

Original Article

The effects of oral supplementation of *spirulina platensis* microalgae on hematological parameters in streptozotocin-induced diabetic rats

Fariba Nasirian^{1*}, Behzad Mesbahzadeh², Saeid Abbasi Maleki³, Mehdi Mogharnasi⁴, Nasroallah Moradi Kor^{5,6*}

¹Department of Animal Sciences, University of Birjand, Birjand, Iran; ²Assistant Professor, Cardiovascular Diseases Research Center, Department of Physiology, School of Paramedical, Birjand University of Medical Sciences, Birjand, Iran; ³Young Researchers and Elite Club, Urmia Branch, Islamic Azad University, Urmia, Iran; ⁴Associate Professor of Exercise Physiology, Department of Sport Sciences, University of Birjand, Birjand, Iran; ⁵Research Centre of Physiology, Faculty of Medicine, Semnan University of Medical Sciences, Semnan, Iran; ⁶Student Research Committee, Faculty of Medicine, Semnan University of Medical Sciences, Semnan, Iran. *Equal contributors.

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Abstract: It is shown that diabetes can change hematological parameters and some microalgae, i.e. *Spirulina platensis*, could improve hematological parameters in non-diabetic rats. The purpose of this study was to investigate the effects of *Spirulina platensis* (SPM) microalgae on hematological parameters in diabetic rats induced by Streptozotocin. Rats, 2.5 months old, were grouped into two sections including healthy and diabetic and received orally 15 and 30 mg/kg body weight SPM for 5 weeks. Control rats received 0.3 ml of distilled water. The experimental groups were as follows; (SH15), healthy rats fed SPM (SH30) 30 mg/kg, diabetic rats fed 15 mg/kg SPM (SD15), diabetic rats fed SPM (SD30) 15 mg/kg, and diabetic control (DC). At the end of the test, blood samples were collected to measure red blood cells, white blood cells, mean corpuscular hemoglobin concentration (MCHC), mean cell volume (MCV) and packed cell volume (PCV). The induction of diabetes decreased RBC, MCHC, PCV, MCV and WBC ($P < 0.05$), but the oral supplement of SPM (30 mg/kg body weight) could improve RBC, WBC, MCHC, PCV and MCV in rats ($P < 0.05$). The oral complement of SPM, at high levels, seems to be an effective strategy against the negative effects of diabetes on hematological parameters.

Keywords: Diabetic rats, mean globular volume, red blood cell, *Spirulina platensis*, white blood cell

Introduction

Diabetes is one of the leading causes of death in the world and needs more attentions, as it differs at the time of recognition and under particular conditions [1]. Based on research in 2009, around 4% of people worldwide were involved with diabetes mellitus, and researchers themselves predicted that diabetes will increase by about 5.4% for 2025 years [2]. Type I diabetes has been known to lack insulin secretion, while type II diabetes is the inability of cells in insulin responses or insulin resistance [3]. It was reported that diabetes can change hematological parameters and the immune system in diabetes mellitus [4]. Anemia, due to hemolysis of red blood cells (red blood cells), is

found in patients with diabetes [5]. It was shown that anemia during diabetes mellitus may be the reason for increased non-enzymatic glycosylation of the membrane proteins of red blood cells that were related to hyperglycemia [6]. Blood leukocytes, polymorphous and monocytic leukocytes, can be activated by advanced glycation products in hyperglycemia [7]. Synthetic drugs applied for the treatment of diabetes cause serious adverse effects [8]. Therefore, the use of natural substances may be a suitable strategy to improve the negative effects of diabetes on hematological parameters.

Natural antioxidants, such as microalgae, have been interested in their ability to reduce the risk

Table 1. Chemical composition of SPM

Components	Amount
Dry matter (%)	95
Ether extract (%)	5.3
Crude protein (%)	61.8
Crude fiber (%)	9.5
Ash (%)	6.9
Ca (mg/100 g)	500
P (mg/100 g)	800
Fe (mg/100 g)	90
K (mg/100 g)	1235

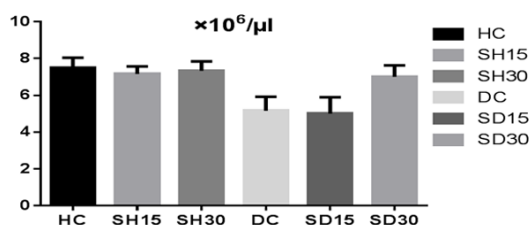


Figure 1. Effects of MPS on the red blood cell count (× 10⁶/μl) in healthy and diabetic rats. SH15 (healthy rats fed 15 mg/kg SPM), SH30 (healthy rats fed 30 mg/kg SPM), DC (diabetic control), SD15 (diabetic rats fed 15 mg/kg SPM) 30 (diabetic rats fed 30 mg/kg SPM).

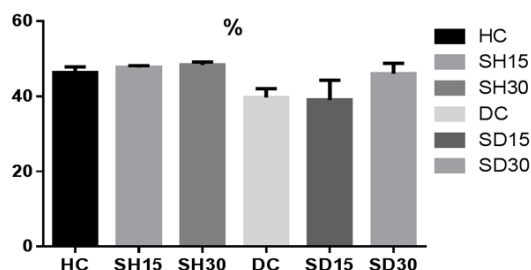


Figure 2. Effects of SPM on hemoglobin concentration (g/dl) in healthy and diabetic rats. SH15 (healthy rats fed 15 mg/kg SPM), SH30 (healthy rats fed 30 mg/kg SPM), DC (diabetic control), SD15 (diabetic rats fed 15 mg/kg SPM) 30 (diabetic rats fed 30 mg/kg SPM).

of chronic diseases and to encourage human health [9]. Microalgae are small photosynthetic components that convert solar energy into biomass. Algae products, especially cyanobacteria, are known to have therapeutic properties [10]. The therapeutic potential of microalgae may be associated with biological compounds such as essential amino acids, vitamins, pigments and lipid profile [11]. Spirulina microalgae (SPM), blue green algae, have two different

species; Spirulina maxima and Spirulina platensis [12]. It is well documented that SPM lipopeptides play an important role in the physiological function, i.e. cytotoxic, antitumor, antiviral, antibiotic, antimalarial, antimycotic, multi-drug resistance, anti-feeding, herbicidal, immunosuppressive factors [13]. It is shown that Spirulina platensis effectively interferes with cellular production of bone marrow and with the cellular immune response and may be cost-effective as adjuvant treatment in anemia or immunodeficiency [14]. It was hypothesized that SPM can efficiently improve hematological parameters in diabetic rats. Therefore, the present study, for the first time, was carried out to investigate the effect of SPM on hematological parameters in diabetic rats.

Materials and methods

Animals

Male Wistar rats (n = 60, weighing 200 ± 20 g at the start of the trial) were kept at room temperature (25°C) and under a light cycle (12 light hours/12 hours dark) for 5 weeks. All rats were ad libitum fed a standard feed diet (Javeneh Khorasan Company) and had free access to water. Chemical analysis of food included 20% protein, 3% fat 4% ash and 6% fiber. The rats were pooled into two sections (healthy and diabetic) and treated orally with microalgae (15 and 30 mg/kg body weight) or distilled water (0.3 ml distilled water: control treatments). The experimental groups were as follows; (SH15), healthy rats fed SPM (SH30) 30 mg/kg, diabetic rats fed 15 mg/kg SPM (SD15), diabetic rats fed SPM (SD30) 15 mg/kg, and diabetic control (DC). Each group or treatment consisted of 10 rats. The SPM was purchased from Ghazaye Sabze Khalij Company (Bandar Abbas-Iran). The chemical composition of the SPM is presented in **Table 1**.

Induction of experimental diabetes

Streptozotocin (STZ: Sigma-Aldrich Company) was used for the induction of diabetes mellitus in rats as explained by Armstrong and Al-Awadi [15]. Diabetes was induced by intraperitoneal injection of STZ (55 mg/kg body weight). First, it was dissolved in citrate buffer, pH 4.5 (0.1 mol/l trisodium citrate, 0.1 mol/l citric acid). Non-diabetic animals were simulated injected with buffer only. Diabetes, three days after STZ

Microalgae in diabetic rats

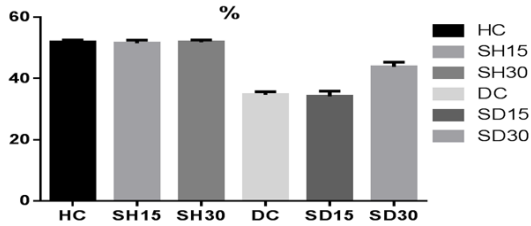


Figure 3. Effects of SPM on percent accumulated cell volume (%) in healthy and diabetic rats. SH15 (healthy rats fed 15 mg/kg SPM), SH30 (healthy rats fed 30 mg/kg SPM), DC (diabetic control), SD15 (diabetic rats fed 15 mg/kg SPM), SD30 (diabetic rats fed 30 mg/kg SPM).

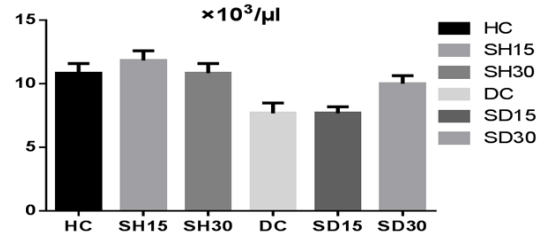


Figure 6. Effects of MPS on white blood cell count ($\times 10^3/\mu\text{l}$) in healthy and diabetic rats. SH15 (healthy rats fed 15 mg/kg SPM), SH30 (healthy rats fed 30 mg/kg SPM), DC (diabetic control), SD15 (diabetic rats fed 15 mg/kg SPM), SD30 (diabetic rats fed 30 mg/kg SPM).

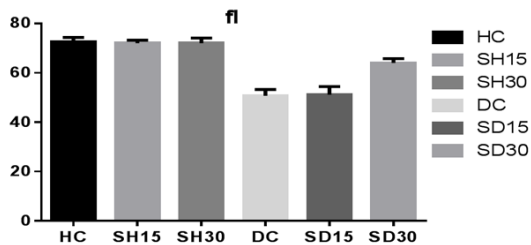


Figure 4. Effects of SPM on mean cell volume (fl) in healthy and diabetic rats. SH15 (healthy rats fed 15 mg/kg SPM), SH30 (healthy rats fed 30 mg/kg SPM), DC (diabetic control), SD15 (diabetic rats fed 15 mg/kg SPM), SD30 (diabetic rats fed 30 mg/kg SPM).

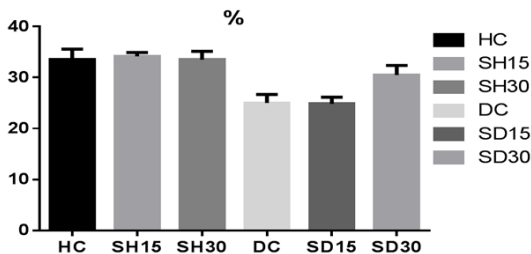


Figure 5. Effects of SPM on the mean corpuscular hemoglobin concentration (%) in healthy and diabetic rats. SH15 (healthy rats fed 15 mg/kg SPM), SH30 (healthy rats fed 30 mg/kg SPM), DC (diabetic control), SD15 (diabetic rats fed 15 mg/kg SPM), SD30 (diabetic rats fed 30 mg/kg SPM).

injection, was approved after measuring blood glucose levels. Rats with serum glucose levels above 250 mg/dl were considered diabetic.

Hematological analysis

At the end of the test, blood samples of 6 rats per treatment were collected into tubes containing heparin and immediately used for the determination of hematological parameters. Total red blood cells (RBC) and white blood cell

counts (WBC) were estimated on the basis of others [16]. The percentage of packed cell volume (PCV) was examined based on the hematocrit procedure [17], while blood hemoglobin (Hb) in all samples was calculated according to the cyanmethemoglobin procedure using Drabkin reagent [17]. The mean cell volume (MCV) and mean corpuscular hemoglobin concentration (MCHC) were measured on the basis of previous studies [16]. Differential WBC counts were measured based on a previous study [18].

Statistical analysis

Blood samples were collected from 6 animals each and blood samples were not pooled. Each blood sample was considered as a data. Each blood sample was divided into different parameters. Data were analyzed by graphically Pad Prism statically software and data were analyzed by analysis of unidirectional variance (ANOVA) with Dennett's multiple-post-test comparison. The treated and untreated rats in each section were compared by multiple Dennett comparisons after the test. In addition, healthy rats were compared to diabetic rats in the same group using the t-test. For example, control of healthy rats was compared to control diabetic rats and data were presented in the Figures. The results are presented as a mean \pm standard deviation. A P value < 0.05 was considered statistically significant.

Results

Our findings showed that DC rats had a lower red blood cell count (Figure 1), Hb concentration (Figure 2), PCV percentage (Figure 3), MCV (Figure 4) and MCHC (Figure 5) compared to As the figures show, oral supplementation of SPM,

Microalgae in diabetic rats

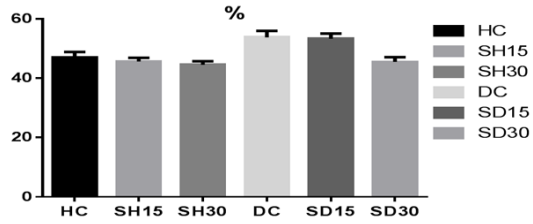


Figure 7. Effects of MPS on neutrophils (%) in healthy and diabetic rats. SH15 (healthy rats fed 15 mg/kg SPM), SH30 (healthy rats fed 30 mg/kg SPM), DC (diabetic control), SD15 (diabetic rats fed 15 mg/kg SPM) 30 (diabetic rats fed 30 mg/kg SPM).

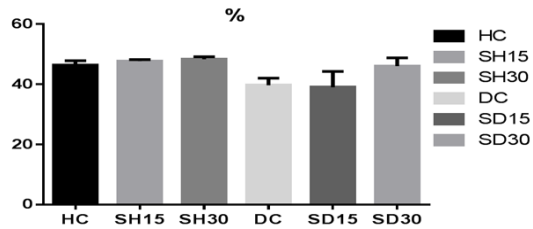


Figure 8. Effects of SPM on lymphocytes (%) in healthy and diabetic rats. SH15 (healthy rats fed 15 mg/kg SPM), SH30 (healthy rats fed 30 mg/kg SPM), DC (diabetic control), SD15 (diabetic rats fed 15 mg/kg SPM) 30 (diabetic rats fed 30 mg/kg SPM).

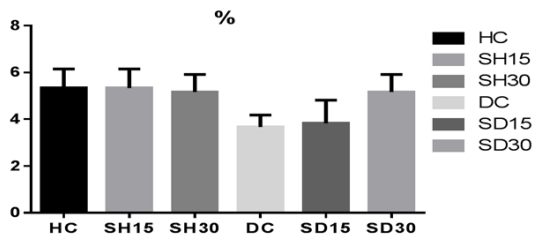


Figure 9. Effects of SPM on monocytes (%) in healthy and diabetic rats. SH15 (healthy rats fed 15 mg/kg SPM), SH30 (healthy rats fed 30 mg/kg SPM), DC (diabetic control), SD15 (diabetic rats fed 15 mg/kg SPM) 30 (diabetic rats fed 30 mg/kg SPM).

the two levels, could not change the parameters mentioned in healthy rats as compared to healthy control rats (Figures 1-5: $P > 0.05$), whereas the other level (30 mg/kg body weight) increased these parameters in diabetic rats compared to diabetic control ($P > 0.05$).

The results showed that diabetic rats showed higher percentages of neutrophils (Figure 7) and percentages of basophils (Figure 11) and lower (Figure 6), lymphocytes (Figure 8) and percentages of monocytes compared to diabetic control (Figure 9: $P < 0.05$). Similarly to

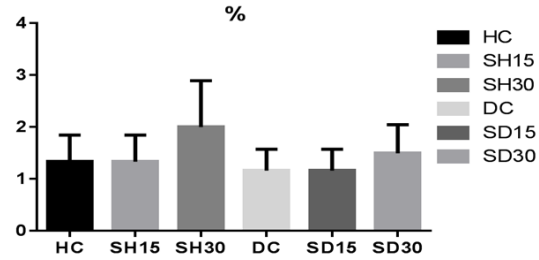


Figure 10. Effects of SPM on eosinophils (%) in healthy and diabetic rats. SH15 (healthy rats fed 15 mg/kg SPM), SH30 (healthy rats fed 30 mg/kg SPM), DC (diabetic control), SD15 (diabetic rats fed 15 mg/kg SPM) 30 (diabetic rats fed 30 mg/kg SPM).

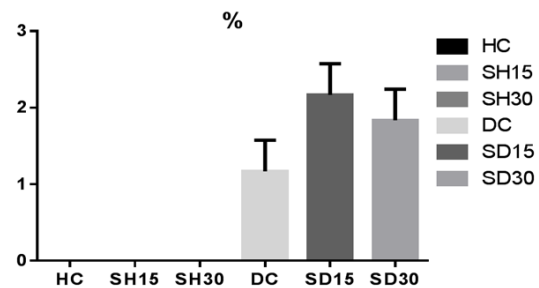


Figure 11. Effects of SPM on basophils (%) in healthy and diabetic rats. SH15 (healthy rats fed 15 mg/kg SPM), SH30 (healthy rats fed 30 mg/kg SPM), DC (diabetic control), SD15 (diabetic rats fed 15 mg/kg SPM) 30 (diabetic rats fed 30 mg/kg SPM).

the RBC parameters, oral supplementation of SPM at both levels did not have a significant effect on the parameters mentioned in healthy rats compared to healthy control ($P > 0.05$). The oral supplement of SPM (15 mg/kg body weight) could not alter these parameters in comparison to the diabetic control ($P > 0.05$), while the other level (30 mg/kg body weight) reduced neutrophils (Figures 6, 7), lymphocytes (Figure 8) and percentages of monocytes (Figure 9). Diabetes and SPM did not have a significant effect on the percentage of eosinophils (Figure 10).

Discussion

In the present study, diabetes could reduce red blood cell count, Hb concentration, and percentage of PCV, MCV, and MCHC. Weiss and Goodnough [19] showed a positive relationship between anemia and chronic diseases, i.e. diabetes. Studies have shown that anemia in patients with diabetes mellitus may be associated with increased non-enzymatic glycosyl-

ation of membrane proteins of red blood cells [6]. It has been shown that anemia has failed erythropoietin production that fails in the kidneys and raises non-enzymatic glycosylation of membrane proteins of red blood cells [20]. Based on the findings, changes in RBC and PCV levels in diabetic animals are the cause of anemia. On the other hand, oxidation of the proteins can raise the production of lipid peroxides which can subsequently raise hemolysis of RBC [20]. On the other hand, high levels of free radicals, during diabetes, cause damage to cellular proteins, membrane lipids and nucleic acids, and cell death. Anemic conditions during diabetes mellitus increase non-enzymatic glycosylation of membrane proteins from red blood cells [6]. Lipid peroxidation increased membrane stiffness and reduced cell deformability, erythrocyte survival and lipid fluidity [21]. Although, lipid peroxide levels of membrane RBC were not measured, the reduced value of MCHC in diabetic rats is a suitable measure for the synthesis of insufficient Hb for blood osmoregulation and plasma osmolarity [22]. The assertion was confirmed by our findings, so diabetic rats produced lower Hb content.

Based on the results, SPM, SD30 group, improved RBC-related parameters in diabetic rats. Food and nutrients play a major role in the normal functioning of the body. It has been shown that the active compounds of human nutrition have enormous health benefits and reduce the risk of chronic diseases, i.e. diabetes [23, 24]. Parallel to our observations, supplementation of *Spirulina platensis* in drinking water for 30 days produces significant positive effects on erythropoiesis in adult rats; this implies in the efficiency of SPM in increasing red blood cell counts and Hb concentration [14]. Previous evidence has been shown that *Spirulina platensis* can decrease the severity of anemia and UV-induced damage to the bone marrow [25, 26], and high blood Hb content [26]. In addition, SPM increased blood Hb content and subsequently improved red blood cell count and other related parameters. We believe that SPM stimulates the formation or secretion of erythropoietin, which stimulates stem cells in the bone marrow to produce red blood cells because the stimulation of erythropoietin increases the rapid synthesis of red blood cells. MCHC is also an optimal one involving the syn-

thesis of RBC [27]. On the other hand, SPM contains high levels of iron (90 mg/100 g) and therefore can provide enough iron for the synthesis of red blood cells.

In our study, diabetes changed the white blood cell count and leukocyte profiles. In the present study, the white blood cell count and the modified leukocyte profiles are criteria for the suppression of the immune system. It is well known that some leukocytes eliminate pathogens by assaulting the larger pathogens or by phagocytosis. The decreased immune system is associated with diabetes mellitus. Previous studies have indicated that diabetes in mice may be associated with moderate neutrophilic leukocytosis and increased neutrophils and monocytes and decreased circulating lymphocytes, which raises susceptibility to infection [28]. In the present study, however, SPM improved the parameters mentioned in SPM30 compared to DC; which demonstrates it modulates in the immune system. It was reported that total counts of leukocytes, neutrophils, and lymphocytes were significantly elevated in rats treated with SPM compared to the control group [14]. It is well known that SPM and other microalgae contain antioxidant pigments and therefore can fight with pathogenic factors found during diabetes and finally improves CMB. SPM effectively interfered with the cellular immune response and may be cost-effective in the treatment of immune deficiency [14]. It is shown that micronutrient deficiencies can have direct effects on the immune system [29]. For example, iron deficiency, present in SPM, is related to reversible abnormalities of immune function [30]. The lowest level of SPM did not improve the WBC and RBC-related parameters. It seems to be related the insufficient rate of SPM that cannot affect the parameters efficiently. On the other hand, it is known that SPM has antioxidant properties [31] and appears to prevent RBC hemolysis and can maintain WBC, platelets, and lymphocytes from reactive oxygen species during diabetes. Studies have reported that some natural antioxidants prevent the structural integrity of immune cells by their antioxidant activity which subsequently maintains the cell membrane of free radical oxidants [32]. Therefore, SPM can help improve hematological parameters in diabetic rats by antioxidant properties.

In conclusion, the findings showed that diabetics reduced red blood cell count, Hb concentration, the percentage of PCV, MCV and MCHC. The oral supplement of SPM, 30 mg/kg body weight, increased these parameters compared to the control of diabetics. Diabetes increased the percentages of neutrophils and basophils and also decreased white blood cell count, lymphocytes, and monocyte percentages. The oral supplement of SPM, at level of 30 mg/kg body weight, decreased neutrophils and increased white blood cell count, lymphocytes, and monocyte percentages. Therefore, dietary supplementation with SPM at a high level is an optimal strategy against the negative effects of diabetes on hematological parameters.

Disclosure of conflict of interest

None.

Address correspondence to: Nasroallah Moradi Kor, Research Centre of Physiology, Faculty of Medicine, Semnan University of Medical Sciences, Semnan, Iran. E-mail: moradikor.nasroallah@yahoo.com

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