RESEARCH ARTICLE

Evaluation of the anti-inflammatory and antioxidant effects of the microalgae *Nannochloropsis gaditana* in streptozotocin-induced diabetic rats

Wassila Nacer[1] · Fatima Zohra Baba Ahmed[1] (iD) · Hafida Merzouk[1] · Ouahiba Benyagoub[1] · Samira Bouanane[1]

Abstract

Purpose This study aims to evaluate the anti-inflammatory and antioxidant effects of *N. gaditana* on streptozotocin (STZ)-induced diabetes mellitus in Wistar rats.

Methods Diabetes was induced in male Wistar rats by single intraperitoneal injection of STZ (45 mg/kg). Male rats were fed on control diet supplemented or not with *N. gaditana* (10%) for a period of 2 months. At the end of the experiment, biochemical parameters and oxidant/antioxidant markers in liver and pancreas tissues, as well as mitochondria isolated from liver of rats, were determined.

Results It was notice that levels of glucose, glycated hemoglobin (HbA_{1c}), lipid profile, kidney functions and liver enzymes in addition to markers of the inflammatory reactions interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF-α) increased significantly ($P < 0.05$) in diabetic rats. Moreover, undesirable alterations of oxidative stress markers of tissue and mitochondria isolated from the liver were noted in these rats. *N. gaditana* supplementation was shown effective in lowering the levels of glucose, HbA_{1c} and improving the renal and hepatic function and also in attenuating the oxidative stress and inflammation in diabetic rats.

Conclusion *N. gaditana* possesses antioxidant properties that might have beneficial effect in treatment of diabetes.

Keywords *Nannochloropsis gaditana* · Diabetes · Mitochondria · Oxidative stress · Inflammation

Introduction

Diabetes mellitus is a group of metabolic disorders which are caused par absolute or relative deficiencies of insulin secretion that is associated with elevated levels of blood glucose and also with disturbances in the metabolism of carbohydrates, lipids, and proteins [1]. Diabetes affects individuals' functional capacities and quality of life, leading to significant morbidity and premature mortality [2].

Streptozotocin (STZ) is a natural diabetic agent that is used to generate diabetic animal models by damaging or destroying the pancreatic β cells; this leads to the cessation of insulin production [3]. On the other hand, STZ has drawn much attention as a potential source of oxidative stress that plays a pivotal role in the development of diabetes and its complications [4, 5].

During diabetes, inflammation can be triggered by increased ROS (reactive oxygen species), which activates several inflammatory signaling cascades. In addition, the condition of diabetes can increase the availability of free fatty acids due to the process of lipolysis. The increase of free fatty acids will activate the immune system for releasing cytokines, such as interleukin-6 (IL-6) and tumor necrosis factor-α (TNF-α) [6].

Nowadays, microalgae constitute a major field of investigation for uncovering new molecules with biological activities likely to further enhance metabolic treatment responses. Microalgae are photosynthetic organisms that convert solar energy to biomass. It is well known that microalgae constitute a source of bioactive compounds with potential applications in functional food supplements as well as in nutraceutical, cosmetic, and pharmaceutical products [7]. Nutritional studies have also revealed that the incorporation of a microalgal

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[1] Laboratory of Physiology, Physiopathology and Biochemistry of Nutrition, Department of Biology, Faculty of Natural and Life Sciences, Earth and Universe, University Abou-Bekr Belkaïd, 13000 Tlemcen, Algeria

biomass and/or its bioactive compounds in foods may reduce the risk of multiple diseases [8].

The pharmacological properties of microalgae exhibit antioxidant, antiviral, and anticancer activity [9]. Microalgae also provide benefits for the treatment of diabetes and its hypoglycemic effect was shown both in alloxan and STZ-induced diabetic rats [10, 11].

Nannochloropsis gaditana is a microalga that belongs to the class of *Eustigmatophyceae*. This marine alga is very rich in lipids and pigments, and is acknowledged for its natural antioxidant potential, and carotenoids pigments, including astaxanthin, β-carotene, canthaxanthin, neoxanthin, violaxanthin, zeaxanthin, and other components [12, 13]. *N. gaditana* is also recognised as a good potential source of EPA (20: 5ω3), and also an important polyunsaturated fatty acid that helps to prevent many diseases [14]. Previous reports have revealed the antioxidant and anticancer activities of *N. gaditana* or its extracts in vitro and in vivo [15, 16].

The present study was undertaken in an attempt to explore the anti-inflammatory and antioxidant properties of the microalgae *N. gaditana* in STZ-induced diabetic rats.

Materials and methods

Plant 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 *N. gaditana*, as shown in Table 1.

Determination of *N. gaditana* composition

Water and dry mater contents were determined in accordance with the AOAC guidelines [17]. In addition, the crude ash content was estimated by incineration in a muffle furnace at 500–600 °C (Heraeus Instruments) according to the AOAC recommendations. The operation was considered as complete when the residues turned white after cooling [18]. Moreover, the total carbohydrates were found using the phenol-sulfuric acid method [19]. The total lipid content of *N. gaditana* was extracted using the Soxhlet extraction method. A quantity of 150 ml of pure n-hexane was used to extract the lipid content, during 6 h, at the rate of 10 refluxes per hour, to achieve maximum extraction efficiency. The amount of lipid extracted was then measured after removing the solvent by means of a rotary vacuum evaporator (Heidolph instruments) for the purpose of evaporating the n-hexane, at 35 °C for 60 min.

Table 1 Composition of the microalgae *N. gaditana*

Components	Amount
Water content	42.9 g/100 g
Dry mater content	57 g/100 g
Ash content/DM	5.74 g/100 g
Protein content/DM	28 g/100 g
Fat content/DM	18.4 g/100 g
Cellulose content/DM	386 mg/100 g
Carbohydrate content/DM	45 g/100 g
Calcium content/DM	4.41 g/100 g
Manganese content/DM	420 mg/100 g
Zinc content/DM	10 mg/100 g
Copper content/DM	5 mg /100 g
Iron content/DM	17 mg/100 g
Beta carotene content/DM	7.85 mg/100 g
Linoleic Acid content	11 mg/100 g
Linolenic Acid content	17 mg/100 g
Oleic Acid content	61 mg/100 g

DM dry material

Afterwards, the lipid content was evaluated [20]. The crude protein content in the samples was determined by the Kjeldahl method; it was calculated using a nitrogen conversion factor of 6.25. The inorganic constituents of the biomass, i.e. calcium, manganese, zinc, copper and iron, were determined by means of a VARIAN AA20 type air-acetylene flame atomic adsorption spectrophotometer. Carotenoids were extracted using the method of Sass-Kiss et al. [21]. The quantity of 20 ml of the mixture of hexane, acetone and ethanol (2: 1: 1) was added to 5 g of the sample under study. Afterwards, the upper phase was recovered after stirring for 30 min. Then, 10 ml of hexane were added for a second extraction. The two phases were then mixed for the purpose of determining the total carotenoids content by spectrophotometry at 450 nm. Carotenoid concentrations were estimated by reference to the calibration curve that uses ß-carotene.

Animals and experimental design

Adult 2 month-old male Wistar rats, weighing between 150 and 200 g, provided by Pasteur's institute (Algeria), were used in this study. The animals were maintained under standard laboratory conditions, i.e. (12 h light-dark cycle), temperature of 25 °C, and relative humidity of (60 ± 5%). They had free access to food (control commercial diet for rats ONAB, Algeria) and water. All aspects of the experiments were conducted according to the guidelines provided by the ethical committee of the experimental animal care at Tlemcen.

Diabetes was induced in rats by STZ. A single dose of STZ (45 mg/kg body weight) dissolved in 0.1 M citrate buffer

(pH 4.5), was administered to each animal by intraperitoneal injection. Three days after STZ injection, the blood glucose level was checked using a glucose meter. Rats with blood glucose level above 120 mg/dL were considered diabetic and were included in the study.

The selected animals were divided into four 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 *N. 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 *N. gaditana*. The microalgae *N. gaditana* dosage used in this study was performed according to the protocol of Markovits et al. [22].

Blood and tissue samples

After eight weeks of the experiment, the rats were anesthetized with intraperitoneal injection of 10% chloral (0.3 ml per 100 g of body weight), after 12 h of fasting. Blood samples were collected from the abdominal aorta in order to determine various biochemical estimations.

Samples of liver and pancreatic tissues were removed, washed with ice-cold saline, quickly blotted and weighed. An aliquot of each sample 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 homogenate was subjected to 6000 g centrifugation at 4 °C for 15 min. The supernatant fractions from the samples were collected and used for the determination of redox status markers.

Another portion of the liver tissue was collected and used for isolation of mitochondria.

Liver mitochondria isolation

Liver mitochondria were isolated as previously described by Frezza et al. [23], with some modifications. A piece of liver with a mass of 10 g was crushed in a pot containing 30 ml of TSE (Tris-Sucrose-EGTA, with 250 mM of Sucrose, 50 mM of Tris, 5 mM of EGTA, pH 7.2), in order to allow the release of mitochondria. Afterwards, the homogenate was subjected to a 1770 rpm centrifugation for a period of 10 min. The resulting supernatant was collected and subjected to 9600 rpm centrifugation for 10 min. The mitochondrial nerve obtained was resuspended in 13 ml of TSE. This suspension was once again subjected to a 9600 rpm centrifugation for 10 min. Afterwards, the mitochondrial nerve was put again in 15 ml of TS buffer (Tris-Sucrose, with 250 mM of Sucrose, 50 mM of Tris, pH 7) and was then centrifuged at 9600 rpm for 10 min. The final pellet was divided into two fractions; the first one was placed into 200 µl of TS buffer in order to get a mitochondrial suspension, and the second was added to the hypotonic solution (25 mM of KH_2PO_4, 5 mM of $Mgcl_2$, pH 7.2) in order to examine the mitochondrial antioxidant enzymes.

Determination of biochemical parameters

Serum glucose, uric acid, urea, creatinine concentrations were determined using colorimetric enzymatic assays (kits from BioAssay Systems, Hayward, CA). Glycated hemoglobin (HbA_{1c}) was determined in whole blood using a kit from Pointe Scientific, Inc., United States. Liquid chromatography method. Serum triglycerides, cholesterol were measured using colorimetric enzymatic kits (Sigma, St. Louis, MO). Serum alanine aminotransferase EC 2.6.1.2 (ALT), aspartate aminotransferase EC 2.6.1.1 (AST), alkaline phosphatase EC 3.1.3.1 (ALP), lactate dehydrogenase EC 1.1.1.27 (LDH) were determined using colorimetric enzymatic kits (Spinreact, Girona, Spain). Serum levels of tumor necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6) were estimated using enzyme-linked immunosorbent assay (ELISA) kits (Genzyme corp, Cambridge, MA, USA).

Determination of markers of the oxidant/antioxidant status

The catalase (CAT) activity was measured by spectrophotometric analysis of the decomposition rate of hydrogen peroxide [24]. The reduced glutathione (GSH) was assayed using Ellman's method [25]. The superoxide dismutase (SOD) activity was assessed following the NADPH oxidation procedure [26]. The activity of glutathione S-transferases (GST) was estimated by investigating its action on the compound 1-chloro-2,4-dinitrobenzene (CDNB), in the presence of glutathione as a co-substrate [27]. The level of malondialdehyde (MDA), which is commonly known as a marker of lipid peroxidation, was determined by considering the reaction of MDA with thiobarbituric acid [28]. Carbonyl proteins (markers of protein oxidation) were assayed by the 2,4-dinitrophenyl hydrazine (DNPH) reaction [29].

Statistical analysis

The results obtained were expressed as mean ± SD (Standart deviation).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

Effects of *N. gaditana* on body weight, food and energy intake

The results of this study showed a significant decrease in the body weight of STZ-induced diabetic rats as compared with normal rats. The group of diabetic rats showed a significant increase in food intake and energy intake as compared to control rats. However, supplementation of the diet of diabetic rats with *N. gaditana* at 10% caused a body weight increase with recovery from these disorders (Table 2).

Effects of *N. gaditana* on biochemical markers

The effects of *N. gaditana* on the levels of glucose, HbA$_{1c}$, triglycerides, cholesterol and markers of renal and hepatic function are shown in Table 3. In diabetic rats, there was a significant increase in the levels of glucose, HbA$_{1c}$, triglycerides, cholesterol, ALP, AST, ALP and LDH, uric acid, urea and creatinine comparatively to the levels found in control rats. However, treatment of diabetic rats with *N. gaditana* significantly decreased levels of these parameters.

Effects of *N. gaditana* on levels of pro-inflammatory cytokines

Serum levels of TNF-α and IL-6 were significantly increased in the diabetic rats as compared with the control rats. However, diabetic rats fed a diet supplemented with *N. gaditana* presented significantly lower levels of TNF-α and IL-6 as compared to diabetic rats fed a control diet (Fig. 1a, b).

Effects of *N. gaditana* on oxidative stress markers

As shown in Fig. 2, the activities of catalase (CAT), reduced glutathione (GSH) and superoxide dismutase (SOD) decreased significantly in the hepatic mitochondria of diabetic rats as compared to control rats; however, when these antioxidant enzymes were measured after dietary supplementation with *N. gaditana*, contradictory results were obtained. In addition, *N. gaditana* was able to reduce malondialdehyde (MDA) and carbonyl proteins levels in liver mitochondria of diabetic rats (Fig. 2a-e).

The levels of MDA and carbonyl proteins in liver tissues were significantly increased in diabetic rats by comparison with control rats. However, diabetic animals fed a diet supplemented with *N. gaditana* presented significantly lower levels of MDA and carbonyl proteins. On the other hand, *N. gaditana* induced a substantial rise in the activities of CAT, GSH, SOD and GST in the liver tissues of diabetic rats (Table 4).

It is worth recalling that diabetic animals fed a diet rich in microalgae *N. gaditana* showed considerably lower contents in MDA and carbonyl proteins with substantially elevated activities of CAT, GSH and SOD in the pancreas, as depicted in Table 5.

Discussion

Diabetes mellitus is a metabolic disorder that is characterized by hyperglycemia due to an absolute or relative deficiency in insulin secretion. Therefore, the present study investigated the anti-inflammatory and antioxidant properties of the microalgae *N. gaditana* and its preventive effects on STZ-induced diabetes and its complications in Wistar rats. The intraperitoneal administration of STZ to normal rats effectively induces diabetes, which is reflected by glycosuria, polyphagia and body weight loss, as previously observed by Akbarzadeh et al. [30]. The current study found out that when STZ-induced diabetic rats are fed a diet supplemented with *N. gaditana*, the above listed diabetic complications are reversed. The body weight increase could probably be attributed to potentiation of insulin secretion by beta cells of the

Table 2 Effects of *N. gaditana* on body weight, food and energy intake

Parameters	Control rats		Diabetic rats		*P* (ANOVA)
	C	CM	D	DM	
Body weight (g)	225 ± 6.21[a]	220.82 ± 5.11[a]	198.17 ± 4.37[c]	210.27 ± 3.21[b]	0.04
Food intake (g/day/rat)	22.41 ± 0.22[c]	23.11 ± 0.92[c]	54.51 ± 0.34[a]	38.34 ± 0.12[b]	0.03
Energy intake (kcal/day/rat)	125.50 ± 10.59[c]	128.27 ± 8.48[c]	206.18 ± 5.63[a]	150.08 ± 16.80[b]	0.001

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%

Values are presented as means ± SD (Standart deviation). Values with different superscript letters (a, b, c, d) are significantly different (ANOVA)

Table 3 Effects of *N. gaditana* on biochemical parameters

Parameters	Control rats		Diabetic rats		P (ANOVA)
	C	CM	D	DM	
Glucose (g/L)	1.17 ± 0.01^{c}	1.20 ± 0.04^{c}	4.41 ± 0.08^{a}	2.44 ± 0.02^{b}	0.010
HbA$_{1c}$ (%)	4.40 ± 0.14^{c}	4.10 ± 0.2^{c}	10.70 ± 0.87^{a}	9.60 ± 0.7^{b}	0.004
Triglycerides (g/L)	1.15 ± 0.01^{c}	1.10 ± 0.01^{c}	2.28 ± 0.04^{a}	1.94 ± 0.03^{b}	0.006
Cholesterol (g/L)	1.53 ± 0.01^{c}	1.44 ± 0.01^{c}	2.43 ± 0.04^{a}	2.02 ± 0.03^{b}	0.004
ALT (IU/L)	42.45 ± 1.47^{c}	40.71 ± 1.47^{c}	52.94 ± 0.88^{a}	48.51 ± 1.91^{b}	0.010
AST (IU/L)	36.86 ± 4.00^{c}	30.8 ± 4.00^{c}	50.51 ± 0.71^{a}	41.94 ± 0.03^{b}	0.007
ALP (IU/L)	83.35 ± 4.15^{b}	81.14 ± 4.15^{b}	90.33 ± 3.83^{a}	84.16 ± 3.48^{b}	0.006
LDH (IU/L)	323.41 ± 159.42^{c}	314.38 ± 166.70^{c}	655.85 ± 191.06^{a}	462.13 ± 286.72^{b}	0.033
Uric acid (mg/dL)	35.38 ± 0.93^{c}	39.18 ± 1.64^{b}	72.76 ± 1.82^{a}	39.44 ± 2.73^{b}	0.010
Urea (mg/dL)	36.62 ± 1.95^{c}	36.15 ± 3.24^{c}	41.26 ± 2.72^{a}	38.45 ± 1.83^{b}	0.010
Creatinine (mg/dL)	65.90 ± 5.55^{c}	64.54 ± 2.07^{c}	90.26 ± 3.29^{a}	87.73 ± 3.25^{b}	0.010

Values are presented as means ± SD (Standart deviation). Values with different superscript letters (a, b, c, d) are significantly different (ANOVA). *HbA$_{1c}$* glycated hemoglobin, *ALT* alanine aminotransferase, *AST* aspartate aminotransferase, *ALP* alkaline phosphatase, *LDH* lactate dehydrogenase

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%

pancreas. Moreover, it has been observed that the incorporation of *N. gaditana* into a diet has remarkable beneficial effects on body weight [31].

In our study, diabetic rats fed a diet supplemented with *N. gaditana* presented significantly lower levels of glucose and glycated hemoglobin, suggesting their antidiabetic effect. Previous studies have tested the antidiabetic activity of microalgae and their anti-glycation properties [32].

It is interesting to note that the most common lipid abnormalities in diabetes are hypertriglyceridemia and hypercholesterolemia [33]. Treatment of diabetic rats with *N. gaditana* markedly reduced cholesterol and triglycerides levels. This study indicated that *N. gaditana* possesses interesting antihyperlipidemic properties. Previous studies reported that the alga *Nannochloropsis* has remarkable hypocholesterolemic effects on rats [34]. It was also revealed that *N. gaditana* has the capacity to improve the lipid metabolism [31].

Furthermore, it has been shown that in general serum enzyme activities (ALT, AST, ALP and LDH) increase in diabetic group; this is mainly attributed to the leakage of these enzymes from the liver cytosol into the bloodstream, which may cause liver dysfunction or damage of liver cells. The
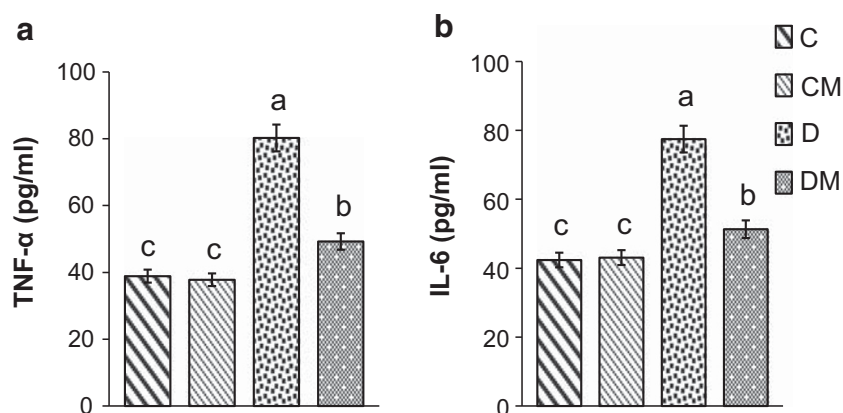
Fig. 1 Effects of *N. gaditana* on TNF-α (**a**) and IL-6 (**b**) levels. TNF-α tumor necrosis factor-alpha, IL-6 interleukin-6, C normal rats fed a control diet, CM normal rats fed a control diet enriched with microalgae at 10%, D diabetic rats fed only with control diet, DM diabetic rats fed a control diet enriched with microalgae at 10%. Values are presented as means ± SD (Standart deviation). Values with different superscript letters (a, b, c, d) are significantly different (ANOVA)

Fig. 2 Effects of *N. gaditana* on CAT (**a**), GSH (**b**), SOD (**c**), MDA (**d**) and carbonyl proteins (**e**) levels in the liver mitochondria. CAT catalase, GSH reduced glutathione, SOD superoxide dismutase, MDA malondialdehyde, 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%. Values are presented as means ± SD (Standart deviation). Values with different superscript letters (**a**, **b**, **c**, **d**) are significantly different (ANOVA)
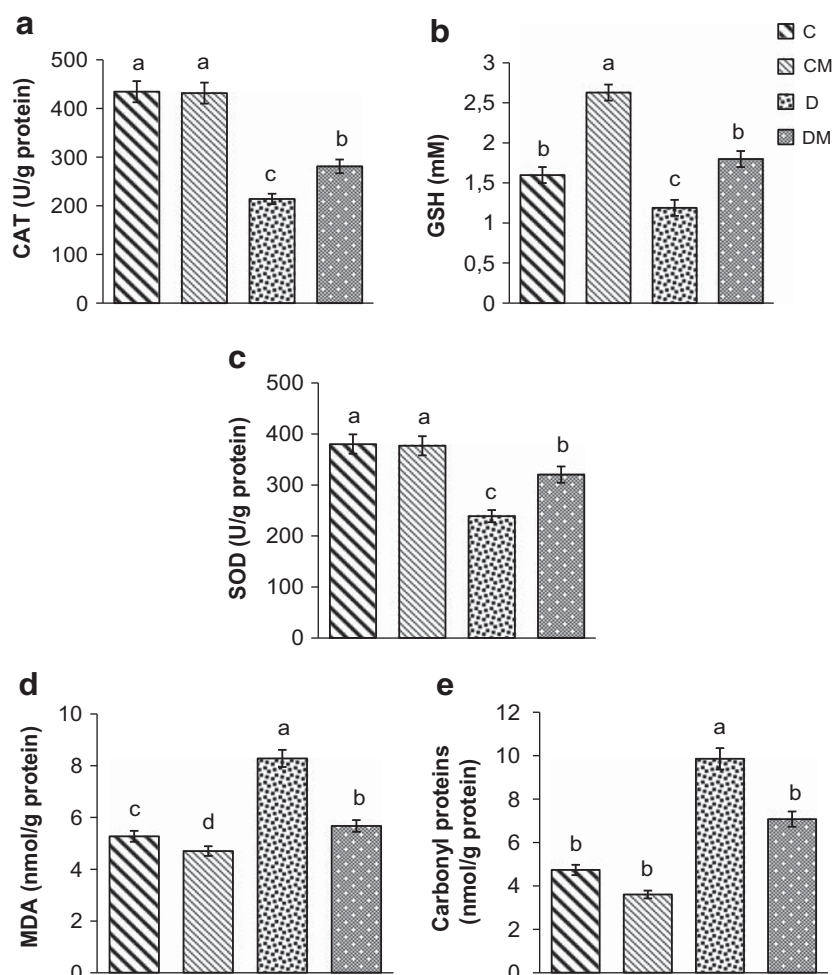
results obtained were found consistent with those reported by Nithiya et al. [35]. Therefore, treatment of diabetic rats with *N. gaditana* caused reduction in the activity of these enzymes, which indicates the hepatoprotective effect of *N. gaditana* in diabetic rats.

Moreover, this study allowed discovering that a diet supplemented with microalgae *N. gaditana* provided good protection against STZ-induced renal dysfunction in diabetic rats because this alga has great potential to normalize the contents of serum uric acid, urea and creatinine in rats with diabetes.

Table 4 Effects of *N. gaditana* on oxidant/antioxidant markers in liver tissues

Parameters	Control rats		Diabetic rats		P (ANOVA)
	C	CM	D	DM	
MDA (μmol/g)	1.30 ± 0.50^c	1.32 ± 0.30^c	3.30 ± 0.94^a	2.15 ± 0.56^b	0.001
Carbonyl proteins (μmol/mg)	2.21 ± 0.56^c	2.27 ± 0.50^c	3.51 ± 0.44^a	2.88 ± 0.56^b	0.001
CAT (U/g)	68.56 ± 4.26^a	65.15 ± 9.20^a	46.53 ± 8.48^c	57.26 ± 3.51^b	0.001
GSH (μmol/g)	5.00 ± 0.60^a	4.98 ± 1.33^a	2.56 ± 0.22^c	3.56 ± 0.28^b	0.001
SOD (U/g)	3.44 ± 0.73^a	3.48 ± 0.99^a	1.61 ± 0.04^c	2.37 ± 0.14^b	0.000
GST (nmol/g)	6.18 ± 0.48^a	5.84 ± 0.94^a	4.36 ± 0.52^c	4.98 ± 1.10^b	0.004

Values are presented as means ± SD (Standart deviation). Values with different superscript letters (a, b, c, d) are significantly different (ANOVA). MDA malondialdehyde, CAT catalase, GSH reduced glutathione, SOD superoxide dismutase, GST glutathione S-transferase

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%

Table 5 Effects of *N. gaditana* on oxidant/antioxidant markers in pancreatic tissues

Parameters	Control rats		Diabetic rats		P (ANOVA)
	C	CM	D	DM	
MDA (μmol/g)	1.47 ± 0.60^c	1.66 ± 0.50^c	3.67 ± 0.83^a	2.03 ± 0.59^b	0.005
Carbonyl proteins (μmol/mg)	2.54 ± 0.25^c	2.60 ± 0.41^c	3.07 ± 0.71^a	2.98 ± 0.14^b	0.001
CAT (U/g)	62.59 ± 5.55^a	60.02 ± 4.35^a	30.07 ± 4.00^c	53.31 ± 8.82^b	0.001
GSH (μmol/g)	5.40 ± 1.19^a	5.92 ± 0.26^a	4.18 ± 0.35^c	5.03 ± 0.19^b	0.002
SOD (U/g)	1.63 ± 0.02^a	1.66 ± 0.01^a	1.31 ± 0.13^c	1.45 ± 0.27^b	0.002

Values are presented as means ± SD (Standart deviation). Values with different superscript letters (a, b, c, d) are significantly different (ANOVA). MDA malondialdehyde, CAT catalase, GSH reduced glutathione, SOD superoxide dismutase

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%

Similar results have been reported about diabetic rats fed a diet supplemented with *Nannochloropsis oculata* [36].

In our study, the serum concentrations of TNF-α and IL-6 were significantly increased in diabetic rats, which is in good agreement with the findings of Mesbahzadeh et al. [37] and Samarghandian et al. [38]. In contrast, *N. gaditana* supplementation was shown effective in lowering the levels of TNF-α and IL-6 in diabetic rats, this suggests that *N. gaditana* supplementation could play a preventive role against inflammation. Moreover, the anti-inflammatory potential of *N. gaditana* could be due to various valuable pigments contained in this microalga such as the carotenoids [39].

It is well known that mitochondria are complex organelles capable of generating intracellular reactive oxygen species [40]. When mitochondrial ROS production exceeds the cellular antioxidant capacity, the increase in ROS levels can lead to oxidative stress [41]. This phenomenon has been shown to play a major role in the development of STZ-induced diabetes mellitus and mitochondria may be the main target of this toxicity [42].

The present work made it possible to show that the concentrations of antioxidant enzymes (CAT, GSH, SOD) increased remarkably in liver mitochondria of diabetic rats fed with *N. gaditana* in comparison with diabetic animals fed an ordinary diet. Indeed, *N. gaditana* reduce contents of carbonyl proteins and MDA in liver mitochondria of diabetic rats. Thus, observed results indicate that *N. gaditana* may improve the antioxidant property in mitochondria, and attenuate the mitochondrial oxidative damage in the liver.

Regarding the oxidant/antioxidant status of liver and pancreas tissues, our results indicate that microalgae *N. gaditana* can help to alleviated oxidative stress in diabetic rats by reducing oxidant markers, such as MDA and carbonyl proteins, and increasing antioxidant defense. These results support the fact that *N. gaditana* has the potential to enhance the antioxidant activities and protect tissues from lipid and protein oxidation during diabetes. This elevation in antioxidants enzymes may be due to the neutralization of reactive oxygen species. To further support our findings, it has been reported that *N. gaditana* has the ability to scavenge free radicals and inhibit lipid peroxidation [43]. Moreover, supplementation with *N. gaditana* reduced the oxidative stress and enhanced antioxidant status in diabetic rats [16].

Conclusion

The present study revealed that *N. gaditana* attenuates hyperglycemia and hyperlipidemia and improves the renal and hepatic functions in STZ-induced diabetic rats. In addition, *N. gaditana* could also attenuate oxidative stress and inflammation and prevent complications associated with diabetes.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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