Revue Agrobiologia
www.agrobiologia.net
ISSN (Print): 2170-1652
e-ISSN (Online): 2507-7627

ANTIHYPERLIPIDEMIC AND ANTIOXIDANT EFFECTS OF THE MICROALGAE *NANNOCHLOROPSIS GADITANA* IN STREPTOZOTOCIN-INDUCED DIABETIC RATS

NACER Wassila[1], BABA AHMED Fatima Zohra[1]*, MERZOUK Hafida[1], BENYAGOUB Ouahiba[1] and BOUANANE Samira[1]

1. University of Tlemcen, Faculty of SNVSTU, Department of Biology, Laboratory of Physiology and Biochemistry of Nutrition, Tlemcen, Algeria.

Reçu le 24/10/2019, Révisé le 28/12/2019, Accepté le 30/12/2019

Abstract

Description of the subject: Marine organisms, such as microalgae, have attracted universal interest in the field of research as an alternative source of therapeutic and biological compounds that can be used for treatment of different diseases.

Objective: The aim of the present study was to investigate the possible hypoglycemic, hypolipidemic and antioxidant effect of the microalgae *Nannochloropsis gaditana* in streptozotocin-induced diabetic rats.

Methods: Diabetes was induced in male Wistar rats by single intraperitoneal injection of streptozotocin (45 mg/kg). Male rats were fed on control diet supplemented or not with *Nannochloropsis gaditana* (10%) for two months. At the end of the experiment, plasma and tissues biochemical parameters and oxidant/antioxidant markers were determined.

Results: The results obtained in this study show that streptozotocin induced animal diabetes characterized by adverse alterations of the metabolic and the balance oxidant /antioxidant. However, *Nannochloropsis gaditana* supplementation resulted in a significant reduction in the level of plasma glycated hemoglobin, plasma and tissue lipids and attenuates oxidative stress and thus improves metabolic status. In addition, it induced a modulation of lipase activities in diabetic rats. On the other hand, due to its antioxidant properties, *Nannochloropsis gaditana* was shown to have the capacity to mitigate the oxidative stress by increasing the antioxidant defense.

Conclusion: This study suggests that the microalgae *Nannochloropsis gaditana* has a beneficial effect in controlling diabetes by reducing blood glucose, lipid profile and oxidative stress, which reduces the risk of developing complications of diabetes.

Keywords: *Nannochloropsis gaditana* ; diabetes ; streptozotocin ; lipid profile ; oxidative stress

EFFETS ANTIHYPERLIPIDÉMIQUES ET ANTIOXYDANTS DE LA MICROALGUE *NANNOCHLOROPSIS GADITANA* CHEZ LES RATS DIABÉTIQUES INDUITS PAR LA STREPTOZOTOCINE

Résumé

Description du sujet: Les organismes marins, tels que les microalgues, ont suscité un intérêt universel dans le domaine de la recherche en tant que source alternative de composés thérapeutiques et biologiques pouvant être utilisés pour le traitement de différentes maladies.

Objectifs: Le but de la présente étude était d'évaluer le possible effet hypoglycémique, hypolipidémique et antioxydant de la microalgue *Nannochloropsis gaditana* chez les rats rendus diabétiques par streptozotocine.

Méthodes: Le diabète a été induit chez les rats Wistar mâles par injection de streptozotocine (45 mg / kg). Les rats mâles ont été nourris avec un régime standard témoin supplémenté ou non en *Nannochloropsis gaditana* (10%) pendant deux mois. À la fin de l'expérimentation, les paramètres biochimiques et les marqueurs oxydants/antioxydants plasmatiques et tissulaires sont déterminés.

Résultats: Les résultats obtenus montrent que la streptozotocine induit chez l'animal un diabète caractérisé par de nombreuses altérations métaboliques et de la balance oxydant/antioxydant. Cependant, la supplémentation en *Nannochloropsis gaditana* provoque une diminution significative de la concentration plasmatique de glucose, l'hémoglobine glyquée, des lipides plasmatiques et tissulaires et atténue le stress oxydatif et améliore ainsi le statut métabolique. De plus, il induit une modulation des activités des lipases chez le rat diabétique. D'autre part, en raison de ses propriétés antioxydantes, *Nannochloropsis gaditana* a la capacité d'atténuer le stress oxydatif en augmentant les défenses antioxydantes.

Conclusion: La présente étude suggère que les microalgues *Nannochloropsis gaditana* ont un effet bénéfique sur le contrôle de diabète par diminution de la glycémie, du profil lipidique et du stress oxydant, ce qui permet de réduire le développement des complications associées au diabète.

Mots clés: *Nannochloropsis gaditana* ; diabète ; streptozotocine ; profil lipidique ; stress oxydatif

* Auteur correspondant: BABA AHMED Fatima Zohra, E-mail:fatimazohra_7@yahoo.fr

INTRODUCTION

Diabetes mellitus is a metabolic disorder characterized by the presence of hyperglycemia as a result of defective insulin secretion, faulty insulin action or both [1]. This metabolic disorder is widely prevalent throughout the world, and is considered today as a major public health issue that seriously threatens human health. Indeed, recent studies have found that this disease affects about 4% of the world population and is expected to rise to 5.4% in 2025 [2, 3].

Dyslipidemia is recognized as one of the most prominent risk factors for cardiovascular diseases due to diabetes; it is characterized by hypertriglyceridemia, low high-density lipoprotein cholesterol levels, and increased small and dense low-density lipoprotein-cholesterol particles [4].

Oxidative stress plays a crucial role in the development of diabetic complications. This phenomenon occurs when the balance between oxidant and antioxidant systems shifts in favour of the former, leading to the production of oxygen free radicals [5]. The involvement of oxidative stress in the pathogenesis of diabetes is suggested not only by oxygen free radicals generation, but also due to nonenzymatic protein glycosylation, auto-oxidation of glucose, impaired glutathione metabolism, alteration in antioxidant enzymes and formation of lipid peroxides [6].

It is worth stating that recently, marine organisms, such as microalgae, have attracted universal interest in the field of research as an alternative source of therapeutic and biological compounds that can be used in pharmaceutical, nutraceutical, antioxidant, anticancer and antiviral applications [7].

Microalgae are microscopic photosynthetic organisms encountered in marine and freshwater environments [8].

Nannochloropsis gaditana is a microalga that belongs to the *Eustigmatophyceae* class, which is known as a source of proteins and polyunsaturated fatty acids [9, 10]. Recently, it has been documented that this marine alga possesses a variety of bioactive compounds that exhibit highly beneficial effects on health through their hypoglycemic and hypolipidemic properties [11, 12].

However, to the best of our knowledge, no prior studies have been reported in the literature so far on the effect of *Nannochloropsis gaditana* on streptozotocin-induced diabetic rats. Consequently, the purpose of the present work consists of exploring the effects of *Nannochloropsis gaditana* on lipid profile, lipase activities and redox status in diabetic rats.

MATERIALS AND METHODS

1. Biological material

The microalgae *Nannochloropsis gaditana* used in our experimental protocol originated from Mediterranean Sea; it came from closed microalgae photo bioreactors (Sidi Bel-Abbes, a northwestern Algerian town). After cultivation, the microalgae biomass was harvested and lyophilized. The resulting lyophilisate was then analyzed in order to determine the physico-chemical composition of *Nannochloropsis gaditana*, as shown in Table 1.

Components	Amount
Water content	42.9 g/100g
Dry mater content	57 g/100g
Ash content / DM	5.74 g/100g
Protein content / DM	28 g/100
Fat content / DM	18.4 g/100g
Cellulose content / DM	386 mg/100g
Carbohydrate content / DM	45 g/100g
Calcium content / DM	4.41 g/100g
Manganese content / DM	420 mg/100g
Zinc content / DM	10 mg/100g
Copper content / DM	5 mg /100g
Iron content / DM	17 mg/100g
Beta carotene content / DM	7.85 mg/100g
Vitamin C content/ DM	0.334 g/100g
Vitamin B1content / DM	610 µg /100 g
Vitamin B2 content / DM	2.17 mg/100 g
Vitamin B3content / DM	830 µg/100 g
Vitamin B12 content / DM	1.92 mg/100 g
Linoleic Acid content	11 mg/100 g
Linolenic Acid content	17 mg/100 g
Oleic Acid content	61 mg/100 g

Table 1: Composition of the microalgae *Nannochloropsis gaditana* DM: dry material

2. Animals and experimental design

Male Wistar rats, eight weeks old and weighing between 150 and 200 g, were obtained from the Pasteur institute in Algiers (Algeria), and were used in this study. All experiments were conducted according to the recommendations of the Committee on the Ethics of Animal Experiments and experimental animal care at the University of Tlemcen, in Algeria. The rats were housed in wood-chip bedded plastic cages and maintained under standard laboratory conditions, i.e. 12:12 h light-dark cycle, temperature of 25 °C and relative humidity of 60±5%. Animals had free access to food (control commercial diet for rats ONAB, Algeria) and water.

Diabetes was induced in rats by a single intraperitoneal injection of streptozotocin (STZ), dissolved in 0.1 M citrate buffer (pH 4.5), at a dose of (45 mg/kg). Diabetes was confirmed three days following the injection, through the estimation of blood glucose level using a glucose meter. It is worth clarifying that rats were considered diabetic only if their blood glucose level exceeded 127 mg/dL; they were then used in the present study.

The selected animals were divided into 4 experimental groups. The first group (control, C, $n = 10$) included normal rats fed a control diet (ONAB); the second group (control microalgae, CM, $n = 10$) contained normal rats fed a control diet enriched with 10% microalgae *Nannochloropsis gaditana*; the third one (diabetic group, D, $n = 10$) involved diabetic rats fed only with control diet; and the fourth and last one (diabetic microalgae, DM, $n = 10$) comprised diabetic rats fed a control diet enriched with 10% microalgae *Nannochloropsis gaditana*. The microalgae *Nannachloropsis gaditana* dosage used in this study was performed according to the protocol of Markovits et *al.* [13].

3. Blood and tissue samples

After the eight week, the experimental animals were fasted overnight and sacrificed. They were anaesthetized with intraperitoneal injection of 10% chloral (0.3 ml per 100 g of body weight). Their blood was then collected from abdominal aorta in two tubes, with and without anticoagulant, for plasma and serum separation. Serum was used for separation of different lipoprotein fractions and plasma was used for biochemical determinations and oxidant/antioxidant status parameters.

After removal of plasma, erythrocytes were washed with isotonic saline and were lysed with ice-cold distilled water and stored at 4 °C for 15 min. The cell debris was removed by centrifugation (2000 g for 15 min). Erythrocyte lysates were assayed for oxidant/antioxidant markers.

Afterwards, the liver, adipose tissue and pancreas were removed, washed with ice-cold saline, quickly blotted and weighed. An aliquot of each tissue was homogenized by means of an Ultra-Turrax homogenizer (Bioblock Scientific, Illkirch, France) in 10 volumes of ice-cold 10 mmol/l phosphate-buffered saline (pH 7.4) containing 1.15% KCl. The homogenates were subjected to a 6000 g centrifugation at 4°C for 15 min. The supernatant fractions were collected and used for tissue triglycerides and total cholesterol assays.

A second aliquot of tissues was homogenized in 0.9% (w/v) NaCl containing heparin (from Sigma in St. Louis, MO, USA) which was used for the determination of the lipoprotein lipase (LPL) activity.

Another portion of the adipose tissue was homogenized in ice cold buffer containing 0.25 M sucrose, 1 mM dithiothreitol and 1 mM EDTA, pH 7.4, supplemented with 20 mg/ml leupeptin, 2 mg/ml antipain and 1 mg/ml pepstatin; it was then used in the adipose hormone sensitive lipase (HSL) assay.

4. Chemical analysis

The plasma glucose level was determined using colorimetric enzymatic assays (kits from BioAssay Systems, Hayward, CA). In addition, the plasma and tissue triglycerides (TG) and total cholesterol (TC) were measured using colorimetric enzymatic kits (Sigma, St. Louis, MO, USA). The liquid chromatography method was employed to quantify the glycated hemoglobin (HbA_{1c}) in whole blood using a kit from Pointe Scientific Inc., United States. Also, the blood cell hemoglobin levels were measured using a hemoglobinometer (HemoCue Ltd., Dronfield, United Kingdom). Serum low density lipoprotein (LDL), very low density lipoprotein (VLDL) and high density lipoprotein (HDL) fractions were separated according to the method of Burstein et al. [14]. Different precipitate fractions were obtained from successive centrifugation with 0.1 ml phosphotungstic acid (30.3 mmol/L) and $MgCl_2$ (100 mmol/L) reagent mixture. HDL-cholesterol, LDL-cholesterol and VLDL-cholesterol concentrations were measured by enzymatic methods (Sigma).

The lipase activity (LPL, EC 3.1.1.34; HSL, EC 3.1.1.79) was measured using the pH-stat technique by titrimetric measurement of fatty acids released after hydrolysis of triglycerides of synthetic substrate with NaOH 0.05 M at pH 8 and at temperature 25 °C. The enzyme activity was expressed in international units (IU). One unit corresponds to the release of one micro-equivalent of fatty acid per minute.

5. Oxidant/antioxidant marker determination

Plasma vitamin C levels were determined using dinitrophenylhydrazine as previously described [15]. Catalase activity was measured on erythrocyte lysate by spectrophotometric analysis of the rate of hydrogen peroxide decomposition at 240 nm [16]. Erythrocyte reduced glutathione (GSH) levels was assayed by colorimetric method based on the reduction of 5,5-dithiobis-2-nitrobenzoic acid by GSH to generate 2-nitro-5-thiobenzoic acid (DTNB or Ellman reagent) [17]. Superoxide dismutase (SOD) activity was measured by the NADPH oxidation procedure [18]. Malondialdehyde (MDA) levels, a marker of lipid peroxidation, were determined in plasma and erythrocyte by the procedure of Ohkawa et al. [19] based on the reaction of MDA with thiobarbituric acid. Carbonyl proteins (CARP) (markers of protein oxidation) in plasma and erythrocyte were assayed by the 2,4-dinitrophenyl hydrazine reaction [20].

6. Statistical analysis

The results obtained were expressed as mean ± standard deviation (SD). The results were tested for normal distribution using the Shapiro–Wilk test. Data not normally distributed were logarithmically transformed. Significant differences among the groups were analyzed statistically by a one-way analysis of variance (ANOVA). When significant changes were observed in ANOVA tests, Fisher least significant difference tests were applied to locate the source of significant difference. The significance level was set at $P < 0.05$. These calculations were performed using Statistica version 4.1 (Statsoft, Tulsa, OK).

RESULTS

1. Effect of Nannochloropsis gaditana on plasma glucose, hemoglobin and glycated hemoglobin

It was found that rats in the diabetic group presented significantly high levels of plasma glucose and glycated hemoglobin, while the hemoglobin level decreased in a significant manner when compared with that of control group rats. However, when microalgae *Nannochloropsis gaditana* was fed to diabetic rats, it tended to bring the above values to near normal (Table 2).

Table 2: Levels of plasma glucose, hemoglobin and glycated hemoglobin in different experimental groups

	Control rats		Diabetic rats		
	C	CM	D	DM	*P* (ANOVA)
Glucose (g/L)	1.17±0.01^{c}	1.20±0.04^{c}	4.41±0.08^{a}	2.44±0.02^{b}	0.001
HbA_{1c} (%)	4.40±0.14^{c}	4.10±0.2^{c}	10.70±0.87^{a}	9.60±0.7^{b}	0.004
Hb (mg/dL)	12.22±0.25^{a}	12.63±0.10^{a}	9.53±0.03^{c}	11.03±0.13^{b}	0.005

Values are presented as means ± standard deviations (SD). C: normal rats fed a control diet; CM: normal rats fed a control diet enriched with microalgae at 10%; D: diabetic rats fed only with control diet; DM: diabetic rats fed a control diet enriched with microalgae at 10%. HbA_{1c}, glycated hemoglobin; Hb, hemoglobin. Values with different superscript letters (a, b, c, d) are significantly different (ANOVA).

2. Effect of Nannochloropsis gaditana on lipid profile

A significant increase in total cholesterol, triglycerides, VLDL-C and LDL-C with significant decline in HDL-C concentrations was observed in diabetic rats as compared to control rats.

The treatment of rats with *Nannochloropsis gaditana* results in an important decrease in the level of total cholesterol, triglycerides, VLDL-C and LDL-C, while HDL-C was considerably increased compared to streptozotocin-induced diabetic rats (Table 3).

Table 3: Lipid profile in different experimental groups

	Control rats		Diabetic rats		
	C	CM	D	DM	*P* (ANOVA)
TC (mg/dL)	153.23±0.01^{c}	144.14±0.01^{c}	243.78±0.04^{a}	202.37±0.03^{b}	0.004
TG (mg/dL)	115.12±0.01^{c}	110.32±0.01^{c}	228.68±0.04^{a}	194.28±0.03^{b}	0.006
HDL-C (mg/dL)	81.06±1.12^{b}	92.75±1.47^{a}	59.06±1.18^{d}	73.08±1.21^{c}	0.001
LDL-C (mg/dL)	55.84±1.42^{c}	45.14±2.38^{d}	90.14±2.38^{a}	69.18±1.40^{b}	0.001
VLDL-C (mg/dL)	38.84±2.5^{c}	40.13±1.59^{c}	70.92±1.06^{a}	59.52±2.90^{b}	0.001

Values are presented as means ± standard deviations (SD). C: normal rats fed a control diet; CM: normal rats fed a control diet enriched with microalgae at 10%; D: diabetic rats fed only with control diet; DM: diabetic rats fed a control diet enriched with microalgae at 10%. TC, total cholesterol; TG, triglycerides; HDL-C, HDL cholesterol; LDL-C, LDL cholesterol; VLDL-C, VLDL cholesterol. Values with different superscript letters (a, b, c, d) are significantly different (ANOVA).

3. Effect of Nannochloropsis gaditana on tissue lipid levels

The group of diabetic rats showed significantly elevated levels of total cholesterol and triglycerides in their liver, pancreas and adipose tissue as compared to those of the control group. However, liver, pancreas and adipose tissue total cholesterol and triglycerides contents were remarkably lower in diabetic rats fed a diet supplemented with *Nannochloropsis gaditana* (Fig. 1).

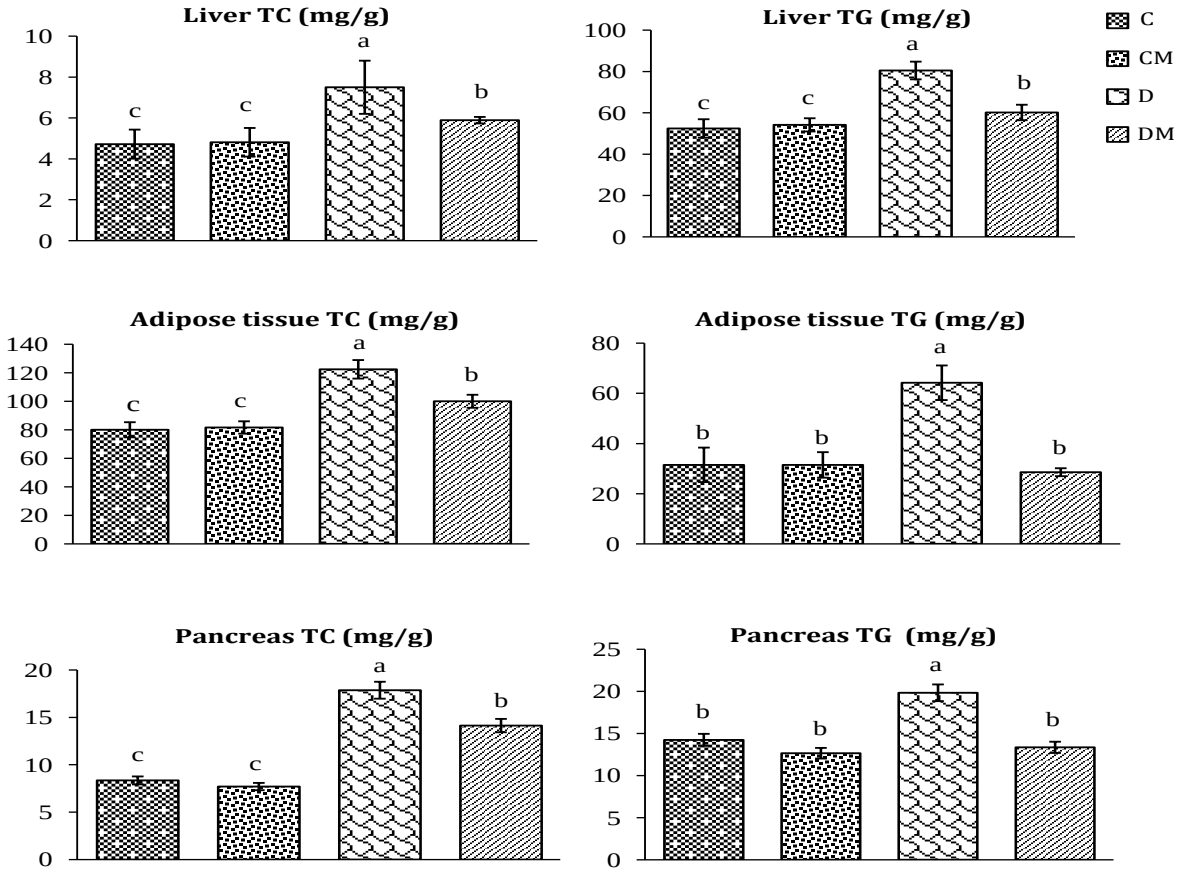

Figure 1: Tissue lipid levels in different experimental groups

Values are presented as means ± standard deviations (SD). C: normal rats fed a control diet; CM: normal rats fed a control diet enriched with microalgae at 10%; D: diabetic rats fed only with control diet; DM: diabetic rats fed a control diet enriched with microalgae at 10%. TC, total cholesterol; TG, triglycerides. Values with different superscript letters (a, b, c, d) are significantly different (ANOVA).

4. Effect of Nannochloropsis gaditana on lipase activities in liver, pancreas and adipose tissue

The LPL activity in liver, pancreas and adipose tissue decreased significantly *i*n diabetic rats by comparison with control rats, whereas higher adipose HSL activity was detected in streptozotocin-induced diabetic rats. It is worth noting that diabetic rats fed a diet rich in microalgae *Nannochloropsis gaditana* markedly improved their lipase activities as compared to diabetic rats fed a control diet (Fig. 2).

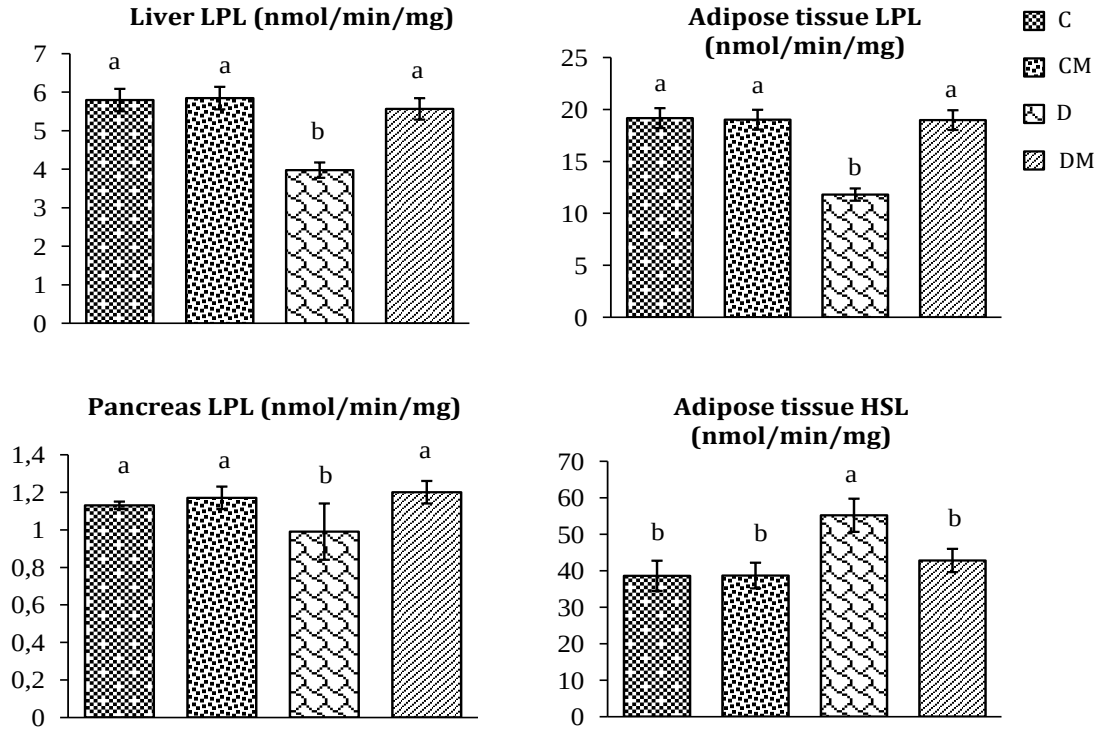

Figure 2: Liver, pancreas and adipose tissue lipase activities in different experimental groups.

Values are presented as means ± standard deviations (SD). C: normal rats fed a control diet; CM: normal rats fed a control diet enriched with microalgae at 10%; D: diabetic rats fed only with control diet; DM: diabetic rats fed a control diet enriched with microalgae at 10%. LPL, lipoprotein lipase; HSL, hormone sensitive lipase. Values with different superscript letters (a, b, c, d) are significantly different (ANOVA).

5. Effect of Nannochloropsis gaditana on oxidative stress markers

In diabetic rats, there was a significant increase in oxidant markers (MDA and carbonyl proteins levels) in plasma and erythrocyte, while antioxidant defense markers (plasma vitamin C, erythrocyte GSH, catalase and SOD) were significantly reduced compared to control rats; however, when these antioxidant enzymes were measured after dietary supplementation with *Nannochloropsis gaditana*, contradictory results were obtained. In addition, *Nannochloropsis gaditana* was able to reduce levels of MDA and carbonyl proteins in plasma and erythrocyte of diabetic rats (Table 4).

Table 4: Plasma and erythrocyte oxidant/antioxidant markers in different experimental groups

	Control rats		Diabetic rats		*P* (ANOVA)
	C	CM	D	DM	
Plasma					
CARP (nmol/l)	1.12±0.23^{c}	1.31±0.37^{c}	4.68±1.98^{a}	2.89±0.56^{b}	0.000
MDA (µmol/l)	4.52±1.08^{b}	4.18±1.23^{b}	8.37±3.50^{a}	6.37±1.49^{b}	0.018
vitamin C (µmol/l)	18.27±4.63^{a}	18.85±3.90^{a}	10.24±1.78^{c}	12.51±1.69^{b}	0.000
Erythrocyte					
CARP (nmol/l)	3.75±1.72^{b}	3.71±1.10^{b}	6.55±1.88^{a}	5.03±0.82^{b}	0.001
MDA (µmol/l)	2.89±0.19^{c}	3.05±0.82^{c}	6.31±1.33^{a}	4.91±0.91^{b}	0.000
GSH (µmol/l)	5.26±0.56^{a}	5.91±1.27^{a}	2.82±0.52^{c}	4.30±0.73^{b}	0.001
Catalase (U/min/ml)	153.67±35.27^{a}	183.00±10.61^{a}	93.56±11.66^{c}	143.79±3.76^{b}	0.000
SOD (mmol/min/ml)	487.25±4.10^{a}	496.17±1.25^{a}	279.81±17.06^{c}	376.19±38.42^{b}	0.000

Values are presented as means ± standard deviations (SD). C: normal rats fed a control diet; CM: normal rats fed a control diet enriched with microalgae at 10%; D: diabetic rats fed only with control diet; DM: diabetic rats fed a control diet enriched with microalgae at 10%. CARP, carbonyl proteins; MDA, malondialdehyde; GSH, reduced glutathione; SOD, superoxide dismutase. Values with different superscript letters (a, b, c, d) are significantly different (ANOVA).

DISCUSSION

Streptozotocin, an antibiotic and anticancer agent, has been widely used for inducing diabetes in animals by effecting degeneration and necrosis of pancreatic β-cells [21]. The present study allowed showing that the intraperitoneal injection of streptozotocin to normal rats resulted in significantly elevated glucose levels, which is in good agreement with the findings of Akberzadah et *al.* [22] and Jin et al. [5]. In contrast, *Nannochloropsis gaditana* supplementation was shown effective in lowering the levels of plasma glucose in streptozotocin-induced diabetic rats. The hypoglycemic action of this microalga might be due to the potentiation of the pancreatic secretion of insulin hormone by pancreatic beta cells. *Nannochloropsis gaditana*, through its ability to scavenge free radicals and inhibit lipid peroxidation [11] can prevent the oxidative stress damage caused by streptozotocin and protect the beta cells, thus resulting in decreased plasma glucose levels. Similar findings have been reported in diabetic rats fed a diet supplemented with *Nannochloropsis oculata* [23].

Low levels of hemoglobin observed in diabetic rats suggest an increased production of glycated hemoglobin. Glycated hemoglobin has always been considered as an important indicator of long-term glycemic control [24]. The excess glucose present in the blood of diabetic animals reacts with hemoglobin to form glycated hemoglobin [25]. According to Jangir et *al.* [26] and Gandhi et *al*. [27], a higher level of glycated hemoglobin was found in the blood of streptozotocin-induced diabetic rats, indicating their poor glycemic control, thus corroborating our findings.

Therefore, treatment of diabetic rats with *Nannochloropsis gaditana* caused reduction in the levels of glycated hemoglobin. This may be attributed to the improved glycemic control in the body.

In general, diabetes mellitus is associated with lipid disorders [28]. It is important to recall that the most common lipid abnormalities in diabetic subjects are hypertriglyceridemia and hypercholesterolemia [29]. Indeed, the high lipid levels observed in diabetic rats are normally linked to an increased mobilization of free fatty acids from the peripheral fat depots [30].

In present study, diabetic rats showed a significant increase in total cholesterol, triglycerides, LDL-C, VLDL-C associated with significant decrease in HDL-C levels.

The results obtained were found consistent with those reported by Kebir et *al*. [31]. However, supplementation with *Nannochloropsis gaditana* at 10% could help to correct the lipid abnormalities observed in streptozotocin-induced diabetic rats. These findings were supported by Aboulthana et al. [23] who found that *Nannochloropsis oculata* decreased the levels of cholesterol, triglycerides, LDL-C but increased the HDL-C content in streptozotocin-induced diabetic rats. It was also revealed that *Nannochloropsis gaditana* has the capacity to improve the lipid metabolism [12]. On the other hand, *Nannochloropsis gaditana* caused a significant reduction in liver, pancreas and adipose tissue total cholesterol and triglycerides contents in diabetic rats. Thus, observed results indicate that *Nannochloropsis gaditana* possesses interesting antihyperlipidemic properties.

In this context, Bendaoud et al. reported that a diet supplemented with 10% *Nannochloropsis gaditana* during a period of 2 months reduced the plasma and tissue triglycerides and cholesterol levels in obese rats subjected to high fat diet [11]. In addition, other studies have discovered that *Nannochloropsis* alga has potential hypocholesterolemic effects on rats fed on high-cholesterol diets [32].

Furthermore, it is well known that lipoprotein lipase (LPL) is a rate-limiting enzyme that catalyzes the hydrolysis of the triglyceride core of the circulating triglyceride-rich lipoproteins, including chylomicrons, with a very low density of lipoproteins [33]. The data obtained revealed a significant decrease in the LPL activities of liver, pancreas and adipose tissue in rats of diabetic groups, as previously reported by Tavangar et *al*. [34] and Ranganathan et *al.* [35]. Regarding the adipose hormone-sensitive lipase, a higher level of HSL was observed in diabetic rats as compared to normal rats. These results were found to be highly consistent with those reported in previous studies. In addition, it was suggested that adipocytes isolated from streptozotocin-induced diabetic rats exhibited elevated lipolytic activity which was accompanied by increased HSL activity [36]. Consequently, treatment of diabetic rats with *Nannochloropsis gaditana* induces an important modulation of lipase activities. Moreover, the results achieved indicate that this microalga has the potential to counteract the development of many metabolic diseases and to prevent a number of complications associated with diabetes. It is interesting to note that previous studies have shown that *Nannochloropsis* is considered as a good potential source of EPA (20: 5ω3), and also an important polyunsaturated fatty acid that helps to prevent many diseases [37].

It has been shown that oxidative stress in diabetes mellitus coexists with a reduction in the antioxidant status, which can increase the deleterious effects of free radicals [25]. In a living organism, antioxidant enzymes as well as nonenzymatic antioxidants are the first line of defense against reactive oxygen species (ROS) induced oxidative damage [38]. In the present experiments, oxidative markers such as MDA and carbonyl proteins were elevated, while the antioxidant markers such as vitamin C, GSH, SOD and catalase activity were significantly reduced in diabetic rats.

These results were completely reversed upon treatment with *Nannochloropsis gaditana*, suggesting their antioxidant effect. In this respect, the previous studies have also supported that *Nannochloropsis gaditana* has the capacity to mitigate oxidative stress and enhance antioxidant status in obese rats subjected to a high fat diet [11]. Moreover, the antioxidant properties of *Nannochloropsis gaditana* could be due to various valuable pigments contained in this microalga such as the carotenoids, which are strong antioxidants and radical scavengers [39].

CONCLUSION

From the present investigation, it can be concluded that *Nannochloropsis gaditana* is effective in improving the lipid profile and modulating the lipase activities in streptozotocin-induced diabetic rats. In addition, these microalgae, because of their antioxidant properties, could also mitigate oxidative stress and prevent complications associated with diabetes.

REFERENCES

[1]. Ozougwu J.C., Obimba K.C., Belonwu., C.D. and Unakalamba C.B. (2013). The pathogenesis and pathophysiology of type 1 and type 2 diabetes mellitus. *J. Physiol. Pathophysiol*, 4(4): 46-57.

[2]. Kim S.H., Hyun S.H. and Choung S.Y. (2006). Anti-diabetic effect of cinnamon extract on blood glucose in db/db mice. *J Ethnopharmacol*, 104: 119-123.

[3]. Pandey A., Tripathi P., Pandey R., Srivatava R. and Goswami S. (2011). Alternative therapies useful in the management of diabetes: A systematic review. *J Pharm Bioall Sci*, 3(4): 504-512.

[4]. Chen S.C. and Tseng C.H. (2013). Dyslipidemia, kidney disease, and cardiovascular disease in diabetic patients. *Rev Diabet Stud*, 10: 88-100.

[5]. Jin L., Xue H.Y., Jin L.J., Li S.Y. and Xu Y.P. (2008). Antioxidant and pancreas-protective effect of aucubin on rats with streptozotocin-induced diabetes. *Eur J Pharmacol*, 582: 162-167.

[6]. Samarghandian S., Borji A., Delkhosh M.B. and Samini F. (2013). Safranal treatment improves hyperglycemia, hyperlipidemia and oxidative stress in streptozotocin-induced diabetic rats. *J Pharm Pharm Sci*, 16(2):352-362.

[7]. Valli Nachiyar V. and Renugadevi K. (2015). Bio-prospecting microalgae for value added products. *RJPBCS*, 6(3): 893-900.

[8]. Priyadarshani I. and Rath B. (2012). Commercial and industrial applications of micro algae- A review. *J. Algal Biomass Utln*, 3(4): 89-100.

[9]. Baba Hamed S., Baba Hamed M.B., Kassouar S. and Abi Ayad S.M.E. (2016). Physicochemical analysis of cellulose from microalgae *Nannochloropsis gaditana*. *Afr. J. Biotechnol*, 15(24): 1201-1207.

[10]. Camacho-Rodríguez J., Cerón-García M.C., González-López C.V., Fernández-Sevilla J.M., Contreras-Gómez A. and Molina-Grima E. (2013). A low-cost culture medium for the production of *Nannochloropsis gaditana* biomass optimized for aquaculture. *Bioresour Technol*, 144: 57-66.

[11]. Bendaoud A., Baba Ahmed F.Z., Merzouk H., Bouanane S. and Bendimerad S. (2018). Effects of dietary microalgae *Nannochloropsis gaditana* on serum and redox status in obese rats subjected to a high fat diet. *Phytothérapie*, 1-11.

[12]. Bendimerad-Benmokhtar S., Bouanane S., Merzouk H., Baba Ahmed F.Z. and Bendaoud A. (2018). Effects of Nannochloropsis fed on serum and tissue lipids metabolism in obese offspring of overfed dams. *Curr Nutr Food Sci*, 14: 1-15.

[13]. Markovits A., Conjeros R., Lopez L. and Lutz M. (1992). Evaluation of marine microalga *Nannochloropsis sp*. As a potential dietary supplement. Chemical, nutritional and short term toxicological evaluation in rats. *Nutr Res*, 12: 1273-1284.

[14]. Burstein M., Fine A., Atger V., Wirbel E. and Girard-Globa A. (1989). Rapid method for the isolation of two purified subfractions of high density lipoproteins by differential dextran sulfate-magnesium chloride precipitation. *Biochimie*, 71(6): 741-746.

[15]. Bouanane S., Merzouk H., Benkalfat N.B., Soulimane N., Merzouk S.A. and Gresti J. (2009). Time course of changes in serum oxidant/antioxidant status in overfed obese rats and their offspring. *Clin Sci*, 116: 669-680.

[16]. Aebi H. (1974). Catalase. In Methods of Enzymatic Analysis, 2nd ed., pp. 673–684 [HU Bergmeyer, editor]. Veinheim: Verlag Chemie.

[17]. Ellman G. (1995). Tissue sulfhydryl groups. *Arch Biochem Biophys*, 82: 70-77.

[18]. Elstner E.F., Youngman R.J. and Obwad W. (1983). Superoxide dismutase. In Methods of Enzymatic Analysis, third ed., pp. 293-302 [HU Bergmeyer Verlag, editor]. Chemie, Weinheim.

[19]. Ohkawa H., Ohishi N. and Yagi K. (1979). Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Anal Biochem*, 95: 351-358.

[20]. Levine R.L., Garland D., Olivier C.N., Amica A., Climent I., Lenz A.G., Ahn B.W., Shantiel S. and Stadtman E.R. (1990). Determination of carbonyl content in oxidatively modified proteins. *Meth Enzymol*, 186: 464-478.

[21]. Merzouk H., Madani S., Chabane D., Prost J., Bouchenak M. and Belleville J. (2000). Time course of changes in serum glucose, insulin, lipids and tissue lipase activities in macrosomic offspring of rats with streptozotocin-induced diabetes. *Clin Sci*, 98: 21-30.

[22]. Akbarzadeh A., Norouzian D., Mehrabi M.R., Jamshidi Sh., Farhangi A., Verdi A.A., Mofidian S.M. and Rad B.L. (2007). Induction of diabetes by streptozotocin in rats. *Indian J Clin Biochem*, 22(2): 60-64.

[23]. Aboulthana W.M., El-Feky A.M., Ibrahim N.E., Sahu R.K. and El-Sayed A.B. (2018). Evaluation of the pancreatoprotective effect of *Nannochloropsis oculata* extract against streptozotocin-induced diabetes in rats. *J App Pharm Sci*, 8(6): 46-58.

[24]. Sirsikar M., Supriya., Mohanty S. and Pinnelli V.B.K. (2016). Role of glycated hemoglobin (HBA1c) as a dual marker to predict glycemic status and dyslipidemia in type II diabetes mellitus. *Int J Res Med Sci*, 4(10): 4524-4529.

[25]. Rajasekaran S., Sivagnanam K. and Subramanian S. (2005). Antioxidant effect of Aloe vera gel extract in streptozotocin-induced diabetes in rats. *Pharmacol Rep*, 57(1): 90-96.

[26]. Jangir R.N. and Jain G.C. (2017). Evaluation of antidiabetic activity of hydroalcoholic extract of *Cassia fistula* Linn. Pod in streptozotocin-induced diabetic rats. *Pharmacogn J*, 9(5): 599-606.

[27]. Gandhi G.R. and Sasikumar P. (2012). Antidiabetic effect of *Merremia emarginata* Burm. F. in streptozotocin induced diabetic rats. *Asian Pac J Trop Biomed*, 2(4): 281-286.

[28]. Shankarprasad D.S., Gundalli S., Mahantesh B., Kashinakunti S.V. and Sunitha P. (2015). Lipid profile in diabetes mellitus. *Indian J Pathol Oncol*, 2(4): 290-294.

[29]. Karan S.K., Mondal A., Mishra S.K., Pal D. and Rout K.K. (2013). Antidiabetic effect of *Streblus asper* in streptozotocin-induced diabetic rats. *Pharm Biol*, 51(3): 369-375.

[30]. Akah P.A., Alemji J.A., Salawu O.A., Okoye T.C. and Offiah N.V. (2009). Effects of *Vernonia amygdalina* on biochemical and hematological parameters in diabetic rats. *Asian J. Med. Sci*, 1(3): 108-113.

[31]. Kebir N.E., Aichouni A. and Zahzeh T. (2017). Raw camel milk properties on alloxan-induced diabetic wistar rats. *Rom J Diabetes Nutr Metab Dis*, 24(1): 41-47.

[32]. Werman M.J., Sukenik A. and Mokady S. (2003). Effects of the marine unicellular alga *Nannochloropsis* sp. to reduce the plasma and liver cholesterol levels in male rats fed on diets with cholesterol. *Biosci. Biotechnol. Biochem,* 67(10): 2266-2268.

[33]. Bensalah M., Bouanane S., Baba Ahmed F.Z., Merzouk H., Merzouk S.A. and Michel N. (2016). Effect of cafeteria diet on lipid metabolism and lipase activities in Wistar rats. *Eur. Chem. Bull*, 5(3): 99-103.

[34]. Tavangar K., Murata Y., Pedersen M.E., Goers J.F., Hoffman A.R. and Kraemer F.B. (1992). Regulation of lipoprotein lipase in the diabetic rat. *J Clin Invest*, 90(5): 1672-1678.

[35]. Ranganathan G., Li C. and Kern P.A. (2000). The translational regulation of lipoprotein lipase in diabetic rats involves the 3′-untranslated region of the lipoprotein lipase mRNA. *J Biol Chem*, 275(52): 40986-40991.

[36]. Sztalryd C. and Kraemer F.B. (1995). Regulation of hormone-sensitive lipase in streptozotocin-induced diabetic rats. *Metabolism,* 44(11): 1391-1396.

[37]. Abirami S., Murugesan S., Sivamurugan V. and Sivaswamy S.N. (2017). Screening and optimization of culture conditions of *Nannochloropsis gaditana* for omega 3 fatty acid production. *J App Biol Biotech*, 5(3): 13-17.

[38]. Sellamuthu P.S., Arulselvan P., Kamalraj S., Fakurazi S. and Kandasamy M. (2013). Protective nature of mangiferin on oxidative stress and antioxidant status in tissues of streptozotocin-induced diabetic rats. *ISRN Pharmacol,* 1-10.

[39]. Zuluaga M., Gueguen V., Pavon-Djavid G. and Letourneur D. (2017). Carotenoids from microalgae to block oxidative stress. *Bioimpacts*, 7(1): 1-3.