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Antihyperglycemic and antihyperlipidemic activities of *Nannochloropsis oculata* microalgae in Streptozotocin-induced diabetic rats

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Abstract: Background: It is well documented that biologically active components of microalgae can be utilized for treatment of different diseases. This study was conducted to evaluate the antihyperglycemic and antihyperlipidemic activities and weight control of *Nannochloropsis oculata* microalgae (NOM) in Streptozotocin-induced diabetic male rats.

Methods: Diabetes was induced by intraperitoneal administration of Streptozotocin (55 mg/kg). Healthy and diabetic rats were divided in to six groups. Healthy and diabetic rats orally received distilled water or NOM (10 and 20 mg/kg) for three weeks.

Results: Oral administration of NOM to diabetic rats significantly reduced the serum concentrations of glucose, cholesterol, triglycerides, LDL and increased the serum concentration of insulin and HDL-C ($P<0.05$). Treatment with NOM had no significant effect on blood parameters in healthy rats ($P>0.05$). Also, NOM maintained body weight in diabetic rats ($P<0.05$).

Conclusion: It can be concluded that NOM has antihyperglycemic and antihyperlipidemic activities in diabetic rats.

Keywords: Diabetic rat, Glucose, Healthy rat, Insulin, *Nannochloropsis* Microalgae

Introduction

Diabetes is one of most common cause of death in the world, that is mainly caused by persistent hyperglycemia due to disturbance in insulin secretion (type 1 diabetes – T1DM and insulin activity (type 2 diabetes- T2DM) [1, 2]. Reactive oxygen species (ROS) are produced in diabetes mellitus [3]. Today researchers are trying to find natural products such as herbs and algae which can be used to normalize the blood glucose and dyslipidemia in diabetes mellitus. Natural antioxidants in microalgae structure have been extensively considered for treatment of diabetes because they decrease the risk of chronic diseases and promote human health [4, 5]. Microalgae are tiny photosynthetic components that convert solar energy to biomass. It is well known that algae products especially cyanobacteria have therapeutic properties [6, 7]. Therapeutic potential of microalgae may be associated to biological compounds such as essential amino acids, vitamins, pigments, and lipid profile [8]. *Nannochloropsis oculata*, marine unicellular microalgae, is a member of the *Eustigmatophyceae* class and are known to have nutritional values [9 - 11]. Nannochloropsis microalgae (NOM) contain some components including soluble and insoluble polysaccharide, protein and eicosapentaenoic acid [12, 13]. Studies have also shown that eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) regulate levels of blood lipids [18 - 20]. In addition, NOM protects the activity of EPA and DHA [14, 15] due to its antioxidant pigments and polyunsaturated fatty acids [13, 16]. On the other hand, previous studies have shown that the EPA in NOM compound can efficiently be used into the blood, liver, and brain lipids of rats [21 - 22]. Thus, NOM prevent EPA and DHA oxidation by their antioxidant properties. Werman et al. [17] reported that male rats fed with *Nannochloropsis oculata* showed lower level of cholesterol in comparison to control group. NOM also contains insoluble fibers that decrease plasma cholesterol by modification of bile acid absorption and metabolism [23]. Moreover, Pandey et al. [24] reported that treatment with other microalgae (*Spirulina maxima*) decreased the serum concentration of glucose in diabetic rats. The *S. platensis* microalga interacts with ROS and quenches them during oxidative process [25, 26]. Since the most studies have used

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Streptozotocin (STZ) in order to induce diabetes [8, 24, 27], as we have used in this study. Therefore, this study aimed to evaluate the antihyperglycemic or antihyperlipidemic activities of NOM in healthy and diabetic rats.

Methods

Experimental animals

The current study was conducted in Birjand University (South Khorasan-Iran). Male Wistar rats (n=60, 200 ± 20 g) were kept at room temperature (25°C). A lighting diet (12h light/12h dark) was considered. All the rats fed a standard pellet diet (Javeneh Khorasan Company) and water *ad libitum*. The rats were separated into two groups (healthy and diabetic) and treated with microalgae (10 and 20 mg/kg BW, orally for three weeks). A control group received distilled water. Thus rats were divided into 6 groups with 10 animals per group. Experimental treatments are presented in Table 1. The *Nannochloropsis oculata* microalga was purchased from Ghazaye Sabze Khalij (Bandar Abbas-Iran). Weight was recorded weekly in order to evaluate the weight changes. All the experiments were approved by the Animal Ethics Committee of Birjand University (Ethics Code: BU/94-D-12661). The experiments were conducted in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

Ethical approval: The research related to animals use has been complied with all the relevant national regulations and institutional policies for the care and was approved by Birjand University Research Ethical Committee (Ethics Code: BU/94-D-12661), Birjand University, Birjand, Iran, on the use of animals, and conformed to the Guide for the Care and Use of Laboratory Animals published by the U. S. National Institutes of Health (NIH Publication No. 85–23, revised 1996) for studies involving experimental animals.

Induction of experimental diabetes

Streptozotocin (Sigma-Aldrich) was dissolved in citrate buffer, pH 4.5 (0.1 mol/l trisodium citrate, 0.1 mol/l citric acid) and used to induce diabetes mellitus (55 mg/kg BW) in rats [27]. Diabetes was confirmed by measuring of blood glucose levels three days after the STZ injection. Animals with serum glucose level higher than 250 mg/dl were considered as diabetic [27].

Biochemical analysis

On 21 d of trial and after a 12-h fasting, animals were anesthetized then decapitated and 3 ml blood of per rat were collected and centrifuged at 3000×g for 15min in order to obtain the sera. Blood samples were analyzed to determine the levels of glucose, insulin, albumin, cholesterol, HDL-C, LDL-C, total protein, total bilirubin, triglycerides, creatinine, alkaline phosphatase (ALP), aspartate aminotransferase (AST), alanine aminotransferase (ALT) and uric acid by auto-analyzer apparatus (Gesun Chem 200. Italy).

Statistical analysis

The analysis was performed using the statistical software package Graph Pad Prism version 5.0 for Windows (Graph Pad Software, San Diego, CA). The data was analyzed using one-way analysis of variance (ANOVA) followed by Dunnett's Multiple Range Test. Animals in healthy group and diabetic group were separately compared by ANOVA way. Healthy and diabetic rats were also compared by dose by T-test. The results show the mean ± standard deviation, with a P value of <0.05 being considered statistically significant.

Results

Our findings show that the serum concentrations of glucose, insulin, triglycerides, cholesterol, LDL-C and HDL-C were not influenced by NOM administration in healthy rats (Tables 3 and 4; $P>0.05$). Administration of NOM in diabetic rats significantly decreased the serum concentration of glucose, triglycerides, cholesterol, and LDL-C in comparison to diabetic control rats. As demonstrated in Tables 2 and 3, HDL and insulin levels remarkably decreased in STZ diabetes in comparison to healthy control. Administration of NOM (20 mg/kg) in diabetic rats effectively prevented HDL and decreased insulin. Significant differences were seen between diabetic rats and healthy rats for total protein, albumin, alkaline phosphatase, aspartate amino transferase and alanine amino transferase ($P<0.05$; Tables 4 and 5). Oral administration of NOM had no significant effects on the mentioned parameters in diabetic rats (Tables 4 and 5). There were no significant differences between diabetic rats and healthy rats for total bilirubin, uric acid and creatinine ($P>0.05$). Results indicated that diabetic rats had lower final weight compared with healthy rats

Table1: Experimental groups (BW= body weight).

Healthy	Diabetic
Control (0.3 ml of distilled water)	Control (0.3 ml of distilled water)
Microalgae 10 mg.kg^{-1} BW	Microalgae 10 mg.kg^{-1} BW
Microalgae 20 mg.kg^{-1} BW	Microalgae 20 mg.kg^{-1} BW

Table 2: Effect of *Nannochloropsis oculata* microalgae (NOM) at levels of 10 and 20 mg.kg^{-1} of body weight on the serum concentrations of glucose and insulin in healthy and diabetic rats.

	Glucose (mg/dl)			Insulin (mIU/ml)		
Groups	Healthy	Diabetic	*P*	Healthy	Diabetic	*P*
Control	180±11^{B}	380±21^{A,a}	*	1.12±0.26^{A}	0.32±0.06^{B,c}	*
NOM 10	175±19^{B}	250±24^{A,b}	*	1.1±0.3^{A}	0.6±0.11^{B,b}	*
NOM 20	173±19	180±18^{c}	NS	1.13±0.14	1.0±0.25^{a}	NS
P	NS	*		NS	*	

$^{A-B}$ shows significant differences at per row at $P<0.05$ and $^{a-c}$ shows significant differences in per column at level of 0.05*. NS: nonsignificant. Column comparisons were performed by ANOVA and row comparisons were done with T-test.

Table 3: Effects of *Nannochloropsis oculata* microalgae (NOM) at rates of 10 and 20mg.kg^{-1} of body weight on the serum concentrations of triglycerides, cholesterol or LDL-C and HDL-C in healthy and diabetic rats.

	Triglycerides (mg/dl)			Cholesterol (mg/dl)			LDL-C (mg/dl)			HDL-C (mg/dl)		
Groups	Healthy	Diabetic	*P*	Healthy	Diabetic	*P*	Healthy	Diabetic	*P*	Healthy	Diabetic	*P*
Control	32±5^{B}	63±4^{A,a}	*	51±4^{B}	80±7^{A,a}	*	32±4^{B}	70±8^{A,a}	*	36±5^{A}	19±3^{B,b}	*
NOM 10	34±7^{B}	60±6^{A,a}	*	53±10	61±9^{b}	NS	34±5^{B}	55±7^{A,b}	*	31±5^{A}	21±5^{B,b}	*
NOM 20	36±4	42±10^{b}	NS	54±9	60±9^{b}	NS	36±6	41±4^{c}	NS	32±4	26±9^{a}	NS
P	NS	*		NS	*		NS	*		NS	*	

$^{A-B}$ shows significant differences at per row at $P<0.05$ and $^{a-b}$ shows significant differences in per column at level of 0.05*. NS: non-significant. Column comparisons were performed by ANOVA and row comparisons were done with T-test.

Table 4: Effects of *Nannochloropsis oculata* microalgae (NOM) at rates of 10 and 20mg.kg^{-1} of body weight on the serum concentrations of total protein, albumin, uric acid and creatinine in healthy and diabetic rats.

	Total protein (g/dl)			Albumin (g/dl)			Uric acid (mg/dl)			Creatinine (mg/dl)		
Groups	Healthy	Diabetic	*P*	Healthy	Diabetic	*P*	Healthy	Diabetic	*P*	Healthy	Diabetic	*P*
Control	3.5±0.5^{B}	5.2±0.6^{A}	*	3.1±0.4^{B}	4.5±0.6^{A}	*	2.1±0.4	2.3±0.4	NS	0.5±0.05	0.5±0.07	NS
NOM 10	3.3±0.4^{B}	5.5±0.5^{A}	*	2.9±0.5^{B}	4.7±0.5^{A}	*	1.9±0.5^{B}	2.2±0.2	NS	0.6±0.1	0.6±0.1	NS
NOM 20	3.6±0.5^{B}	5.0±0.6^{A}	*	2.8±0.3^{B}	5.0±0.5^{A}	*	2.2±0.8	2.4±0.2	NS	0.7±0.2	0.7±0.1	NS
P	NS	NS		NS	NS		NS	NS		NS	NS	

$^{A-B}$ shows significant differences at per row at $P<0.05$ *. NS: non-significant. Column comparisons were performed by ANOVA and row comparisons were done with T-test.

Table 5: Effects of *Nannochloropsis oculata* microalgae (NOM) at rates of 10 and 20mg.kg^{-1} of body weight on the serum concentrations of total bilirubin, alkaline phosphatase (ALP), aspartate amino transferase (ASAT) and alanine amino transferase (ALAT) in healthy and diabetic rats.

	Total bilirubin (mg/dl)			ALP (U/l)			ASAT (U/l)			ALAT (U/l))		
Groups	Healthy	Diabetic	*P*	Healthy	Diabetic	*P*	Healthy	Diabetic	*P*	Healthy	Diabetic	*P*
Control	0.17±0.04	0.17±0.05	NS	86±15^{B}	431±19^{A}	*	97±25^{B}	420±31^{A}	*	80±5.5^{B}	154±6.4^{A}	*
NOM 10	0.17±0.05	0.18±0.05	NS	89±11^{B}	440±18^{A}	*	101±14^{B}	430±65^{A}	*	75±5.3^{B}	156±8.6^{A}	*
NOM 20	0.18±0.05	0.19±0.04	NS	97±17^{B}	483±85^{A}	*	113±28^{B}	442±67^{A}	*	84±9.1^{B}	153±6.0^{A}	*
P	NS	NS		NS	NS		NS	NS		NS	NS	

$^{A\text{-}B}$ shows significant differences at per row at $P<0.05$ NS: non-significant. Column comparisons were performed by ANOVA and row comparisons were done with T-test.

Table 6: Effects of *Nannochloropsis oculata* microalgae (NOM) at rates of 10 and 20mg.kg^{-1} of body weight on final body weight (g) in healthy and diabetic rats.

	Final Weight (g)		
Groups	Healthy	Diabetic	*P*
Control	225±13^{A,c}	170±13^{B,b}	*
NOM 10	250±13^{A,b}	185±11^{B,a}	*
NOM 20	270±17^{A,a}	190±13^{B,a}	*
P	*	*	

$^{A\text{-}B}$ shows significant differences at per row at $P<0.05$ and $^{a\text{-}c}$ shows significant differences in per column at level of 0.05*. NS: non-significant. Column comparisons were performed by ANOVA and row comparisons were done with T-test.

($P<0.05$). Our findings in Table 6 show that treatment with NOM increased final weight in healthy rats and helped to maintain the weight in diabetic rats ($P<0.05$).

Discussion

There were significant differences between healthy and diabetic rats (control and NOM10) for insulin and glucose concentration. It is well known that STZ increases glucose concentration. Our findings were confirmed by other researchers who showed that administration of STZ increased glucose and decreased insulin concentration in rats [21, 23, 28]. Montilla et al. [29] believed that hyperglycemia induced injuries in tissues and brain, urinary glucose excretion and decreased plasma insulin. These injuries create physiologic changes in some tissues, where oxidative stress induced by hyperglycemia plays an important role in the etiology [25, 30, 31]. In this regard, Gu et al. [32] stated that STZ damages pancreatic β-cells by induction of ROS and thus it reduces capability of insulin secretion. ROS play a key role in development and progression of diabetes and levels of ROS were increased in pancreatic islets of diabetic rats [27]. Our results showed that NOM has protective effects on insulin and glucose concentration, but significant differences were not observed between diabetic rats treated with NOM 20 and healthy. Unfortunately, we could not find any study showing effects of NOM on glucose and insulin in healthy and diabetic rats. Other studies showed that treatment of diabetic rats with *Spirulina* microalgae [28, 29] and *Chlorella* microalgae [30, 31] reduced glucose levels and increased insulin concentration. It seems NOM reduces glucose levels by insulin secretion from β-cell islet or prevention of pancreatic injuries or increase transport of glucose to peripheral tissues. The idea was supported by Yanardag et al. [33] who showed that treatment with insulin had protective effects against STZ in rats. We believed that antioxidant pigments present in NOM [34], protect cellular damage induced by ROS and may increase insulin secretion in diabetic rats. In the current study, NOM not only contains antioxidant pigments but also contains selenium and possible antioxidant vitamins which may protect pancreas. Selenium modulates the antioxidant system through cooperating in glutathione peroxidases, thioredoxin reductase, and iodothyronine deiodinase structure. Our theory is confirmed by other researchers who showed that *S. platensis* microalgae interacts with ROS during the oxidative process and quenches free radicals [6, 22]. Glucose and lipid metabolism are associated other. Similar to glucose and insulin findings, administration of STZ increased lipid profile in rats when compared with normal rats (healthy control vs diabetic

control), i.e. an increase in the serum concentrations of triglycerides, cholesterol, LDL-C and a decrease in HDL-C.

In present study, diabetic rats showed abnormalities in lipid metabolism as proved by increased the plasma total triglycerides, cholesterol, LDL-C and decreased HDL-C levels. Treatment with NOM, for three weeks, markedly reduced plasma TG, total cholesterol, and LDL-C associated with significant increased HDL-C levels in diabetic rats indicating its potent antihyperlipidemic and anti-atherogenic activity. Insulin suppresses the release of free fatty acids by inhibition the activity of hormone sensitive lipases and subsequently inhibits lipolysis. Since the activity of this enzyme is to be increased in diabetes, more fatty acid releases to the circulation by lipolysis, therefore more β-oxidation of FA which produces more acetyl-CoA and cholesterol in diabetics. The hypoinsulinemia in diabetes plays an important role in disturbance of lipid profile. This study indicated that NOM supplementation improved lipid profile in diabetic rats. The hypocholesterolaemic activity of NOM may be due to the prevention of absorption of cholesterol from the intestines (similar to *Spirulina)* [10, 35 - 38] or inhibition of oxidation and the uptake of LDL-C. There are reports documenting an improvement in lipid profile and oxidative status in diabetic animals and humans treated with other microalgae [28, 39 - 43]. Markovits et al. [44] reported that dietary fibers present in *Nannochloropsis*, especially insoluble fibers, prevent intestinal cholesterol absorption [45] and have anti-hypercholestromic activity. EPA is extensively founds in NOM and has protective effects against atherogenesis and lipid abnormalities [11]. Moreover, EPA and DHA regulate levels of blood lipids [18, 19, 20]. In addition, Mokady and Sukenik [30] reported that EPA and DHA act as lowering lipid and cholesterol in blood, liver, and brain. Some studies showed that hypoinsulinemia caused significant changes in lipids turnover [29, 41] and since NOM increased insulin concentration and an increase in insulin concentration may be factor for improvement in lipid profile in diabetic rats treated with NOM (20 mg/kg). On the other hand, oxygen free radical initiates peroxidation of lipids, which it stimulates glycation of protein and inactivation of enzymes. There are clinical studies that show production of ROS is to be increased in both types of diabetes. Normally, the level of oxidative stress is modulated by antioxidant defense systems [37]. Thus, present antioxidant pigments in NOM may decrease negative effects of ROS on lipid profiles. The oxygen species, as oxidants are known to increase the lipids and glucose. Antioxidant pigments, vitamins and minerals may interact with oxidants and thus decrease their negative effects on lipid parameters.

In the present study STZ increased the serum concentrations of albumin and protein in diabetic rats ($P<0.05$) without significant changes in uric acid, total bilirubin, and creatinine concentrations ($P>0.05$). Administration of NOM did not have any effect on albumin and protein in diabetic rats. Although diabetes mellitus disturbs albumin and protein metabolism [43], but Montilla et al. [29] did not observe significant changes in albumin and creatinine in STZ-induced diabetic rats. Partly similar to our observation, Kagan et al. [45, 46] showed EPA-rich polar lipid obtained from NOM had no significant effect on the plasma concentration of albumin, protein, bilirubin and creatinine in male rats.

In our STZ induced hepatotoxicity which is proved by increased levels of ALP, ALT and AST in the serum [47]. Our findings were confirmed by other researchers who showed STZ increases levels of ALP, ALT and AST in serum [44] and kidney [48]. Treatment of rats with NOM had no significant effect on mentioned parameters. Parallel to our findings, Kagan et al. [45] reported that EPA-rich polar lipid oil, produced from NOM, does not change ALP, ALAT and ASAT concentration in male rats.

Diabetic rats (control group) showed lower body weight compared with control healthy rats ($P<0.05$). Our observations were confirmed by others [23, 31, 49]. It is well known that diabetes is accompanied with some metabolic diseases that increase weight loss [29, 41]. Oral administration of NOM prevented weight loss in diabetic rats. In contrast to our findings, Nuno et al. [26] documented that NOM administration did not change weight in diabetic rats. In agreement with our findings, treatment with other microalgae (*Spirulina* microalgae; 5-15 $mg.kg^{-1}$ BW) maintained weight in diabetic rats [50 - 54]. NOM may maintain weight by increased insulin and decreased glucose level that alleviate metabolic disorders. In addition, antioxidant pigments and EPA, present in NOM, may maintain levels of glucose, insulin and lipid profile that subsequently improve metabolism in body, resulting in weight maintain. Interestingly, NOM increased weight in healthy rats without affecting on blood biochemical parameters. It is may be related to NOM components that increases body weight and prevents disturb in blood parameters.

Conclusion

In conclusion, STZ decreased body weight and the serum concentration of HDL-C or insulin and also increased other lipid parameters, glucose, ALP, AST and ALT concentrations in diabetic rats when compared with

healthy rats, but NOM reduced lipid profile except HDL-C, glucose and it also increased insulin and HDL-C in diabetic rats. Thus, NOM has anti-hyperlipidemic and anti-hyperglycemic activity in diabetic rats. Oral administration of NOM controlled body weight in diabetic rats. These potentials may be explained by some components present in NOM such as fibers, lipid profile and antioxidant pigments. These findings suggest using NOM for treatment of diabetes mellitus. The relation between NOM and diabetes will be needed more studies.

Abbreviations

NOM: *Nannochloropsis oculata* microalgae
T2DM: Type 2 Diabetes Mellitus
AST: Aspartate aminotransferase
ALT: Alanine aminotransferase
DHA: Docosahexaenoic acid
EPA: Eicosapentaenoic acid
ALP: Alkaline phosphatase
STZ: Streptozotocin
BW: Body weight
HR: Healthy rats
DR: Diabetic rats

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Authors' contributions: NM & HS designed and supervised the study. FN conducted all experiments, statistical analysis, interpreted the results and wrote a draft of the manuscript while NM & HS critically revised and finalized the manuscript for submission. All authors equally contributed. They read and approved the final manuscript.

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