Research Article

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The effects of supplementation of Nannochloropsis oculata microalgae on biochemical, inflammatory and antioxidant responses in diabetic rats

https://doi.org/10.1515/bmc-2022-0025
received September 15, 2022; accepted October 11, 2022

Abstract: Diabetes is accompanied by inflammation and oxidation. Supplementation of anti-inflammatory and antioxidant compounds can prevent the progression of diabetes. This study aimed to investigate the effects of supplementation of Nannochloropsis oculata microalgae (NOM) on the inflammatory and antioxidant responses in diabetic rats. Sixty male rats were divided into six groups as diabetic and non-diabetic rats receiving 0, 10 and 20 mg/kg of body weight of NOM daily for 21 days. Body weight, the serum concentrations of insulin and glucose and the tissue concentrations of interleukin-6 (IL-6), tumor necrosis factor-alpha (TNF-α), nuclear factor kappa B (NF-κB), interleukin-1β (IL-1β), malondialdehyde (MDA), ferric reducing antioxidant power (FRAP), superoxide dismutase (SOD), glutathione peroxidase (GPx) were assessed. The results showed that induction of diabetes significantly reduced the body weight, the serum concentrations of insulin and the tissue concentrations of SOD, FRAP and GPx while increasing the concentrations of glucose, MDA, IL-1β, IL-6, NF-κB and TNF-α. Daily oral administration of NOM (10 and 20 mg/kg) significantly maintained the body weight, the serum concentrations of insulin and the tissue concentrations of SOD, FRAP and GPx while preventing the increase in the concentrations of glucose, MDA, IL-1β and TNF-α. In conclusion, diabetes caused inflammation and oxidation while NOM worked as a natural anti-inflammatory and antioxidant compound.

Keywords: antioxidants, diabetes, inflammation, insulin, rat

Introduction

Diabetes is one of the most complicated diseases which influences human health, welfare and the economy worldwide [1]. It is diagnosed with signs such as hyperglycemia higher than a threshold blood glucose concentration (250 mg/dL) and microvascular end-organ complications [2]. Chronic hyperglycemia in diabetes leads to faults and involves the retina, kidneys, nerves, heart and blood vessels [3]. Studies have shown the involvement of oxidative stress, inflammation cytokines, chemokines, apoptosis and ferroptosis as important factors in the progression of diabetic complications [4,5]. Diabetes also induces oxidative stress and adversely influences insulin activity via several interactive pathways and the production of reactive oxygen species (ROS) [6]. The increase in ROS causes a second messenger and regulates the biological activities of several molecules for the development of diabetes [7]. The induction of diabetes increases the concentration of malondialdehyde (MDA) and decreases the total antioxidant capacity [2,8]. On the other hand, inflammatory factors increase the pathogenesis of diabetes. The involvement of interleukin-1β (IL-1β) as a key cytokine in insulin signaling, glucose uptake, lipogenesis and the expression of type 4 has been elucidated [9]. Tumor necrosis factor alpha (TNF-α) is closely related to obesity and diabetes and correlates with hemoglobin A1C [10]. Nuclear factor kappa B (NF-κB) is a transcription factor that is responsible for an increase in the expression of inflammatory proteins in diabetes [11]. Interleukin-6 (IL-6) plays an important role in the early phases of inflammation and inflammatory response [12]. Since inflammation and oxidative stress are involved in the pathogenesis of diabetes, supplementation of antioxidant and anti-inflammatory compounds are efficient
for the prevention of diabetes. Several studies have suggested supplementing of natural antioxidants and anti-inflammatory combinations for the prevention of diabetes [13–15].

Microalgae have long been considered as replacements for unconventional protein sources and food supplements for animal and human nutrition [16]. *Nannochloropsis* (Ochrophyta, Eustigmatophyceae) is fast-growing microalgae used for biotechnology applications owing to its compounds such as high triacylglycerol content and omega-3 fatty acids [17]. It is known to have antioxidant properties in DPPH, H2O2 and ABTS assays [18,19]. *Nannochloropsis oculata* microalgae (NOM) had anti-inflammatory properties via modulation in the expression of pro-inflammatory cytokines [20]. Several studies have investigated the efficiency of NOM in diabetes via lipid profile and intestinal morphology [21], blood biochemical parameters [22] and enzymatic mechanisms [23]. However, a study investigating the effects of NOM on inflammation and antioxidant responses in diabetic rats was not found. This study aimed to study the effects of NOM on inflammatory and antioxidant responses in diabetic rats via assessing the concentrations of IL-6, NF-κB, IL-1β, TNF-α, MDA, ferric reducing antioxidant power (FRAP), superoxide dismutase (SOD), glutathione peroxidase (GPx), insulin and glucose.

**Materials and methods**

**Animals**

In the current study, 60 male adult Wistar rats (*Rattus norvegicus allivias*) were randomly assigned into six groups (*n* = 10). The rats were first divided to non-diabetic and diabetic groups. They were again grouped into three groups. Experimental groups included 1–3 non-diabetic rats receiving daily 0 (C–H), 10 (H-10) and 20 mg/kg body weight NOM (H-20) and 4–6 diabetic rats receiving daily 0 (C–D), 10 (D-10) and 20 mg/kg body weight NOM (D-20). Animals that did not receive NOM were considered as control. Animals were kept under standard conditions for humidity, temperature and access to water and feed. The microalgae package was prepared from Green Food Company (Bandar-Abbas city) in powdered form. Microalgae were dissolved in water and daily administered orally for 21 days. Control rats daily received 20 mg/kg body weight of normal saline. Body weight was also measured after 21 days.

**Ethical approval**: The research related to animals’ use has been complied with all the relevant national regulations and institutional policies for the care and use of animals. The ethical Committee of Islamic Azad University approved all the treatments and procedures in this study (No. IR.IAU.PS.REC.1400.178).

**Preliminary bioassay**

A preliminary bioassay was conducted as reported by other studies [21]. In summary, 30 rats were grouped into eight groups and daily administered with 0, 5, 10, 20, 40 and 60 mg/kg body weight of NOM for 1 week. The animals were monitored during the intervention and 1 week after modulation for any signs of toxicity, fever, diarrhea, vomiting, lethargy and mortality. Since no toxicity signs and mortality were seen, doses of 10 and 20 mg/kg of body weight were selected.

**The induction of diabetes**

Diabetes was induced as reported by other studies with the help of streptozotocin [22]. Briefly, the rats were administrated with 55 mg/kg of body weight streptozotocin (Sigma-Aldrich) dissolved in citrate buffer. The blood samples were collected after 1 week and animals with a serum concentration of glucose higher than 250 mg/dL were considered diabetic.

**The preparation of blood and tissue samples**

On Day 21 and after 12 h fasting, animals were anesthetized using ketamine (80 mg/kg) and xylazine (6 mg/kg) and euthanized using CO₂. The blood samples were collected from the heart and then centrifuged at 1,700 rpm for 15 min. Liver samples were obtained and stored at −20°C until further studies.

**The investigation of parameters**

The serum concentrations of insulin and glucose were assessed using commercial kits of Pars Azmoon
Company (Tehran, Iran) using a Gesan56hem.200 auto-analyzer. To assess MDA, 1.5 mL phosphoric acid 1.00%, 0.5 mL TBA and 250 µL of the sample were mixed, transferred into Bain-Marie bath for 45 min and cooled. Four milliliters of N-butanol was added and centrifuged at 3,000 rpm for 10 min. Upper solution was investigated for MDA using ELISA microplate reader at 535 nm wavelength. To assess FRAP, 1.5 mL reagent was mixed with 50 µL of sample at 37°C for 5 min and investigated at 593 nm. To evaluate the other factors, liver samples were homogenously prepared. The concentration of inflammatory factors was assessed using commercial kits of Abcam company based on producer company recommendations. The antioxidant enzyme concentration was assessed using commercial kits of ZELLBIO company and with the help of a spectrophotometer at a wavelength of 412 nm.

**Data analyses**

The data were investigated for normal distribution by Kolmogorov–Smirnov test in SPSS software (version 23). Since the data were normally distributed, ANOVA pathway was used, and figures were illustrated using Graph-Pad Pad Prism software (version 6.07).

**Results**

**Body weight**

Figure 1 depicts the results for the effects of daily supplementation of NOM on body weight. The results showed that glucose (a) and insulin serum concentrations (b) were significantly higher and lower in diabetic rats compared with non-diabetic rats, respectively (P = 0.000). There were no significant differences between non-diabetic rats for glucose (P = 0.843) and insulin (P = 0.189). However, the supplementation of NOM significantly decreased the serum concentration of glucose (P = 0.0001) while increasing insulin concentration (P = 0.0001). The effects of NOM on glucose and insulin were in a dose-dependent manner.

**Antioxidant status**

As depicted in Figure 3 the effects of NOM on the antioxidant status of the rats showed significant differences between diabetic and non-diabetic rats for the concentrations of GPx (P = 0.000), SOD (P = 0.000), FRAP (P = 0.000) and MDA (P = 0.000). The concentrations of GPx, SOD and FRAP were significantly higher in non-diabetic rats compared to diabetic rats, while the concentration of MDA was higher in diabetic rats. Non-diabetic rats receiving NOM did not show significant differences for GPx (P = 0.851), SOD (P = 0.498), FRAP (P = 0.536) and MDA (P = 0.445). The administration of NOM caused significant differences among diabetic rats. The rats receiving NOM showed higher concentrations for GPx, SOD and FRAP (P = 0.000) and lower concentration for MDA (P = 0.000) in comparison with the control diabetes. The effects were considerable in the rats receiving 20 mg/kg body weight of NOM versus other groups.
Figure 2: Effects of daily administration of NOM on the serum concentrations of glucose (a) and insulin (b) in diabetic and non-diabetic rats. Different superscripts (a–d) show significant differences between the groups. The results did not show significant differences between healthy animals. The administration of the NOM increased insulin and decreased glucose compared with diabetic control in diabetic groups. C–H, H-10 and H-20 are healthy rats receiving 0, 10 and 20 mg/kg NOM, while C–D, D-10 and D-20 are diabetic rats receiving 0, 10 and 20 mg/kg NOM.

Figure 3: Effects of daily administration of NOM on the concentrations of GPx (a), SOD (b), FRAP (c) and MDA (d) in diabetic and non-diabetic rats. Different superscripts (a–d) show significant differences between the groups. The results did not show significant differences between healthy animals. The administrations of the NOM increased FRAP and decreased MDA compared with diabetic control in diabetic groups. C–H, H-10 and H-20 are healthy rats receiving 0, 10 and 20 mg/kg NOM, while C–D, D-10 and D-20 are diabetic rats receiving 0, 10 and 20 mg/kg NOM.
The tissue concentrations of pro-inflammatory cytokines

The results for the effects of the tissue concentrations of pro-inflammatory cytokines are illustrated in Figure 4. The issues showed that the tissue concentrations of IL-1β (a), TNF-α (b), IL-6 (c) and NF-κB (d) were significantly greater in diabetic rats compared to non-diabetic rats ($P = 0.000$). There were no significant differences between non-diabetic rats for IL-6 ($P = 0.785$), NF-κB ($P = 0.812$), IL-1β ($P = 0.213$) and TNF-α ($P = 0.499$). However, the supplementation of NOM significantly diminished the serum concentrations of IL-6 ($P = 0.00$), NF-κB ($P = 0.00$), IL-1β ($P = 0.000$) and TNF-α ($P = 0.000$). The effects of NOM on pro-inflammatory cytokines were in a dose-dependent manner.

Discussion

This study aimed to investigate the effects of supplementation of NOM on the inflammatory and antioxidant responses in diabetic rats. Diabetes had adverse effects on the body weight of rats. The results are in agreement with other studies for the effects of diabetes on body weight [24,25]. Indeed, the decreased body weight of rats could be attributed to lower metabolism of diet glucose and/or a decrease of food consumption [26]. The findings showed that diabetes disturbed glucose metabolism, which is proof for the effects of diabetes on glucose metabolism and body weight. However, supplementation of NOM could improve body weight in a dose-dependent manner that agrees with the results reported by other studies on the effects of *Nannochloropsis* on the body weight under diabetes [22,27]. There is no clear mechanism for the effects of NOM on body weight, but it could be explained by the effects of NOM on glucose and insulin. Seemingly, NOM works as an anti-diabetic compound and reduces serum glucose. The improved glucose metabolism leads to maintain body weight under diabetic conditions.

The results showed that diabetes lowered the serum concentrations of insulin while raising the serum concentrations of glucose in rats. The results are parallel with the results reported by other studies for the effects of

| Figure 4: Effects of daily administration of NOM on the concentrations of IL-1β (a), TNF-α (b), IL-6 (c) and NF-κB (d) in diabetic and non-diabetic rats. Different superscripts (a–d) show significant differences between the groups. The results did not show significant differences between healthy animals. The administration of the NOM decreased the concentration of cytokines compared with diabetic control. C–H, H-10 and H-20 are healthy rats receiving 0, 10 and 20 mg/kg NOM, while C–D, D-10 and D-20 are diabetic rats receiving 0, 10 and 20 mg/kg NOM. |
diabetes on glucose [28, 29]. Diabetes is known with signs such as increased blood glucose either due to a defect in insulin synthesis, secretion, binding to the receptor or an augmentation of insulin resistance [30]. In fact, hyperglycemia resulting from the administration of streptozotocin causes injuries in tissues. Gu et al. [31] believed that diabetes induces damages on pancreatic β-cells via induction of oxidative stress and decreases the capability of insulin secretion. Oxidative stress plays important roles in the development and progression of diabetes [32]. The results of the current study as well showed that diabetic rats had lower antioxidant capacity that confirms the idea for the effects of diabetes on glucose via antioxidant system. In the current study, supplementation of NOM in a dose-dependent manner could spare insulin and lower the glucose in comparison with control diabetic rats. The mechanism of action of NOM on insulin and diabetes is unknown. The results for the effects of NOM on insulin and glucose concur with the results reported by Nasirian et al. [22]. The effects of NOM on glucose and insulin could be explained by its efficiency on oxidant and antioxidant parameters. Our findings showed that NOM functions as an antioxidant and spares antioxidant enzymes, protecting tissues from damage.

The findings showed that diabetes adversely affected antioxidant enzymes and FRAP and increased MDA. The results are parallel with the results reported by other studies [33–35]. SOD is an enzyme catalyzing the dismutation of the superoxide radical into ordinary molecular oxygen and hydrogen peroxide. The biochemical activity of GPx decreases lipid hydroperoxides to their corresponding alcohols and free hydrogen peroxide to water. FRAP is a method for determining the kinetics of diffusion via tissue or cells. MDA is commonly known as a marker for assessing the oxidative stress and the antioxidant status in the patients. Free radicals produced during the oxidative process are involved in the genesis of diabetes and play a pivotal role in the development of diabetes and complications [36]. In the current study, oral gavage of NOM significantly improved the antioxidant status in diabetic rats. The results for antioxidant activity of NOM are in agreement with the previous studies [37–39]. Seemingly, compounds of NOM work as an antioxidant and maintain GPx and SOD. The improved SOD and GPx prevent to increase MDA.

Diabetes significantly induced the increase in the serum concentrations of IL-1β and TNF-α [40, 41]. Hyperglycemia contributes to aggravated inflammatory responses [42]. TNF-α is commonly produced by immune cells, such as macrophages and mast cells [43]. IL-1β is also a pro-inflammatory cytokine involved in inflammatory responses [11]. NF-κB increases the expression of inflammatory proteins, such as IL-6 [11] and IL-6 participates in the early phases of inflammation and inflammatory response [12]. Furthermore, the current study showed that the administration of NOM worked as an anti-inflammatory compound. The results for the effects of NOM on inflammation concur with the other studies [27, 44, 45]. The anti-inflammatory potential of NOM could be explained by valuable pigments containing carotenoids [27]. As mentioned previously, there is a close relation between hyperglycemia and inflammation. Our findings showed that glucose reduction can reduce inflammation, as shown for pro-inflammatory cytokines.

Conclusion

In conclusion, oral supplementation of NOM in a dose-dependent manner decreased glucose and prevented the decrease of insulin via antioxidant system in a murine model. In addition, the administration of NOM lessened glucose and subsequently inflammatory responses. In summary, NOM exhibited antioxidant and anti-inflammatory properties in a rat model and can be administered to prevent the progression of diabetes in humans after clinical studies.

Conflict of interest: Authors state no conflict of interest.

Data availability statement: The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

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