 mg/Kg of body weight (BW). The second dose was at 100mg/kg BW after 48 hrs of the first injection. Finally, the last dose was applied after 5 days of the second dose with alloxan of 100mg/kg BW. The second and third doses were used to ensure the induction of diabetes through the experimental duration. The animals could drink 5% glucose solution overnight to overcome the hypoglycemia shock since alloxan monohydrate can produce fatal hypoglycemia as a result of massive insulin release from the pancreatic cells. Control rats were injected with equivalent amount of citrate buffer alone. After the final dose, the rats were fasted overnight for collection of blood samples and postprandial blood glucose determination; where these samples were drawn from the animal's tail tips. Glucose check was done by Fine test Blood Glucose Monitoring System (Vanamedica compony, Cairo, Egypt.) using test strips, and rats with a blood glucose levels overhead 250mg/dl were considered diabetic. Treatment using the most promising extract was started on the seventh day after the alloxan injection and this was considered the first day of the treatment.

Experimental design

The experiment was taken place for 40 days with oral administration of *S. platensis* methanolic extract at two different concentrations (doses). The dried cyanobacterial extract was dissolved in distilled water to prepare the tested concentrations.

Forty-nine male albino rats were selected for this study and divided into 7 groups of 7 rats in each group (El-Baz et al., 2013; Motshakeri et al., 2014) as follows: Group 1: Normal healthy control rats (NC), daily depends on tap water along the experiment; Group 2: Normal rats orally treated with 15mg/kg BW of *S. platensis* methanolic extract; Group 3: normal rats orally treated with 10mg/kg BW of *S. platensis* methanolic extract*;* Group 4: Treated alloxanized-diabetic control; Groups 5: Diabetic rats orally treated with 15mg/kg BW of *S. platensis* methanolic extract; Group 6: Diabetic rats orally treated with 10mg/kg BW of *S. platensis* methanolic extract; Group 7: Diabetic rats orally administered diabenor 0.3mg/kg BW as an antidiabetic reference drug. Body weights and blood glucose levels were monitored weekly after treatments in all diabetic and normal animal sets.

Samples collection and preparation

After 40 days of treatment, rats in the experiment were fasted overnight (12-14hrs.), anesthetized by sodium pentobarbital (Gadah et al., 2020) and blood samples were collected in EDTA tubes for plasma collection by cardiac puncture with gently shaking and in non-additive test tubes for serum samples. The serum tubes were left for 10min to clot, then centrifuged at 3000rpm for plasma and serum separation. The separated plasma and serum samples were used for hematological and biochemical analysis by using commercially diagnostic kits, as will be detailed in the following section.

Hematological and biochemical analyses

Hemoglobin (Hb) levels: Blood samples were assayed using Swelab Alfa Basic device for hemoglobin measurement.

Liver enzyme activities: The activity of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) enzymes was assayed using BioMed kits protocol (Henry, 1964; Tietz, 1976).

Liver function indices: Quantitative determination of total bilirubin (Burtis & Ashwood, 1999) and albumin in serum (Doumas & Biggs, 1976) were determined using BioMed-Total Bilirubin Assay Kit and BioMed-Albumin Reagent protocols, respectively.

Lipid profile analysis: Quantitative determination of cholesterol was assayed using BioMed-Cholesterol reagent. For the quantitative determination of triglycerides (Fossati & Prencipe, 1982), a BioMed reagent was used. And for High-Density lipoprotein cholesterol, a BioMed HDL cholesterol reagent was used (Young & Friedman, 2001).

Kidney function markers: A BioMed reagents for the quantitative determination of creatinine and urea (Henry, 1974; Vassault et al., 1986), respectively.

Tissue collection and histopathological analysis

The histopathological analysis of the tested rats was performed for demonstration of the histopathological changes due to diabetes induction and the effectiveness of *S. platensis* treatment to ameliorate these pathological features. After blood collection, the liver and pancreas tissues of the sacrificed rats from each group were removed immediately and a part of it was fixed utilizing 10% formalin for histopathological observations (Abdollahi et al., 2011; Tekeleselassie et al., 2013). Both organs were washed with standard saline then fixed utilizing 10 % neutral buffered formalin. For dehydration process, the fixed organs were cut into small sizes and were placed in an identified cassette containing sequence of different concentrations of ethanol 70, 95, and 100% for 5 times, respectively. The organs were rinsed with xylene two times beforehand they were included in paraffin mold. Each cooled paraffin block was cut up to 4mm thicknesses utilizing a microtome. Each piece was floated on the water bath at 45°C and putted up on a glass microscope slide. The slices were then dried on a heater at 60°C for 15min to smelt the wax layer and to lock the slices promptly on the glass slide. For the hematoxylin and eosin (H and E) staining, the sections were de-paraffinized using xylene and rehydrated through a sequence of diluted alcohol concentration. Hematoxylin (H) stains the nuclei of the cell and Eosin (E) is able to stain the cytoplasmic components. Slides were then dehydrated again like previously described and then examined under light microscope.

Statistical analysis

Statistical analysis was performed using one-way ANOVA, two-way ANOVA, compare means in cross-tabulation (IBM SPSS software package version 25, 2017). All results were expressed as mean ± standard deviation (SD) at a significance levels of $P \leq 0.05$ or $P \leq 0.01$.

Results and Discussion

Spirulina platensis culture

The biomass of *S. platensis* was collected after 21 days and estimated to be 3.05±0.53g dry wt./L. For the methanolic extract, 0.5g of the powdered material was obtained after methanol evaporation.

In vitro analyses

Antioxidant assays and total phenolic content

Results in Table 1 showed that radical scavenging activity of DPPH was the maximum for the methanol extract of *S. platensis* with an inhibition percentage of 59.44% and an estimated IC_{50} value of 27.39mg/mL. The same extract exhibited potent reducing activity of 2.85mg AAE/g DW and total antioxidant activity of 3.85 mg AAE/g DW. Oppositely, the water extract of *S. platensis* recorded the lowest reducing power activity (0.65 mg AAE/g DW) and the smallest total antioxidant capacity (0.78mg AAE/g DW) with a concurrent minimum DPPH radical scavenging activity of 14.02% (Table 1). The outcomes of all scavenging activities of the studied

S. platensis using different extracts showed great variance depending on the used solvent polarity. In accordance with these results, *S. platensis* butanol extract displayed the maximum DPPH scavenging activity with an IC50 of 61.7µg/mL (Gouda et al., 2015). In this connection, alkaloid, phenols and flavonoids active compounds were noticed in the dietary supplement of diabetic rats with different *S. platensis* extracts which exhibited antioxidant and antidiabetic activities (Emami & Olfati, 2017). Also, Santos-Sánchez et al. (2019) reported that, microalgae are considered to be the chief source of human antioxidants.

TABLE 1. Antioxidant activity and total phenolic content of different *S. platensis* extracts

Assay	Extracts			
	Water	Methanol	Ethanol	Acetone
DPPH (Inhibition %)	$14.02^{d} \pm 0.38$	$59.94^{a} \pm 0.68$	$55.89^{b} \pm 0.35$	$41.39^{c} \pm 0.52$
Reducing Power Activity (mg AAE/g DW)	$0.65^{d} \pm 0.011$	$2.85^{a} \pm 0.005$	$2.35^{b} \pm 0.014$	$0.88^{c} \pm 0.011$
Total Antioxidant Capacity (mg AAE/g DW)	$0.78^{d} \pm 0.008$	$3.85^{a} \pm 0.008$	$3.11^{b} \pm 0.009$	$1.67^{c} \pm 0.012$
Total Phenolic content (mg GAE/g DW)	$2.14^{d} \pm 0.026$	$5.85^{a} \pm 0.02$	$4.69^{b} \pm 0.04$	$3.74^{c} \pm 0.03$

- Values were mean ± SD of three replicates (n = 3).
- Different small superscripts letters in the same row are significantly different at $P \leq 0.05$.

In a related context, total phenolic contents of various tested extracts in this study ranged between 2.14 to 5.86mg GAE/g DW. Herein also, the methanol extract of *S. platensis* recorded the highest phenolic content (5.86 mg GAE/g DW) followed by the ethanol, acetone and finally, the water extract (Table 1). These results showed the existence of considerable phenols quantities in the different tested extracts of *S. platensis.* This finding was in conformity with that reported by Scaglioni et al. (2018) who stated that, the aqueous extracts of *Spirulina sp.* and *Nannochloropsis sp.* enclosed a high phenolic content which can act as antioxidant. Moreover, our results indicated strong correlation R^2= 0.998 (at P≤ 0.05) between the antioxidant activity using DPPH, reducing power and total antioxidant activity with the estimated total phenolic content of *S. platensis* different extracts. In the same direction, Zaid et al. (2015) and Scaglioni et al. (2018) confirmed the antioxidant activity of the phenolic substances existing in *S. platensis* water extract.

In vitro antidiabetic activity

α- amylase and α-glucosidase inhibition activity

A significant *α*-amylase inhibition activity (at P≤ 0.05) was recorded for *S. platensis* extracts depending on the used solvent. As illustrated in Figs. 1 (a & b), the methanolic extract of *S. platensis* exhibited the maximum *α*-amylase enzyme inhibition activity of 96.46% with an IC_{50} value of 13.31mg/mL compared to Acarbose standard drug which recorded 1.59mg/mL. The same extract was also found to have a strong α-glucosidase inhibitory activity of 97.42% and an IC_{50} value of 9.56mg/mL compared to Acarbose standard drug which recorded 1.03mg/mL. Similar findings were recorded by Gouda et al. (2015) who reported inhibition of α- glucosidase activity by *Spirulina* butanol extract with an IC_{50} of 23µg/mL. Controlling of postprandial hyperglycemia can be done by inhibiting the hydrolyzing enzymes of carbohydrate such as α- amylase and α- glucosidase in the digestive system, thus decreasing the absorption of glucose in the intestine (Pirian et al., 2017). In addition, Okechukwu et al. (2019) confirmed the antidiabetic activity of *Spirulina* by inhibiting both α-amylase and α- glucosidase enzymes. The authors attributed this effect to the direct correlation between the phenolic content and α- glucosidase inhibitory action, excessive production of reactive oxygen species (ROS) or imbalanced antioxidant protection mechanisms (Baynes, 2003), that resulted in diabetes and led to oxidative impairment of pancreas β cells. Supplementation, in dietary meals, with enough amounts of such antioxidant components may be effective in governing diabetic complications (Mohapatra et al., 2016; Nasirian et al., 2018).

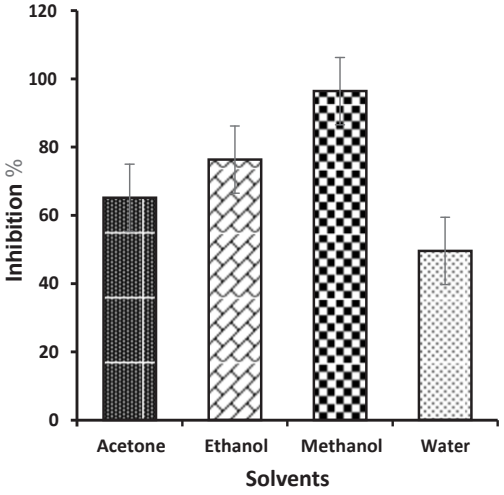

Fig. 1 (a) *In vitro* α-amylase inhibitory activity (at 540nm) of different extracts of *Spirulina platensis*

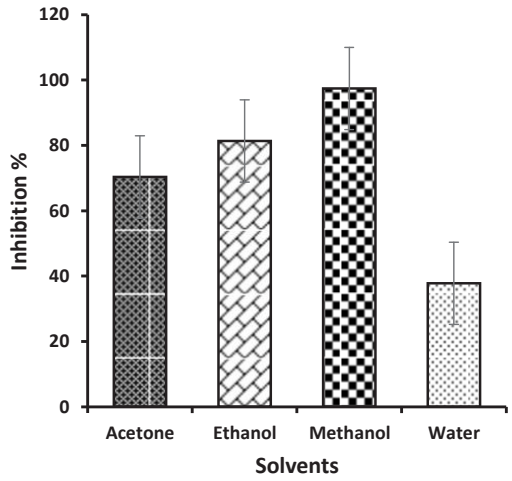

Fig. 1 (b) *In vitro* α-glucosidase inhibitory activity (at 405nm) of different extracts of *Spirulina platensis*

Cytotoxicity assay

The results graphed in Fig. 2 showed that *S. platensis* methanolic extract was safe on human prostatic stromal myofibroblast normal cell line (WPMY-1) at 24 and 48hrs. of incubation period. By increasing the extract concentration, the cell inhibition slightly increased. At minimum

concentration (250µg/mL), the cell proliferation percentage was 100% after 24 and 48hrs., respectively. The percentage of viability was decreased to 62.24% and 54.08% at the maximum extract concentration of 1000 µg/ml, after 24 and 48hrs. of incubation, respectively (Fig. 2). The recorded IC_{50} value for the extract was above the maximum tested concentration (1000µg/mL) which supports the safety of the extracts.

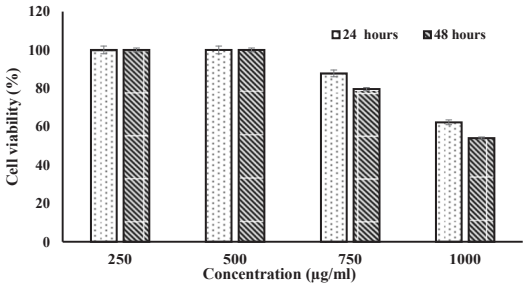

Fig. 2. Cytotoxicity on WPMY-1 normal cell line treated with different concentrations of *Spirulina platensis* methanolic extract

Gas chromatography-mass spectrum (GC-MS) analysis of S. platensis extract

GC-MS analysis of *S. platensis* methanol extract identified different compounds from their corresponding peaks (Fig. 3) which might be responsible for its antioxidant and hypoglycemic properties. The major bioactive compounds were phytol (100%), 1- monolinoleoylglycerol trimethylsilyl ether (71.31%), and the steroid compound cholestan-3-ol, 2-methylene-(3β, 5α) (54.62%). In addition to 9, 12, 15- octadecatrienoic acid, 2, 3-dihydroxypropyl ester (28.21%), hexadecanoic acid methyl ester (23.23%), and the heterocyclic organic compound methenamine (23.21%). These recorded bioactive compounds were listed in Table 2, and may acted synergistically, especially, the phytol, phenolic compounds and the fatty acids methyl esters, to exert their biological role including antidiabetic related activities, α-amylase inhibitory activity as well as antioxidant and hypocholesterolemia activity (Dr. Duke's Phytochemical and Ethnobotanical Databases, 1992-2019).

Because of these promising *in vitro* properties antidiabetic of *S. platensis* methanolic extract, it was further investigated to assess its antidiabetic activity *in vivo.* Alloxan (a β-cytotoxin compound) has been frequently used to induce 'chemical diabetes' in different animals by destructive of the pancreas insulin-secreting cells and changes serum biochemical parameters (Oberley, 1988). It is thus a toxic agent for pancreas β cells that involves over production and decreased utilization of glucose by the tissues (Latner, 1958) thus boosting a hyperglycemia effect in diabetes mellitus. In the present study, injection of alloxan intraperitoneal with a dose of 150mg/kg of body weight effectively induced diabetes in normal rats. Diabetes was diagnosed in the rats when their plasma glucose concentration reaches a value of or more than ≥ 250mg/dL, after 2hrs. (postprandial) of a meal intake.

In vivo study: Effect of S. platensis methanolic extract (ME) on the physiological and biochemical parameters of normal and diabetic - induced rats

Effect of S. platensis methanolic extract on blood glucose level

S. platensis ME at 15 and 10mg/kg body weight doses were orally supplemented daily for 40 days. Postprandial blood glucose level of diabetic control rats surpassed the normal levels (82.33mg/dL) in the control rats to record 250mg/dL after last dose of the injection (before the initiation of the experiment). There was a continuous increase in the postprandial blood glucose level of these alloxan diabetic rats from the first day of the experiment (468.67mg/dL) till the last day (565mg/dL). On the other side, the antidiabetic impact of *S. platensis* ME was significantly observed after 40 days. The diabetic rats treated with *S. platensis* ME at 15mg/kg dose of body weight showed a higher effect in the reduction of glucose level than at 10mg/kg dose estimating 189 and 227mg/dL, respectively. The percentage of glucose reduction was up to 55.49% at 15mg/kg dose. However, this decrement value was close to that estimated for Diabenor drug (at 0.3mg/kg dose) counter levels that was capable to reduce the blood glucose with a percentage of 75.14% by the end of the experiment (Table 3).

Many previous studies supported these results, Venkataraman (1998) reported that using medicinal *Spirulina* powder to treat alloxan-induced diabetic mices resulted in the activation of ß-cells and insulinogenic effects. The hypoglycemic effect may be as a result of excessive secretion of insulin from the β-cells of pancreas, i.e. pancreatic trophic action (El-Alfy et al., 2005). The hypoglycemic effect of *Spirulina maxima* or *S. platensis* powder aids in the diabetes treatment by controlling the increased blood glucose levels in the streptozotocin (STZ) induced diabetic animals (Pandey et al., 2011;

Ripa et al., 2018). Moreover, the administration of 400 mg/Kg of *S. platensis* powder could reduce the adverse effect of plasma hyperglycemia in the alloxan induced diabetic rats as recommended by Hussaini et al. (2018) and Okechukwu et al. (2019). The possible mechanism by which *Spirulina* brings about its antihyperglycemic action may be through improving the pancreatic secretion of insulin from *β*-cell islets or due to enhanced transportation of blood glucose to the peripheral tissue in the *Spirulina* -treated diabetic rats (El-Baz et al., 2013).

Effect of S. platensis methanolic extract on the body weight

Results in Table 3 revealed that diabetic control rats showed a remarkable constant lose in body weight up to a percentage of -23.09% at the 40th. Diabetic rats treated with Diabenor standard drug exhibited a slight improvement in their weight loss recording -2.24%. Notably, treatment of diabetic rats with *S. platensis* ME at both doses caused no loss of body weight along the experiment. Alternatively, the rats showed weight-regain with an estimated overall percentage of -2.57 and -2.98, respectively. These values were comparable to that of the body weight gain recorded for the Diabenor treated diabetic rates (-2.24%) which implying the feasibility and safety of the extract.

Induction of diabetes by alloxan is accompanying with a progressive body weight loss, which may be due to augmented muscle wasting or over breakdown of proteins in the tissues (Chatterjea & Shinde, 2002). In the present study, normal treated rats exhibited slight increment in their body weight whereas untreated diabetic animals showed a contentious reduction in their body weight**.** The administration of *S. platensis* ME as well as Diabenor drug tended to reverse the loss of body weight**.** Significant restoring of the body weight was achieved by taking different oral doses of *S. platensis* ME as treatments comparable to the diabetic control rats (Table 3). In this connection, diabetic rats treated with *Spirulina maxima* showed a regain in their body weight which may be explained by the increased insulin secretion or the increased food consumption (Pandey et al., 2010). Likewise, oral administration of *S. platensis* aqueous extract to diabetic rats for 50 days led to an obvious regain in their body weight loss, suggesting general health status and metabolic mechanisms improvement by effective control of glycemia or reversing of gluconeogenesis (Aissaoui et al., 2017; Hussaini et al., 2018).

Effect of S. platensis methanolic extract on the hemoglobin (Hb) levels

The hemoglobin level is affected by the glucose presence in the blood. An excessive reduction in the hemoglobin levels in the diabetic control rats was observed recording 7.77g/dL (Table 4), after induction of diabetes by alloxan. Alternatively, for the diabetic rats, significant enhancement in the hemoglobin levels of 13.33 and 12.83g/dL was evaluated when orally administrated with 15 and 10mg/kg doses of *S. platensis* ME, respectively. These results were in agreement with that reported by Layam & Reddy (2007) who explained that, the improvement in the Hb level with *Spirulina* administration may be due to the decreased level of blood glucose, that would automatically lead to a decrease in HBA1C. Another reason might be that *Spirulina* is a respectable source of iron which may contributed to the raise of Hb levels (Ripa et al., 2018).

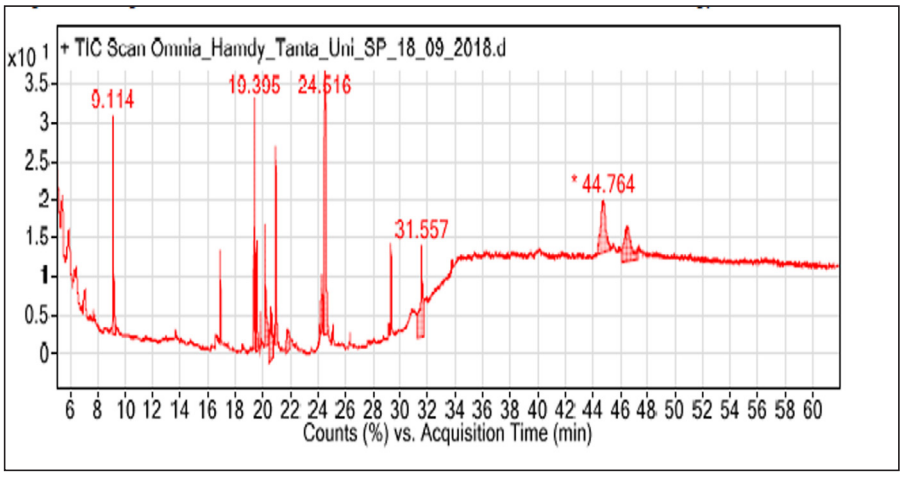

Fig. 3. GC-MS chromatogram of *Spirulina platensis* methanolic extract

TABLE 2. GC-MS analysis of the bioactive compounds existed in *Spirulina platensis* methanolic extract

RT	Compounds name	PA%	MF	Biological activity **
9.114	Methenamine	23.21	$C_6H_{12}N_4$	Antibacterial
19.395	Cholestan-3-ol, 2-methylene-, (3β,5α)-	20.44	$C_{28}H_{48}O$	Anti-Inflammatory, Cytotoxic Activity, Anticancer Antinociceptive, Antioxidant, Antimicrobial, Diuretic and Chemo-Preventive Properties
19.548	Cholestan-3-ol, 2-methylene-, (3β,5α)-	12.76	$C_{28}H_{48}O$	As mentioned
20.176	Cholestan-3-ol, 2-methylene-, (3β,5α)-	21.42	$C_{28}H_{48}O$	As mentioned
20.6	9,12,15-Octadecatrienoic acid, 2,3- dihydroxypropyl ester, (Z,Z,Z)	17.32	$C_{21}H_{36}O_4$	Analgesic, Antipyretic, Anticonvulsant, Antiseptic, Antiviral, Anti- obesity, Anti-inflammatory and CNS depressant activity.
20.956	Hexadecanoic acid, methyl ester	23.23	$C_{17}H_{34}O_2$	Antibacterial, Antifungal, Anti-Inflammatory, Cancer Preventive, Hepatoprotective, Antihistaminic, Antieczemic, Antiarthritic, Anticoronary, Antioxidant, Hemolytic, Hypocholesterolemia, Antiandrogenic, Alpha Reductase Inhibitor, Amylase Inhibitory Activity and Antidiabetic.
24.277	9,12,15-Octadecatrienoic acid, 2,3- dihydroxypropyl ester, (Z,Z,Z)-	10.89	$C_{21}H_{36}O_4$	As mentioned.
24.516	Phytol	100	$C_{16}H_{30}O_2$	Antioxidant, Anticancer, Anti-inflammatory, Anti-diuretic, Antimicrobial, Vaccine Formulations, Anti-malarial, Antinociceptive, and Chemo-preventive Properties
31.557	1-Monolinoleoylglycerol trimethylsilyl ether	32.75	$C_{27}H_{54}O_4Si_2$	Antiarthritic, Anticancer, Hepatoprotective, Antimicrobial, Antiasthma, Anti-Diuretic, Antioxidant, Anti-Inflammatory and Anti-Diabetic
44.764	1-Monolinoleoylglycerol trimethylsilyl ether	38.56	$C_{27}H_{54}O_4Si_2$	As mentioned.

RT: Retention Time; PA: Peak Area; MF: Molecular Formula. ** (Source: Dr. Duke's Phytochemical and Ethnobotanical Databases).

TABLE 3. Effect of *Spirulina platensis* (ME) methanolic extract on blood glucose level and body weight of normal and diabetic induced rats

Treatments (Dose)	Postprandial blood glucose level		Body weight (g)		
	0 day	40 days	0 day	40 days	Weight gain %
Normal control rat	81.67^{aA} ±2.90	82.33^{aA} ±1.00	210.00^{aA} ±10.00	216.67^{aA} ±15.28	4.15^{A}
Normal + S. platensis ME (15mg/kg)	88.33^{aA} ±7.60	86.67^{aA} ±9.70	220.67^{aA} ±20.03	227.67^{aA} ±13.65	3.92^{A}
Normal + S. platensis ME (10mg/kg)	86.83^{aA} ±2.9	84.17^{aA} ±4.00	224.67^{aA} ±15.50	233.33^{aA} ±11.59	3.83^{A}
Diabetic control rat	468.67^{aC} ±16.29	565.00^{bC} ±18.33	201.67^{aA} ±2.89	154.33^{bB} ±5.51	-23.09^{C}
Diabetic + Diabenor (0.3mg/kg)	464.00^{aC} ±21.93	115.33^{bA} ±6.03	208.33^{aA} ±11.93	203.67^{aA} ±4.73	-2.24^{B}
Diabetic + S. platensis ME (15mg/kg)	424.67^{aB} ±30.92	189.00^{bB} ±10.15	194.33^{aA} ±10.69	189.33^{aA} ±2.52	-2.57^{B}
Diabetic + S. platensis ME (10mg/kg)	455.67^{aC} ±9.29	227.33^{bB} ±8.50	190.00^{aA} ±9.17	184.33^{aA} ±6.66	-2.98^{B}

- Values in the same row with the same superscript small letters are insignificantly different at $P \leq 0.05$.
- Values in the same column with the same superscript capital letters are insignificantly different at $P \leq 0.05$.

TABLE 4. Effect of *Spirulina platensis* methanolic extract on hemoglobin, liver and kidney function parameters of blood serum in different groups of rats*

Treatments (Dose)	GPT/ALT (U/L)	GOT/AST (U/L)	Urea (mg/dL)	Creatinine (mg/dL)	Total protein (g/dL)	Albumin (g/dL)	Total bilirubin (mg/dL)	Hemoglobin (g/dL)
Normal control rats	21.00^{a}± 1.00	24^{a}± 1.00	24^{a}± 1.00	0.81^{a}± 0.02	8^{a}± 0.1	4.27^{a}± 0.25	0.44^{a}± 0.03	15.43^{a}± 0.06
Normal + *S. platensis* ME (15mg/kg)	22.33^{a}± 0.58	25^{a}± 1.00	21.33^{a}± 1.53	0.81^{a}± 0.02	7.63^{a}± 0.15	4.27^{a}± 0.25	0.44^{a}± 0.02	15.33^{a}± 0.12
Normal +*S. platensis* ME (10mg/kg)	23.33^{a}± 1.16	28^{a}± 1.00	24.67^{a,c}± 3.79	0.83^{a}± 0.02	7.77^{a}± 0.25	4.20^{a}± 0.25	0.43^{a}± 0.01	15.00^{a}± 0.21
Diabetic control rats	87.33^{c}± 2.08	99.33^{d}± 0.58	90.33^{d} ± 7.23	3.00^{c}± 0.1	4.77^{c}± 0.25	3.00^{d}± 0.10	1.87^{d}± 0.15	7.77^{c}± 0.25
Diabetic + Diabenor (0.3mg/kg)	48.33^{b}± 1.53	53.33^{b}± 1.53	42.67^{b}± 2.08	1.03^{b}± 0.05	6.83^{b}± 0.06	3.60^{c}± 0.36	0.87^{b}± 0.04	12.30^{b}± 0.26
Diabetic +*S. platensis* ME (15mg/kg)	33.33^{a}± 1.53	41.33^{c}± 3.21	31.67^{c}± 1.53	1.03^{b}± 0.04	7.03^{b}± 0.0.6	3.93^{b}± 0.06	0.90^{c}± 0.02	13.33^{b}± 0.23
Diabetic +*S. platensis* ME (10mg/kg)	38.00^{b}± 1.00	46.00^{b}± 1.00	44.33^{b}± 2.08	1.42^{b}± 0.01	6.80^{b}± 0.10	3.63^{c}± 0.38	0.95^{c}± 0.02	12.83^{b}± 0.32

* Values in the same column with the same superscript small letters are insignificantly different at P≤ 0 .05.

Effect of S. platensis methanolic extract on GPT (ALT) and GOT (AST) liver-- function enzymes

As shown in Table 4, a significant increase (at P≤ 0.05) in liver function enzymes glutamate-pyruvate transaminase **(**GPT) and glutamic oxaloacetic transaminase (GOT) (alternatively; ALT: Alanine aminotransferase and AST: Aspartate aminotransferase, respectively) of the diabetic control rats was measured to equal 87.33 U/L for ALT and 99.33 U/L for AST compared to the values measured for normal control rats. Regarding the diabetic rats treated with *S. platensis* ME at 15 and 10mg/kg doses, the results showed a significant reduction in the enzyme functions values of ALT (33.33 and 41.33 U/L) and AST (38.00 and 46.00 U/L) at both doses, respectively. These values were comparable to that measured for Diabenor (0.3mg/kg) standard drug which was evaluated to be 48.33 U/L for ALT and 53.33 U/L for AST enzymes.

AST or ALT levels are valuable primarily aid in the diagnosis of liver disease as liver toxicity markers and also reflecting hepatocellular necrosis (Setorki et al., 2010). The activities of these amino transferase enzymes increase under deficiency of insulin leading to augmented ketogenesis and gluconeogenesis during diabetic disorders (Ohaeri, 2001). The mechanism by which the serum of both aminotransferases are elevated in diabetic animals may involve amplified release of these enzymes from tissues, mainly liver, owing to oxidative stress, progressive formation of glycosylation end products or due to liver dysfunction (Mori et al., 2003). It may be also due to liver tissue induced necrosis in diabetic rats which causes the leakage of these enzymes from the cytosol of the liver into the blood stream and thus gives a sign of hepatotoxic effect (Ohaeri, 2001). Alloxan injection, as a diabetic inducer, has a negative effect on the hepatic tissues, accompanied by an increase in GOT and GPT enzymes (Aissaoui et al., 2017) (Table 4). Treatment of diabetic rats with 15mg/kg BW dose of *S. platensis* ME was more significant in reduction of hepatic transaminase levels compared to the diabetic rats. This may be attributed to the hepato-protective properties of *S. platensis* extract because of its anti-inflammatory, antioxidant, membrane-stabilizing and immune correcting actions as recommended by Panigrahi et al. (2010). Our results were also supported by those mentioned by El-Baz et al. (2013), Nasirian et al. (2018) and Okechukwu et al. (2019). All previous studies endorsed the therapeutic effect of *S. platensis* as extracts, powder or as dietary supplementation in reducing serum hepatic levels of AST and ALT of diabetic treated rats compared to the control untreated ones.

Effect of S. platensis methanolic extract on total bilirubin concentration

The concentration of total bilirubin in the diabetic control rats increased significantly to reach 1.87mg/dL. On the other hand, administration of S. platensis ME at 15 and 10mg/Kg doses showed a potent reduction effect on the total bilirubin of 0.90 and 0.95mg/dL, respectively. These values were significantly related to that of Diabenor (0.87mg/dL) for the reduction of total bilirubin concentrations in diabetic rats, as shown in Table 4. In diagnosis, the concentrations of bilirubin may indicate the status of the liver and its damage type (Yakubu et al., 2005). According to the results obtained in this study, the bilirubin value was significantly increased after inducing alloxan in rats. Alternatively, total bilirubin level exhibited a significant reduction in diabetic rats treated with *S. platensis* ME at different doses. The possibility of restoring liver- execratory function in the treated rats may be due to the administration of insulin like protein in *Spirulina* extract (Kuriakose & Kurup, 2010).

Effect of S. platensis methanolic extract on the urea and creatinine levels

The results in Table 4 listed the estimated effect of *S. platensis* ME on the urea levels of rats. The diabetic control rats showed a significant increment in urea levels (90.33mg/dL) comparable to that recorded for normal control rats (24.00mg/dL). At the same time, *S. platensis* ME at 10mg/kg dose and Diabenor drug seems to have the same effect in decreasing the urea levels in diabetic treated rats and estimating 44.33 and 42.67mg/dL, respectively. A more effective reduction in urea level was measured for *S. platensis* ME at 15 mg/Kg dose recording 31.67mg/dL. In a related manner, the results in Table 4 revealed that creatinine levels in the diabetic control rats exhibited a significant increase of 3.00mg/dL compared to its level in the normal control rats (0.81mg/dL). In addition, significant reduction in the creatinine level of the diabetic treated rats was estimated to be 1.03mg/dL followed by 1.42mg/dL at 15 and 10mg/kg doses of *S. platensis* ME, respectively. Obviously, these values were closely weighed to that recorded for Diabenor.

Alloxan augmented the manufacture of reactive oxygen species, enhanced protein carbonylation and lipid peroxidation related with decreased intracellular antioxidant defense in the kidney tissue (Brito et al., 2011) which eventually led to a significant rise in serum urea and serum creatinine in the diabetic rats. According to the results in the present study, the diabetic control rats displayed a significant increment in the creatinine and urea levels, reflecting a drop in the glomerular filtration rate and induction of renal dysfunction (Table 4). The elevated levels of urea and creatinine were reduced significantly after the treatment with different doses of *S. platensis* methanolic extract (Table 4). These findings were in convention with those reported by Salem et al. (2014) and Abbas et al. (2015). Moreover, Ripa et al. (2018) indicated that, the mitigation effect may be due to the potential antioxidant properties of *S. platensis* extract that improved the renal function via attenuation of the oxidative stress-mediated decline in kidney function. This was in agreement with Okechukwu et al. (2019) who reported that, diabetic rats treated with *Spirulina* showed a pronounced reduction in the renal functions through decreasing lipid peroxidation and elevating the antioxidant levels, thus considerably modify the renal damage.

Effect of S. platensis methanolic extract on total protein and albumin levels

The concentration of total protein in the diabetic control rats were significantly declined to 4.77g/dL compared to that of normal control rats (8.00g/dL). On the other hand, treatment of diabetic rats showed significant reverse in the protein level to reach 7.03 and 6.80g/dL at 15 and 10mg/kg doses of *S. platensis* ME, respectively. These values were feasibly compared to the diabetic rats treated with Diabenor (6.83g/dL). In a parallel direction, the albumin level of the diabetic control rats decreased significantly much more than its levels in the normal control rats which were evaluated to equal 3.00 and 4.27g/dL, respectively (Table 4). Administration of *S. platensis* ME caused an elevation of the albumin level in the diabetic treated rats of 3.93 and 3.63g/dL at both doses, respectively. These values were notably more effective than that recorded by Diabenor drug (3.60g/dL).

The recorded noteworthy reduction in the total protein content of the diabetic control rats was observed. This may be resulted from the reduction in protein secretion, its intracellular

transport and protein discharge, or due to an obvious increase in the protein excretion (Alderson et al., 2004). An amelioration of the protein profile has been observed in the diabetic rats treated with 15mg/kg of *S. platensis* ME. This improvement was possibly due to the immuno-stimulatory and the antioxidant properties of *S. platensis* and its role in boosting the hepatic function and/or its abundance of proteins (Venkataraman, 1998). Furthermore, *S. platensis* is composed of various amino acids, it may have become a direct source of protein for the rats and produce numerous metabolic effects (Belay, 2002). Similar findings were presented by Senthilkumar & John (2008). Related to the reduction of total protein, hypoalbuminemia is a common problem in the animals with diabetes-associated hypoproteinemia. This may be resulted from the decreased protein synthesis, increased protein degradation, and/or increased urinary excretion of proteins (American Diabetes Association, 2009). In this study, the diabetic control rats exhibited a great reduction in the albumin levels which was reversed by treating the diabetic rats with *S. platensis* ME (Table 4). In the same direction, oral administration with 15mg/kg of *S. platensis* as ethanolic extract or 15mg/kg of *S. platensis* as powder could ameliorate the reduced levels of albumin in the diabetic rats as informed by El-Baz et al. (2013) and Salem et al. (2014), respectively.

Effect of S. platensis methanolic extract on the lipid profile

The data in Fig. 4 illustrated the cholesterol (TC), triglyceride (TG), high-density lipoprotein (HDL) and low-density lipoprotein (LDL) levels in different groups of rats under *S. platensis* ME administration. High increment values were noticed in the TC, TG and LDL levels of the diabetic control rats recording 198.33, 171.00 and 143.47mg/dL, respectively. The same parameters recorded 88.67, 71.00 and 29.80mg/dL, respectively in the normal control rats. In contrast, a highly significant reduction in HDL level was estimated to equal 20.67mg/dL in diabetic rats. The results also showed that, the standard drug Diabenor (0.mg/kg) was effective in the reduction of TC (141mg/dL), TG (144.33mg/dL) and LDL (79.80mg/dL) concentrations in the serum, and oppositely the increase in the concentration of HDL (32.33mg/dL). A more pronounced effect was evaluated for these parameters in the treated diabetic rats which was related to the dose. At 15mg/kg dose, reduction of TC, TG and LDL in the serum concentration recorded 99.00, 92.33 and 39.53mg/dL, respectively, while evaluated 127.00, 137.33 and 64.53mg/dL, respectively, at 10mg/kg dose. A reverse effect on the serum concentration of HDL was estimated to equal 41.00mg/dL and 35mg/dL at both doses, respectively (Fig. 4).

Diabetes commonly triggers abnormal lipid metabolism, in addition to the irregular glucose metabolism, which is reflected as further metabolic diabetic complications. The concentration of the LDL-C (low-density lipoprotein cholesterol) particles increased by increased activation of lipoprotein lipase and lecithin acyl- cholesterol transferases enzymes (Sethi et al., 2004); while it was accompanied by a declined level of cardio-protective HDL-C (high-density lipoprotein cholesterol) (Ma, 2012). Under normal situations, insulin stimulates lipoprotein lipase which hydrolyzes TG while insulin deficiency results in failure of this enzyme activation while elevated the cortisol hormone level, thus causing hyper-triglyceridemia (increase of TG levels in blood) and fat accumulation (hyperlipidemia) leading to hepatomegaly (Shirwaikar et al., 2004). High cortisol levels help the free fatty acids to release from obese tissues into the blood. Moreover, under insulin lack, lipolysis of fat depots increased the outflow of free fatty acids into plasma, which trigger cardio-vascular threat (El-Baz et al., 2013).

Therefore, in the present study, hyperlipidemia estimated in diabetic rats (Fig. 4) may be due to insulin deficiency or the oxidative stress exerted by diabetes and affected lipid metabolism Administration of *S. platensis* ME at both doses significantly decreased serum lipid profile level of TG, TC, LDL as well as reversed the effect of the reduced HDL level (Fig. 4). This hypolipidemic activity may result from the existence of phenolic compounds in the extract which activate lipoprotein lipases enzymes in the muscles while decrease its activity in the adipose tissues. Similar results were obtained by Salem et al. (2014) when orally administrated 15mg/kg BW of *S. platensis* powder for diabetic induced rats as treatment.

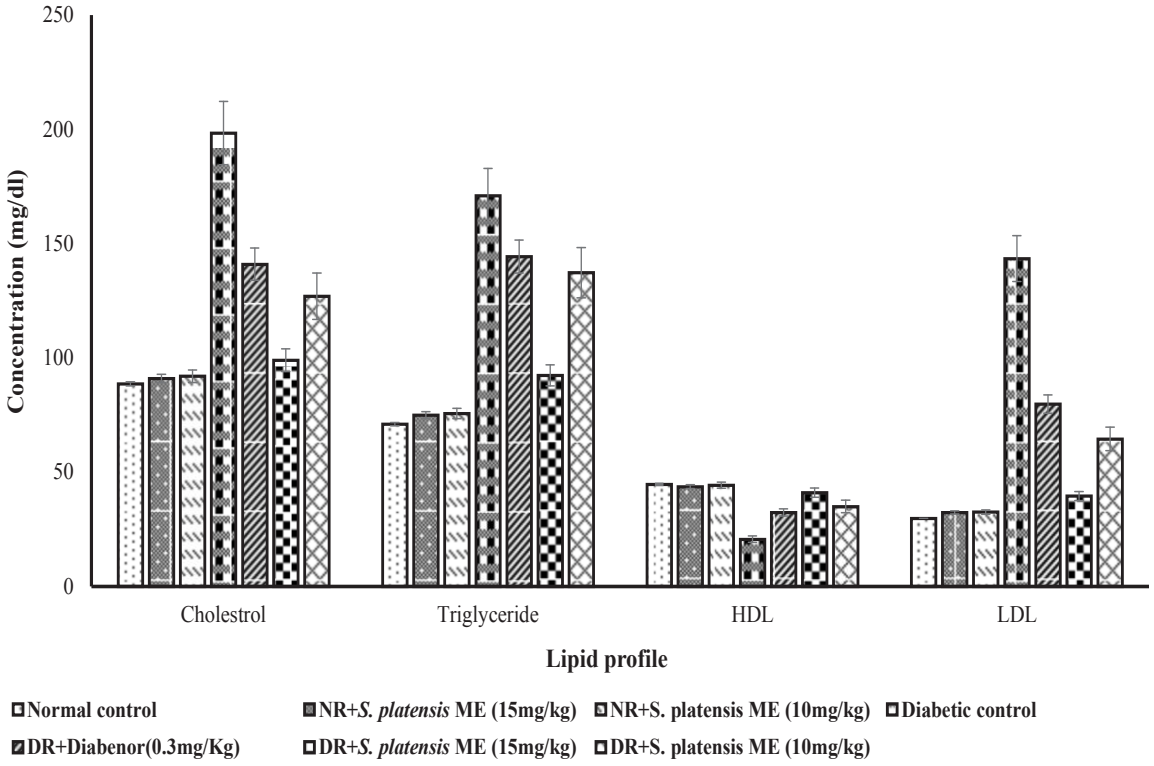

Fig. 4. Effect of *Spirulina platensis* methanolic extract on the lipid profile of normal and diabetic- induced rats

It should be regarded that, according to the obtained results, there was no significant difference in all previously estimated biochemical parameters between normal control rats values and the treated normal rats values at both *S. platensis* ME doses of 15 and 10mg/kg, respectively, indicating the safety of the extract.

Effect of S. platensis methanolic extract on histopathological examinations of normal and diabetic-induced rats

Liver examinations: The histological investigation of the untreated normal rats' liver (normal control) showed that the liver lobe is made up of hepatic lobules. The lobules are shaped hexagonally and consist of hepatocytes plates arising from a central vein with central nucleus. The central vein joins to the hepatic vein to float blood from the liver. The portal triad is a distinguishing constituent of a lobule and run along each corner of the lobule. The portal triad is made up of five structures: a branch of the hepatic artery, a branch of the portal hepatic vein, a bile duct, as well as lymphatic vessels (Fig 5a). Interestingly, liver sections of the normal rats treated with *S. platensis* ME at 15 and 10 mg/Kg doses almost retained the normal structure of liver hepatocytes with rounded vesicular nuclei and homogenous cytoplasm (Figs. 5 b & c).

For diabetic control rats, the liver sections showed distortion in its architecture with degenerative changes such as vacuolated hepatocyte with pyknosis of some nuclei. Also, lacking cytoplasm and destruction of some blood capillaries leading to cell necrosis. Sever cellular infiltration with massive amounts of collagen fibers and inflammatory cells in the portal area surrounding dilated congested central vein were also observed (Fig 5 d).

Alternatively, for liver sections of the diabetic rats treated with *S. platensis* ME (15mg/kg), normal recovery of liver structure was noticed with mild dilatation of some blood vessels. Similar regaining of normal hepatocytes was recorded in the liver sections of diabetic rats treated with *S. platensis* ME at 10 mg/Kg; yet mild cell infiltration and inflammatory cells were still noticed (Figs. 5 e & f). The same amelioration of the liver tissues damage was observed in liver sections of the diabetic rats treated with standard drug Diabenor (Fig. 5 g).

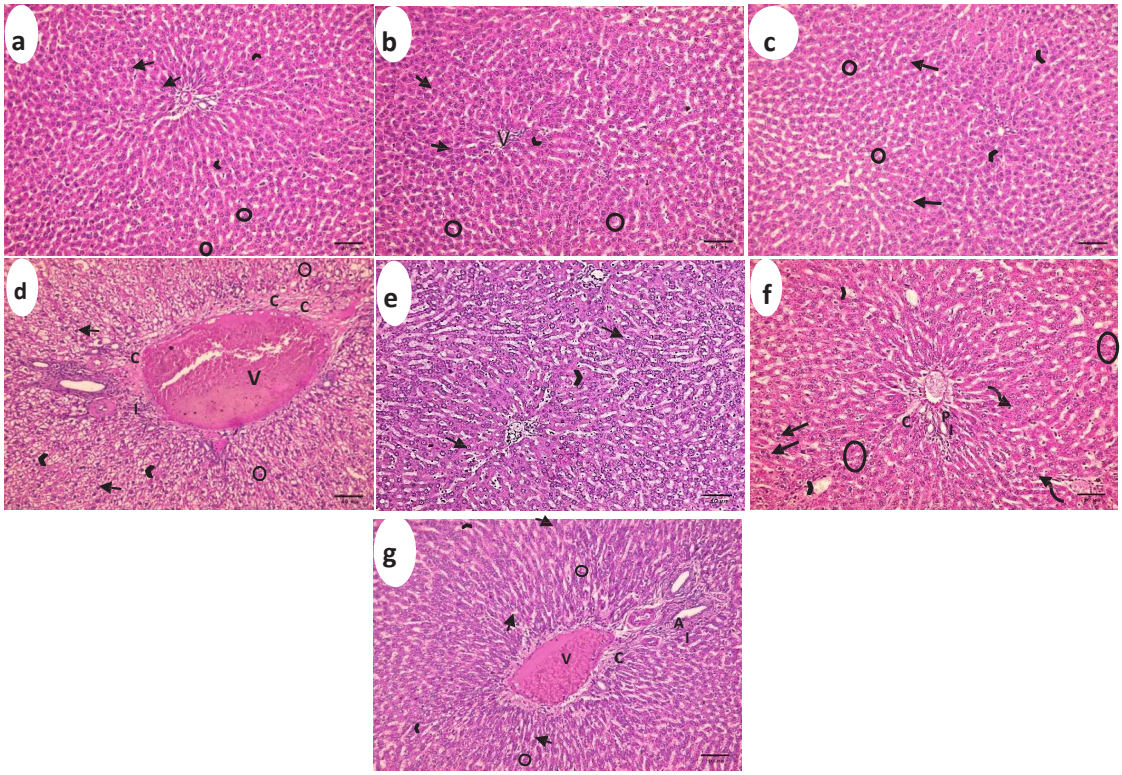

Fig. 5. Effect of different doses of *S. platensis* methanolic extract on albino rats' livers. Photomicrograph of liver sections from: (a) Untreated normal rat showing normal architecture and hepatocytes radiating from the portal area, central vein with acidophilic cytoplasm (arrowhead) and central rounded vesicular nuclei (arrow). Some of the cells appear bi-nucleated (circle), (b) Normal rat treated with 15mg/kg of *S. platensis* ME showing almost normal architecture of central vein (V), rounded nucleus (arrow), homogeneous cytoplasm (arrowhead) and normal blood capillaries (circle), (c) Normal rat treated with 10mg/kg of *S. platensis* ME showing most probably normal architect ure of hepatocytes with central rounded vesicular nuclei (arrow) and homogenous acidophilic cytoplasm (arrowhead). Some of the cells appear bi-nucleated (circle), (d) Alloxan diabetic control rat showing distortion of hepatocytes architecture with destruction of some blood capillaries, massive cell infiltration with collagen fibers (C) and inflammatory cells (I) around portal area. Also, other degenerative changes in the form of rarified cytoplasm (arrowhead) and vacuolated hepatocyte (circle), pyknosis of some nuclei (arrow), dilated congested portal vein (V), (e) Diabetic rat treated with 15mg/kg of *S. platensis* ME showing restore of hepatocytes normal architecture with acidophilic cytoplasm (arrowhead) and central rounded vesicular nuclei (arrow), (f) Diabetic rat treated with 10mg/kg of *S. platensis* ME showing mild cell infiltration with collagen fibers (C) and inflammatory cells (I) around portal area (P), most of the surrounding hepatocyte more or less normal, distortion of some hepatocytes with destruction (circle) of some blood capillaries (arrowhead) , vacuolated hepatocyte (curved arrow) and pyknosis of some nuclei (arrow), (g) Diabetic rat treated with 0.3mg/kg of standard drug Diabenor showing moderate distortion of hepatocytes architecture (arrow) with destruction of some blood capillaries (arrowhead), moderate cell infiltration with collagen fibers (C) and inflammatory cells (I) surrounded the portal area ((central vein (C) and central artery (A)). Also, other degenerative changes in the form of rarified cytoplasm and vacuolated hepatocyte (circle) and pyknosis of some nuclei (curved arrow). (H&E x250)

Liver plays an imperative role in the excretion and elimination of undesirable substances from the body. The results of the current study showed histopathological changes in the liver sections of diabetic control rats compared to *Spirulina* extract and Diabenor treated animals. Similar observations were also informed by Zhou et al. (2008) and Aboonabi (2014) who investigated the oxidative damage in the liver tissue of diabetes induced rats. As shown in Fig. 5 e, liver of diabetic rats treated with 15mg/kg BW dose of *S. platensis* ME turned most likely as normal histological structure. This

may be due to the antioxidant activity of the phenolic compounds present in *Spirulina* extract or its proved free radical-scavenging activity (Abdel-Daim et al., 2013, 2016). Therefore, this cyanobacterium can act as anti-hepatotoxicity agent (Abdel-Daim, 2014). These results were in conformity with those described by El-Baz et al. (2013) who displayed the effective role of *S. platensis* ethanol extract at 15mg/kg BW to reverse the hepatic histological changes resulting from diabetes. Similarly, Abbas et al. (2015) described apparent normal histological structure of liver tissues of diabetic rats treated with 200mg/kg BW of *Spirulina* powder, except a marked apoptosis of hepatocytes.

Pancreas examinations: pancreas is a mixed gland formed of exocrine and endocrine parts. The exocrine part includes the pancreatic acini which secret enzymes and the endocrine part that includes the islets of Langerhans which secret pancreatic hormones. The histological investigation of the normal pancreas from the untreated normal control rats showed the acinar pancreatic cells comprise pyramidal shape including basal rounded nuclei. The head of each pyramidal cell holds acidophilic granules and the basal portion is basophilic. The islets of Langerhans are rounded or oval, rich in blood capillaries and distributed between acinar cells (Fig. 6 a). On the same direction, the pancreatic section of normal rats treated with *S. platensis* ME (at 15 and 10mg/kg doses) showed closely normal appearance of acini, islets of Langerhans with the blood vessels almost like those of normal one (Figs. 6 b & c).

For diabetic control rats, histological damages were apparent in pancreas sections such as vacuolization in Langerhans islets cells, inflammation and dilation of many blood capillaries. Also, distorted acini with darkly stained condensed irregular nuclei of acinar cells accompanied by the reduction of islets size (atrophy), and cellular disintegration (Fig. 6 d).

On the other side, the diabetic rats treated with *S. platensis* ME showed a remarkable recovery of the pancreatic islets and acinar cells. Slight improvement in the pancreatic acini, moderate infiltration with inflammatory cells, and minimization of vacuolated islets cells were observed (Figs. 6 e & f) which were mostly triggered with 15mg/kg dose compared to 10mg/kg dose. Concerning sections of diabetic rats treated with Diabenor, similar amendment of the pancreatic structure was noticed such as mild congestion of some blood vessels with vacuolization of islets although distortion of some acinar cells was yet found (Fig. 6 g).

Pancreas plays a crucial role in the regulation of the metabolism of micronutrient. Hence its tissues may be damaged by alloxan induced diabetes. The intracellular deteriorations in the pancreatic β-cell function and on the pancreatic islets morphological changes during the progress of diabetes have inadequate available information, due to the absence of visual observation techniques (Nugent et al., 2008). Moreover, any alterations in the systemic metabolism connect to insensitivity, secretion of insulin, and the loss of glycemic control ability are reflected as alternations in the structure, size, or function of the islets (Nugent et al., 2008). The histopathological investigation of the pancreas revealed that diabetic rats treated with 15mg/kg BW *S. platensis* ME significantly improved the histological architecture of the islets with a mild vacuolation compared to the diabetic control ones (Fig. 6 e). In this connection, Abbas et al. (2015) revealed that, diabetic rats treated with *S. platensis* powder showed hyperplasia in β-cell of the pancreatic tissue and increased the number of Langerhans islet. As well, *S. platensis* supplementation has a potent activity on the free radical scavenging and reduced various indicators of toxicity such as tissue damage in treated rats (Abdel-Daim et al., 2015; Aissaoui et al., 2017).

It was interesting that, according to our results, the standard drug Diabenor exhibited the lowest effect in the improvement of liver and pancreas damage in the diabetic rats compared to that exerted by *S. platensis* ME at different doses. In addition, oral administration of normal rats with *S. platensis* ME at both doses (Figs. 6 b & c) displayed no poisonous effects on liver tissues when compared to the normal control rats (Fig. 6 a). The same behavior has been detected in the pancreatic tissue of *S. platensis* ME treated rats comparing to normal control ones. Collectively, these observations well-matched with our results of the MTT cytotoxicity assay and the biochemical parameters which proved the safety of both tested doses with no detected difference between the treated rat parameters and the normal control ones. In this context, several clinical trials have designated that diet supplementation of *Spirulina* can constitute a beneficial strategy for hazardous health problems related to free radicals including hyperglycemia and atherosclerosis (Belay, 2002; Alam et al., 2016).

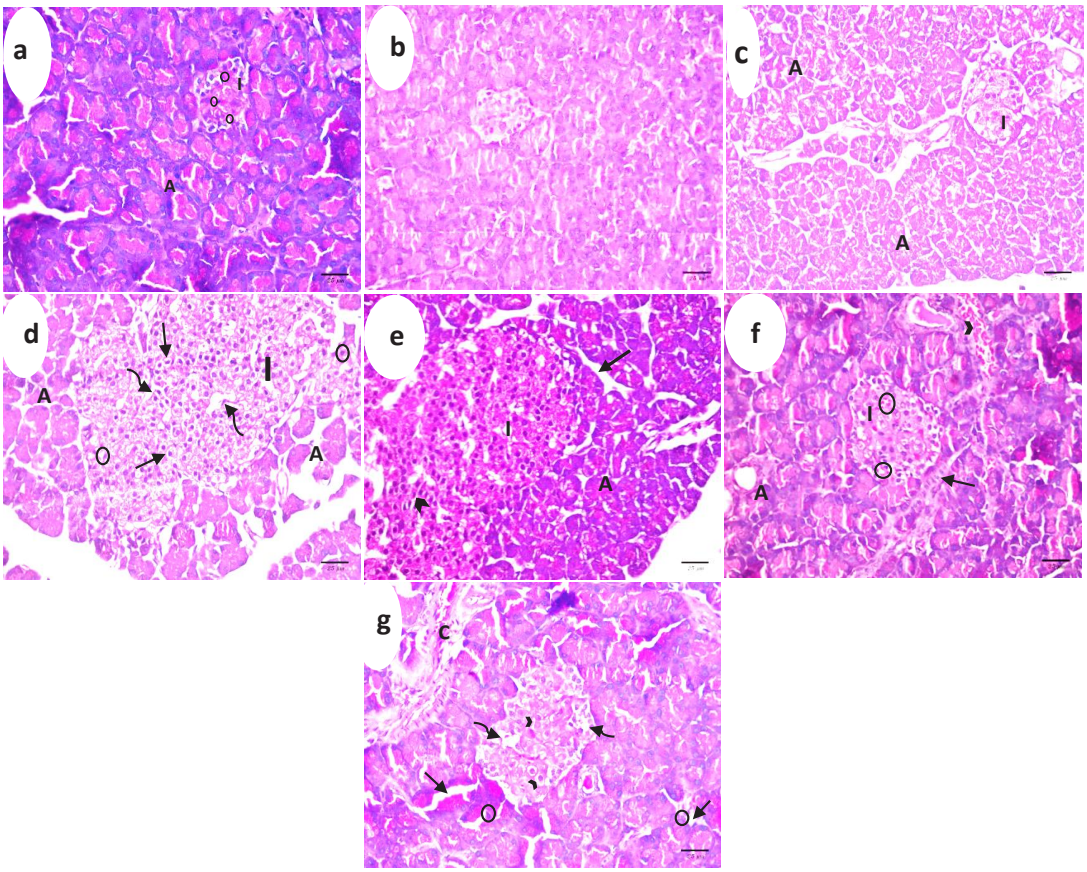

Fig. 6. Effect of different doses of *S. platensis* methanolic extract on albino rats' pancreas. Photomicrographs of pancreas sections from (a) Untreated normal rat showing an islet (I) rich in capillaries (circle) surrounded by many normal pancreatic acini (A); (b) Normal rat treated with 15mg/kg of *S. platensis* ME showing normal islets in which the exocrine part shows normal acini with rounded normal nucleus; (c) Normal rat treated with 10mg/kg of *S. platensis* ME showing the exocrine part with normal acini and mild vacuolation, while the islet is more or less normal; (d) Untreated alloxan diabetic rat showing an islet (I) with dilated capillaries (curved arrow) surrounded by many pancreatic acini (A). Most of islet cells show vacuolation (arrow), while other cells show small darkly stained condensed nuclei (circle); (e) Diabetic rat treated with 15mg/kg of *S. platensis* ME showing the exocrine part of restored normal acini (A) with rounded normal nucleus, while the islet (I) is almost normal; (f) Diabetic rat treated with ME *S. platensis* 10mg/kg showing an islet (I) rich in capillaries (circle) surrounded by many pancreatic acini (A), moderate infiltration with inflammatory cells (arrow) between acini and congestion of some capillaries (arrowhead) are still observed; (g) Diabetic rat treated with 0.3mg/kg of standard drug Diabenor showing the exocrine part with congested (C) blood vessels and distorted acini (arrow) with darkly stained condensed irregular nuclei (circle) of some acinar cells. The islet is vacuolated (arrowhead) in between two type of cells while focal lytic area (curved arrow) can be observed in the islet (H&E x 400).

Conclusion

In the existing study, *S. platensis* methanolic extract showed the highest antioxidant and the maximum activity in the inhibition of both diabetic enzymes. The antioxidant capacity was significantly correlated to the phenolic content of all extracts. GC-MS analysis confirmed the occurrence of bioactive ingredients, especially phytol, phenolic compounds and the fatty acids methyl esters**,** which may be responsible for its antioxidant and antidiabetic activities. The cytotoxicity test on normal cells indicated the safety of *S. platensis* ME. According to the *in vivo* investigations, the diabetic rats treated with *S. platensis* ME caused a notable decrease in the

elevated glucose level, liver functions, kidney functions, total bilirubin and lipid profile. Also, caused a re-gain in the body weight loss, protein profile, albumin, hemoglobin and HDL levels compared to the diabetic rat's group.

Regarding the histopathological studies, *S. platensis* ME treatment caused a substantial improvement and restored the histological tissue damage related to diabetes induction in the liver and pancreas tissues without causing any side effects. Therefore, *S. platensis* methanolic extract has a potential health beneficial applications as antioxidant and antidiabetic drugs for pharmaceutical industry.

Conflict of interest: The authors declare that there is no conflict of interest.

Author contribution: All authors contributed to the study protocol. S. F. Gheda to the design and interpretation of the results; A. M. Abo Shady to the editing and directing of the study; O.H. Abdel-Karim to the practical and statistical analysis; G.A. Ismail to the conception and methodology organization. All authors commented on the previous versions of the manuscript and approved its final form.

Ethical approval: All protocols and procedures adopted for the present investigation agreed with the approval of the Institutional Animal Ethics Committee of National Research Center and in accordance with the recommendation of the appropriate care and usage of laboratory animals, regulated by Faculty of Science, Tanta University, under the license No: IACUC-SCI-TU-0115; 30/3/2019.

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النشاط المضاد للأكسدة ولفرط سكر الدم من المستخلص الميثانولى لطحلب أرثرواسبيرا بلاتنسيس (أسبيرولينا بلاتنسيس): دراسة فى المختبر وعلى الكائن الحى

سالي فاروق غيده، عاطف محمد أبوشادي، أمنية حمدي عبد الكريم، جيهان أحمد إسماعيل
قسم النبات - كلية العلوم - جامعة طنطا - طنطا - مصر.

فى هذه الدراسة تم تقييم المستخلصات المختلفة لطحلب أسبيرولينا بلاتنسيس كمضاد للأكسدة و لمرض السكرى. وقد أوضحت النتائج أن كل مستخلصات الأسبيرولينا لها تأثير مضاد للأكسدة بأستخدام ثلاث طرق مختلفة بالأضافة لتأثيرها على مرض السكر والتأثير على حسب المذيب المستخدم. وقد وجد أن المستخلص الميثانولى لأسبيرولينا أعطى أعلى مضاد أكسدة فى كل الطرق و كذلك أعلى تأثير مثبط لأنزيم ألفا أميليز بنسبة (96.46%) وأنزيم ألفا جليكوسيداز بنسبة (97.42%). ووجد أيضا أن المستخلص الميثانولى لأسبيرولينا ليس له أى سميه على الخلايا الخطيه بأستخدام MTT assay.

وبالتحليل الكروماتوجرافى للمستخلص الميثانولى لأسبيرولينا وجد أنه يحتوى على العديد من المواد الفعالة وهذه المركبات تعمل بطريقه تكاملية وهى المسبب الرئيسى للتأثير المضاد للأكسدة والسكر.

وفى الكائن الحى: المستخلص الميثانولى لأسبيرولينا بتركيزات (10 و15 ملجرام لكل كيلوجرام) سبب تقليل جلوكوز الدم المرتفع وأيضا وظائف الكبد و الكليه و نسب الدهون المرتفعة المتعلقه بمرض السكرى. ونفس المستخلص تسبب فى أستعادة نقص الجسم و زيادة البروتين والهيموجلوبين فى فئران التجارب مقارنة بالفئران المصابة بالسكرى بمادة الألوكزان.

وأيضا معالجة الفئران المصابة بالسكرى عن طريق المستخلص الميثانولى للأسبيرولينا أدى إلى تحسين التشوهات الهستولوجيه المحدثه فى الكبد و البنكرياس والمتعلقه بمرض السكر.

وتوصى هذه الدراسة بأستخدام المستخلص الميثانولى للأسبيرولينا فى التحضيرات الطبية لعلاج مرض السكرى والأعراض المصاحبة له.