Original Article

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Effects of Dietary Supplementing of Spirulina Platensis and Chlorella Vulgaris Microalgae on Hematologic Parameters in Streptozotocin-Induced Diabetic Rats

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Abstract

Background: Microalgae are known to have beneficial effects on health. On the other hand, studies have reported negative effects of diabetes on hematological parameters. Effects of Spirulina platensis (SP) and Chlorella vulgaris (CV) microalgae, singly and combined, on hematological parameters in diabetic rats are not still studied. Thus, the present study was conducted to investigate the effects of SP and CV microalgae on hematological parameters in Streptozotocin-induced diabetic rats.

Materials and Methods: This interventional study was conducted in Tabriz University of Medical Science between October and November 2016. Diabetes was induced by intraperitoneal administration of Streptozotocin. Thirty-six Wistar rats were treated with CV, SP and their combination for 28 days. Two groups including diabetic control and healthy control were also considered. Blood samples were collected to investigate the levels of hemoglobin (Hb), packed cell volume (PCV), red blood cell count (RBC), white blood cell count (WBC), platelet count, lymphocytes, neutrophils, plasma iron and selenium and glutathione peroxidase (GPx) activity in erythrocytes at 14 and 28 days of trial.

Results: Results showed that dietary supplementing of CV and SP microalgae, alone and combination form, could increase the reduced levels of RBC, WBC, platelet, PCV, GPx activity, and selenium in diabetic rats on 28 days ($P<0.05$).

Conclusion: It can be concluded that dietary supplementing of CV and SP microalgae, especially combined form, may partly improve deleterious effects of diabetes on hematological parameters.

Keywords: Diabetic rat, Hematologic parameters, Microalgae

Introduction

Diabetes mellitus (DM), a common metabolic disorder, is characterized by defaulted carbohydrate, protein, and lipid metabolism. DM increased risk of atherosclerotic arterial disease by 2 to 6-fold (1). It is estimated to be involving by 366 million individuals with DM in 2030 years (2). A study showed relation between hematological parameters and the immune system during the course of diabetes (3). It is shown that diabetes may change platelet activity and increase thrombus formation (4). Studies have shown that DM can change hematological parameters (5, 6, 7). Thomas et al (8) introduced some factors as influencing factors on anemia in patients involving with DM, including glomerular filtration rate, urinary albumin excretion rate, and glycated hemoglobin levels. DM could lyse blood cells and subsequently reduce hemoglobin level (Hb), packed cell volume (PCV) and red blood cell count (RBC) by reactive oxygen species (ROS) (9, 10), which the changes are accompanied with anemia in diabetic patients (11). Much attention in this regard has been paid in applying functional foods, i.e. medicinal plants or microalgae for treatment of diabetes.

Microalgae are considered as functional foods because of rich sources of fibers and phytochemicals. Spirulina platensis (SP)

microalga, a helical blue-green alga, is shown to have 65 to 70% protein and high fiber (12) and minerals and vitamins (13). Beneficial effects of SP microalga for treatment of malnutrition, obesity and diabetes mellitus have been reported (14). An animal study showed that diet supplementing of SP microalga could reduce toxic effects of arsenic on PCV and Hb (15). Studies have reported that blue-green algae can increase antioxidant activity and also improve hematological markers (16).

Chlorella vulgaris (CV) microalga, unicellular marine alga, contains protein, chlorophyll, fiber, and some minerals and vitamins (17). Beneficial effects of CV microalga have been reported for growth of cells and growth and development of children (18). The CV microalga is considered as an antioxidant, since it also contains extensive range of antioxidants, bioactive substances, chlorophylls, etc. (19, 20). Thus, it was hypothesized that combination of two microalgae can efficiently show antioxidant properties and also alleviate deleterious effect of diabetes on hematological parameters. In addition, effect of microalgae on hematological parameters is not still investigated. Therefore, the present study was conducted to investigate the effects of CV and SP microalgae on hematological parameters in diabetic rats.

Materials and Methods

This interventional study was conducted in Tabriz University of Medical Science between October and November 2016. Young male Wistar rats, 80 days of age and weighing 250±20 gr, were purchased from the Pasteur Institute (Tehran, Iran). The rats were kept in polyethylene cages (n=8) and temperature was maintained at 22± 2°C. Animals had free access to water and feed. All the ethical principles were in agreement with National Institutes of Health guide for the care and use of Laboratory animals (NIH Publications No. 8023, revised 1978).

The both microalgae, SP and CV, were purchased in form fine powder from Ghazaye Sabze Khalij Company (Bandar Abbas-Iran). These microalgae had been cultured by Pulz et al (21) method, using sunlight and photobioreactor PBR 4000 for animal studies. The compositions of CV and SP microalgae are presented in Table I. Minerals were analyzed using an atomic absorption spectrometer as previously described by others (22). Two gr CV and 2 gr SP, separately, were transferred to furnace for 16 h at 500°C until produce the ash. After 16 h, 2 drops distilled water was added to ash and then transferred to Bain Marie for 1 h at 80°C and 10 ml chloric acid (2 N) was added to it. One hundred ml distilled water was added to it and the solution was analyzed for selenium using an atomic absorption spectrophotometer. This procedure was repeated for other elements.

Diabetes was induced by Sancheti et al (23) method. A single dose, 65 mg/Kg body weight of streptozotocin (STZ) was dissolved in 0.1 cold citrate buffer (pH= 4.5) and it was intraperitoneally (IP) administrated to rats. Blood samples were collected from the tail vein to measure the blood glucose levels by ACCU-Check glucose meter 72 h after the STZ administration. The animals with level of fasting blood glucose >300 mg/dL were considered as diabetic and used for continuing study.

Forty-eight Wistar rats were divided into 6 groups (8 rats per group) including 1) healthy rats receiving the control diet (HC), 2) diabetic rats receiving the control diet (DC), 3) diabetic animals fed the CV (2% diet: CV), 4) diabetic animals receiving the SP (2% diet: SP), 5) diabetic rats treated with SP and CV (2% diet, 1% CV+1% SP: CV+SP), and 6) diabetic animals receiving the SP and CV (4% diet, 2% CV+2% SP: CV2+SP2). The control diet included casein, corn starch, dextrin, sucrose, soy oil, cellulose, and mixture of minerals and vitamins. All diets were iso-protein and isocaloric.

Blood samples were collected from tail veins (6 animals each group) and transferred into tubes containing 100 IU heparin/mL of blood at 14 and 28 days. Part of blood was used for the measurement of hematological parameters and glutathione peroxidase (GPx) activity in erythrocytes. Other part was centrifuged at 2500 rpm for 12 minutes to measure the minerals.

Hemoglobin level (Hb), packed cell volume (PCV), red blood cell count (RBC), white blood cell count (WBC), platelet, lymphocytes, and neutrophils counts were analyzed using auto analyzer (Tech¬nicon-H1).

Erythrocytes were separated from whole blood by Beutler et al (24) method and then washed for three times by 0.9% NaCl and finally suspend in volume of 0.9% NaCl.

The GPx activity was assessed by Rotruck et al (24) procedure. The activity of GPx was reported as µmoles of glutathione oxidized/min/mg Hb. The levels of selenium and iron in plasma were investigated using atomic absorption spectrophotometer as previously described by Perkin (26).

The data were analyzed by SPSS (SPSS Inc., Chicago, IL, USA) (version 17), and one-way analysis of variance (ANOVA) with Dennett's multiple comparison post-test. The results were presented as mean ± standard deviation. $P<0.05$ was considered as significant difference.

Results

The hematological data are shown in Tables II and III. Hb level, PCV, RB and WBC, platelet and lymphocytes counts were reduced in diabetic animals ($P<0.05$) at 14 days of trial, but dietary inclusion of CV and SP could not improve these parameters ($P>0.05$). The data for hematological parameters on 28th day are presented in Table III. Comparing HC and DC groups showed lower Hb level, PCV, RB, WBC counts, platelet, and lymphocytes counts ($P<0.05$). Applying CV, SP, and their combination at lower levels (CV+SP) could increase the reduced Hb level, PCV, RBCs, WBC, platelet and lymphocytes counts in diabetic control. The best response was found in groups treated with CV and SP at high levels (CV2+SP2 group), but the values were lower in CV2+SP2 group compared with healthy rats ($P<0.05$). Neutrophils count was not influenced by diabetes and treatments ($P>0.05$). Findings in Table IV show that diabetes can lower the plasma concentrations of iron and selenium and GPx activity in erythrocytes on 14th and 28th days. Results showed that dietary inclusion of CV and SP cannot change iron level and also GPx activity in erythrocytes on 14th day ($P>0.05$). Dietary supplementing of CV and SP, especially combined form at high levels, increased the plasma concentration of selenium and also GPx activity in erythrocytes, but, the values were lower compared with healthy rats.

Table I: Chemical composition of SPM and CV microalgae

Components	SP	CV
Dry matter (%)	95	95
Crude Fat (%)	5.3	5.41
Crude protein (%)	61.8	44.65
Crude fiber (%)	9.5	8.51
Ash (%)	6.9	5.98
Ca (mg/100 g)	500	620
P (mg/100 g)	800	760
Fe (mg/100 g)	15	21
Se (mg/100 g)	8	10
Vitamins (g/100 g)	5	7.3

Table II: Hematological parameters at 14 days

Parameters	HC	DC	SP	CV	CV+SP	CV2+SP2	*P*
RBC ($\times10^{12}$/ L)	7.03±0.19[a]	6.16±0.26[b]	6.16±0.18[b]	6.03±0.16[b]	6.13±0.16[b]	6.03±0.15[b]	<0.0001
WBC ($\times10^{9}$/ L)	8.71±0.68[a]	7.43±0.74[b]	7.71±0.57[b]	7.56±0.77[b]	7.30±0.92[b]	7.43±1.01[b]	0.049
Hb (g/dL)	12.50±1.40[a]	9.83±1.03[b]	10.67±0.40[b]	10.17±0.81[b]	10.08±0.91[b]	10.17±0.75[b]	0.0001
% PCV	33.17±1.94[a]	27.33±1.75[b]	28±1.78[b]	29.33±2.16[b]	29.33±1.03[b]	28.83±1.83[b]	<0.0001
Platelet ($\times10^{9}$/ L)	421±17.23[a]	346±10.33[b]	335±11.68[b]	338±7.52[b]	323±15.06[b]	338±11.69[b]	<0.0001
Lym ($\times10^{9}$/ L)	4.5±0.54[a]	3.16±0.40[b]	3.50±0.54[b]	3.33±0.51[b]	3.50±0.54[b]	3.50±0.54[b]	0.0023
Neu ($\times10^{9}$/ L)	1.83±0.75	1.73±0.72	2.00±0.89	2.00±0.63	2.00±0.63	2.00±0.83	0.994

Lym: lymphocyte: Neu: neutrophil. Superscript, a-b, show significant differences each row at $P<0.05$

Table III: Hematological parameters at 28 days

Parameters	HC	DC	SP	CV	CV+SP	CV2+SP2	*P*
RBC ($\times10^{12}$/ L)	7.05±0.25[a]	5.01±0.18[d]	5.66±0.19[c]	5.73±0.43[c]	5.61±0.38[c]	6.36±0.21[b]	<0.0001
WBC ($\times10^{9}$/ L)	8.65±0.92[a]	6.18±0.58[d]	7.31±0.29[c]	7.35±0.32[c]	7.31±0.34[c]	7.91±0.55[b]	<0.0001
Hb (g/dL)	12.58±0.66[a]	8.33±0.40[d]	10.42±0.66[c]	10.67±0.75[c]	10.42±0.49[c]	11.57±0.66[b]	<0.0001
%PCV	33.15±1.16[a]	24.33±1.21[d]	27.17±1.94[c]	27.15±1.63[c]	27.17±2.13[c]	30.67.83±1.75[b]	<0.0001
Platelet ($\times10^{9}$/ L)	431±9.83 [a]	316±8.16[d]	333±10.38[c]	343±15.60[c]	343±8.16[c]	365±10.49[b]	<0.0001
Lym ($\times10^{9}$/ L)	4.33±0.81[a]	2.16±0.40[d]	3.16±0.40[c]	3.00±0.63[c]	3.16±0.45[c]	3.66±0.51[b]	<0.0001
Neu ($\times10^{9}$/ L)	2.16±0.40	2.33±0.52	2.00±0.63	2.33±0.51	2.16±0.53	2.33±0.51	0.917

Lym: lymphocyte: Neu: neutrophil . Superscript, a-d, show significant differences each row at $P<0.05$

Table IV: Plasma iron and selenium and GPx activity in erythrocytes at 14 and 28 days

Parameters	HC	DC	SP	CV	CV+SP	CV2+SP2	*P*
			14 days				
Iron (µmoL/L)	29.33±0.81[a]	18.00±3.48[b]	18.33±2.19[b]	19.67±2.13[b]	20.50±1.68[b]	21.83±2.41[b]	<0.0001
Selenium (µmoL/L)	13.67±0.51[a]	8.83±0.75[b]	9.33±0.51[b]	9.16±0.75[b]	9.00±0.65[b]	9.50±1.05[b]	<0.0001
GPx activity	15.17±0.75[a]	12.17±0.75[b]	11.67±0.81[b]	11.50±1.04[b]	11.50±1.04[b]	11.67±1.21[b]	<0.0001
			28 days				
Iron (µmoL/L)	30.83±1.47[a]	18.33±1.36[b]	18.83±2.13[b]	20.17±1.72[b]	18.87±2.25[b]	20.00±2.75[b]	<0.0001
Selenium (µmoL/L)	13.83±0.75[a]	8.33±1.21[d]	10.50±0.83[c]	10.00±1.23[c]	10.17±1.16[c]	11.50±0.54[b]	<0.0001
GPx activity	14.33±0.85[a]	10.83±0.72[d]	12.33±0.53[c]	12.50±0.54[c]	12.33±0.57[c]	13.50±0.41[b]	<0.0001

Lym: lymphocyte: Neu: neutrophil . Superscript, a-d, show significant differences each row at $P<0.05$. GPx activity was expressed as µmoles of glutathione oxidized/min/mg Hb

Discussion

Findings showed that diabetes can lower Hb level, PCV, RBC, WBC, platelet, and lymphocytes counts. With regard to HC and DC, changes can be found (6.16 vs. 5.01 for RBC: 7.43 vs 6.18 for WBC: 9.83 vs 8.33 for Hb: 27.33 vs 24.33 for PCV: 346 vs 316 for platelet: 3.16 vs 2.16). Erukainure et al., (27) reported that diabetes lower Hb level, PCV, RBC, WBC, platelet, and lymphocytes counts. Defaulted erythropoietin production in kidneys and increased non-enzymatic glycosylation of RBC membrane proteins have been reported as factors for anemia (28). Decreased levels of WBC, platelet, and lymphocytes are criteria for immune system suppression (29, 30). It is well known that WBC, platelet, and lymphocytes can attack to pathogens by contact and phagocytosis (29). Decreased WBC, platelet, and lymphocytes levels implicates on relations between diabetes and immune system. Similarly, another study showed relation between hematological parameters and the immune system during the course of diabetes (3). Results showed that the both microalgae, especially combined form at high levels, could increase the Hb level, PCV, RBC, WBC, platelet, and lymphocytes counts in diabetic rats. Considering mean and standard deviation in SP, CV and CV+SP groups, it can be stated that these groups kept the values from 14-28 days, but CV2+SP2 group could increase values at 28 days compared with 14 days of trial; however, the values were lower than healthy rats. It seems that microalgae may efficiently act at more time intervals. The role of foods and nutrients, especially dietary fiber, in management of chronic disorders, i.e. diabetes, has been previously reported (30, 31). As shown in Table I, the both microalgae have about 9% fiber. It can be claimed that higher fiber can be considered as main reason for better improvement in hematological parameters in CV2+SP2 group compared with other groups. Preventing RBCs of oxidation can inhibit hemolysis in RBCs (30). It has been also reported that some micronutrients such as selenium can help immune system (33). CV and SP are known to have antioxidant properties (16, 19, and 20). It seems that microalgae may prevent hemolysis of RBC and maintain WBC, platelets and lymphocytes from negative effects of ROS during diabetes, because of their antioxidant properties. This claim was partly confirmed by findings in Table IV, because GPx activity was increased in rats fed the microalgae. Blue-green algae are known to have antioxidant properties which can help increase the activity of antioxidant enzymes and subsequently improve hematological markers (16). Slavin (32) believes that some essential oils can prevent the structural integrity of immune cells and also maintain cell membrane from free radicals damage by their antioxidant properties. It can be concluded that CV and SP can improve deleterious effect of diabetes on hematological parameters by their antioxidant properties.

Results also showed that diabetes reduces the plasma concentrations of iron and selenium, although microalgae can improve selenium concentration on 28th days of trial. As shown in Table I, the both microalgae have lower levels of selenium and higher levels of iron; however, the plasma concentration of selenium was increased. Higher bioavailability of selenium in microalgae may be reason for its high concentration in plasma. Future studies are needed to investigate the relation between trace minerals in microalgae and diabetes.

Studies have reported a relation between dietary inclusion of selenium and functional biomarkers, i.e. GPx in erythrocytes (34). RBCs are sensitive to oxidative damage because of high concentration of polyunsaturated fatty acids, iron, and oxygen in its structure (35). Thus, it can be stated that microalgae can provide enough selenium for GPx

synthesis which are important in diabetic rats and they can finally inhibit oxidation. Hypothesis was confirmed that combination of two microalgae can alleviate deleterious effect of diabetes on hematological parameters on 28th day of trial, since dietary inclusion of SP and CV could increase the reduced hematological parameters, selenium concentration, and GPx. Moreover, the best response was found in rats receiving the combined of 2% SP and 2% CV; showing that higher levels may efficiently alleviate deleterious effects of diabetes on hematological parameters during more time intervals. The additives could not improve hematological parameters on 14th day. It can be also stated that SP and CV maintain RBCs and WBCs by increasing GPx activity during diabetes.

Conclusion

This study for first time reported efficiency of CV and SP in improving the hematological parameters in diabetic rats, but future studies will be needed to investigate the selenium level in erythrocytes.

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Conflict of interest

Authors declare that authors have no conflict of interest.

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