Effect of Spirulina on Lipid Profile, Glucose and Malondialdehyde Levels in Type 2 Diabetic Patients

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The study aimed to assess possible spirulina effects on lipid profile, glucose, and malondialdehyde levels in new cases of type 2 diabetes. The subjects consisted of 30 new cases of type 2 diabetes that divided into two groups; each consisted of 15 diabetic patients. Group I did not take any functional food or supplement and received no spirulina supplementation. Group II or experimental group also did not take any functional food or supplement but received spirulina supplementation. Analysis of data was done using SPSS 16.0. The Kolmogorov-Smirnov test, paired t-test, Wilcoxon test, and Spearman correlation analysis were used to analyze the data. After eight weeks of spirulina supplementation, significant differences were shown in the serum levels of total cholesterol, low-density lipoprotein (LDL)-cholesterol, triglyceride, and malondialdehyde. The serum fasting blood glucose, lipid profiles, and malondialdehyde levels at baseline were negatively and positively correlated with changes in these parameters. Spirulina supplementation may have a beneficial effect on lipid profile and malondialdehyde (MDA) levels through an interventional 8 weeks. This effect may protect subjects against free radicals and the development of some diseases such as atherosclerosis. The spirulina supplementation also showed a potential lipid-lowering effect on new case type 2 diabetic patients which may help the diabetics to have control on lipid levels. In addition, spirulina may be used as a functional food for the management of lipid profiles and MDA levels.

Keywords: Spirulina. Glucose. Lipids. Malondialdehyde. Diabetes Mellitus, Type 2.

INTRODUCTION

Diabetes mellitus (DM) affects different organs in the human body. It is the third cause of death worldwide (Thornalley, 2002). DM can cause dysfunction in the metabolism of carbohydrates, fat, and proteins. Different epidemiological studies and reports by the World Health Organization have indicated the increasing prevalence of DM in the world (Akbarzadeh et al., 2007). Different agents have been used to hinder the beginning of diabetes in pre-diabetic subjects or animals (Sprietsma, Schuitemaker, 1994). Many studies have been conducted to find natural sources that can affect the carbohydrate level in diabetic patients (Wolf, 1987). Spirulina is a cyanobacterium that grows in high temperatures and alkaline conditions. In 1967, the International Association of Applied Microbiology accepted spirulina as a source of excellent food for the future (Anitha, Chandralekha, 2010). Spirulina contains high protein content (60–70%), carotenoids, vitamin E, phycocyanine, and chlorophyll (Layam, Reddy, 2006). It has been reported as a new functional food that may help patients with chronic diseases such as diabetes. Some studies have shown an association between spirulina...
(as a functional food for humans) and cholesterol-regulatory properties, the host immune system modulation, and antioxidant effect (Hirata et al., 2000; Qureshi, Kidd, Ali, 1996). Moreover, toxicological studies have revealed the safety of spirulina (Salazar et al., 1996). Many findings have performed to assess the effect of spirulina on human beings’ health and different metabolic disorders (Kim, Kim, 2005; Parikh, Mani, Iyer, 2001; Rodriguez-Hernandez et al., 2001). Spirulina may decrease blood lipids in healthy (Park, Kim, 2003), heart disease (Ramamoorthy, Premakumari, 1996), and diabetic individuals (Mani, Desai, Iyer, 2000). Spirulina also shows antioxidant properties that can inhibit lipid peroxidation (Benedetti et al., 2004). Some studies have found that spirulina reduces plasma triglycerides, total-and LDL-cholesterol, and blood pressure in healthy elderly people (Park et al., 2008; Park, Kim, 2003). Spirulina shows many biological activities such as anemia prevention (Hemalatha et al., 2012), stopping of herpes simplex infection (Ferreira-Hermosillo et al., 2011), elevated production of antibodies (Premkumar et al., 2004), hypoglycemic (Abdel-Daim, Abuzead, Halawa, 2013), hypolipemic (Jarouliya et al., 2012), antihypertensive characteristics (Ponce-Canchihuaman, et al., 2010), reduction of lipid profiles of the liver and lipid peroxidation products (El-Baky, El Baz, El Baroty, 2009). The aim of this study was to assess the possible spirulina effects on lipid profile, glucose, and malondialdehyde (MDA) concentrations in type 2 diabetic patients.

**MATERIAL AND METHODS**

Subjects were chosen from patients with diabetes mellitus type 2 who referred to the health center in Behshar, Mazandaran Province, Iran. The subjects consisted of 30 new cases of types 2 diabetes (with fasting blood glucose ≥ 126 mg/dl). The subjects were divided into two groups; each consisted of 15 diabetic patients. Group, I did not take any functional food or supplement and received no spirulina supplementation. Group II or experimental group also did not take any oral hypoglycemic drugs, insulin or functional food and any other supplementation, but received spirulina supplementation (provided from Dana Med Pars Company, Tehran, Iran). The nutrition profile of spirulina powder is shown in Table I. Spirulina supplementation as pills were given for two months (4 grams/day) to the experimental group. The regular diets and physical activity of study subjects were maintained during the study. Diabetic patients were asked to maintain the usual diet and prevented to take any functional foods or dietary supplements. Follow up was continuously performed by phone calls twice a week. Exclusion criteria contain pregnant women, coronary artery diseases, peripheral vascular disease, cerebrovascular disease, liver disease, and impaired organ functions.

The 12-hr overnight fasting blood samples were collected for determination of fasting blood glucose, triglyceride, cholesterol, HDL-cholesterol, LDL-cholesterol, and MDA in the Metabolic Disorders Research Center, Golestan University of Medical Sciences. Blood samples were collected at the beginning and after eight weeks of the study groups. Serums were separated by centrifugation at 1000 g for 10 minutes. Before and after treatment with spirulina, the levels of biochemical parameters and MDA (nano mol/L) were determined by commercial kits and Kei Satoh (Satoh, 1978) method using spectrophotometry (JENWAY6305), respectively. The serum insulin level was determined by the enzyme-linked immunosorbent assay. The study was approved by the Research Deputy of Golestan University of Medical Sciences Ethics Committee (IR.GOUMS.REC.1394.34). From all study subjects, a written agreement was obtained before blood collection and the start of the experiments. All subjects were weighed using digital scales. Study subjects were with a minimal cloth and without shoes. A tape meter was used to measure height when subjects were in standing position. Calculation of Body Mass Index (BMI) was done as weight (in kilograms) divided by height (in meters squared). Subjects were classified in to different categories of body mass index. Study subjects with BMI equal to 25.0-29.9 Kg/m² and BMI higher or equal to 30 Kg/m² were taken into account as overweight and obese subjects, respectively (WHO, 1998).
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### TABLE I - Nutrition profile of spirulina powder

| Nutritional composition Per 100 g | Amounts |
|----------------------------------|---------|
| **General**                      |         |
| Total fat (g)                    | 5       |
| Saturated fat (g)                | 2.2     |
| Cholesterol (mg)                 | 0       |
| Total carbohydrates (g)          | 16      |
| Dietary fiber (g)                | 7       |
| Sugars (g)                       | 0       |
| Protein (g)                      | 67      |
| **Vitamins**                     |         |
| Vit A (as Beta carotene) (IU)    | 375000  |
| Vitamin E (IU)                   | 7       |
| Vitamin K1 (μg)                  | 2000    |
| Vit K2 (μg)                      | 500     |
| Thiamin (B1)( μg)                | 117     |
| Riboflavin (B2)( μg)             | 4667    |
| Niacin (B3)( μg)                 | 13333   |
| Vit (B6) (μg)                    | 10000   |
| Folate (μg)                      | 200     |
| Vit B12 (μg)                     | 300     |
| Biotin (μg)                      | 33 <    |
| Pantothenic acid (μg)            | 150     |
| **Minerals**                     |         |
| Calcium (mg)                     | 333     |
| Iron (mg)                        | 217     |
| Phosphorous(mg)                  | 1100    |
| Iodine (μg)                      | 500     |
| Magnesium (mg)                   | 500     |
| Zinc (mg)                        | 3       |
| Selenium (mg)                    | 30      |
| Copper (mg)                      | 0.7     |
| Manganese (mg)                   | 13      |
| Chromium (μg)                    | 13333   |
| Sodium(mg)                       | 1000    |

### TABLE I - Nutrition profile of spirulina powder

| Nutritional composition Per 100 g | Amounts |
|-----------------------------------|---------|
| Potassium (mg)                    | 2000    |
| **Carotenoids & Phytonutrients**  |         |
| Gama linolenic Acid (GLA) (mg)    | 1067    |
| Zeaxanthin (mg)                   | 300     |
| Total carotenoids (mg)            | 500     |
| Chlorophyll (mg)                  | 1000    |
| c-phycocyanin (mg)                | 8000    |
| Superoxide dismutase (units)      | 36000   |

### Data Analysis

Analysis of data was done using SPSS 16.0 (SPSS Inc., Chicago, IL, USA). Data were shown as mean with standard deviation. The Kolmogorov-Smirnov test was used to check the normal distribution of biochemical parameters. A comparison of mean differences of biochemical parameters was done by the paired t-test at the beginning and after eight weeks of intervention. MDA levels in both groups were compared and analyzed by the Wilcoxon test. Spearman correlation analysis was used to investigate the association between baseline blood profiles, fasting blood glucose and MDA levels, and changes in the biochemical parameters. Statistical significant was considered as P-value < 0.05.

### RESULTS

The mean age of the spirulina treated type 2 diabetic patients and diabetic controls were 46.70±8.10 and 47.30±8.80, respectively. Body Mass Index of the spirulina treatment group and diabetic controls were 28.27±2.05 and 27.21±1.83, respectively. Baseline data of two groups’ type 2 diabetic patients are shown in Table II. Significant differences are considered in the baseline data between the spirulina and control subjects in terms of fasting blood glucose, triglyceride, and MDA levels. No differences were found in other parameters (Table II). Table III shows the blood profile of the baseline and after eight weeks of intervention data between the
spirulina treated group and control subject. Comparison between baselines and after eight weeks of intervention in spirulina treated diabetic patients were shown significant decreases in the total cholesterol, LDL-cholesterol, triglyceride, and MDA serum levels (P<0.05, Table III) but other tested parameters was not revealed any significant changes. Therefore, spirulina supplementation shows a significant effect on the level of total cholesterol, triglyceride, and MDA levels in diabetic patients with higher triglyceride, total cholesterol, and MDA levels after 8 weeks of intervention with spirulina (p<0.01, Table III).

### TABLE II - Baseline characteristics of study subjects

| Parameters                        | Spirulina treated diabetic patients (n=15) | Diabetic controls (n=15) | P-value |
|-----------------------------------|-------------------------------------------|--------------------------|---------|
| Age (years)                       | 46.70±8.10                                | 47.30±8.80               | 0.262   |
| Gender (male/female, n)           | 6/9                                        | 5/10                     |         |
| BMI (kg/m²)                       | 28.27±2.05                                | 27.21±1.83               | 0.171   |
| Fasting blood glucose (mg/dl)     | 186.60±36.86*                             | 174.07±33.40             | 0.040   |
| Insulin (ng/dl)                   | 6.52±5.21                                 | 5.45±5.54                | 0.152   |
| Total cholesterol (mg/dl)         | 193.40±36.52                              | 199.20±21.88             | 0.066   |
| LDL-cholesterol (mg/dl)           | 106.33±36.75                              | 119.13±20.25             | 0.081   |
| HDL-cholesterol (mg/dl)           | 40.46±10.93                               | 40.80±9.42               | 0.251   |
| Triglyceride (mg/dl)              | 231.13±86.31*                             | 195.33±47.19             | 0.020   |
| MDA (nmol/l)                      | 6.0±1.43*                                 | 5.02±0.97                | 0.030   |

BMI= Body mass index, LDL-cholesterol: Low Density Lipoprotein-cholesterol, HDL-cholesterol: High Density Lipoprotein-cholesterol, Malondialdehyde = MDA

### TABLE III - Blood profile of the study subjects after eight weeks intervention

| Parameters                        | Spirulina treated diabetic patients (n=15) | Diabetic control (n=15) | P-value |
|-----------------------------------|-------------------------------------------|--------------------------|---------|
| Fasting blood glucose (mg/dl)     | 186.60±36.86*                             | 180.93±29.49             | 0.11    |
| Insulin (ng/dl)                   | 6.52±5.21                                 | 6.18±3.50                | 0.59    |
| Total cholesterol (mg/dl)         | 193.40±36.52                              | 168.46±23.98*            | 0.001   |
| LDL-cholesterol (mg/dl)           | 106.33±36.75                              | 93.86±27.07*             | 0.001   |
| HDL-cholesterol (mg/dl)           | 40.46±10.93                               | 41.26±10.91              | 0.271   |
| Triglyceride (mg/dl)              | 231.13±86.31*                             | 171.33±69.36*            | 0.001   |
| MDA (nmol/l)                      | 6.0±1.43*                                 | 4.88±1.16*               | 0.020   |

LDL-cholesterol: Low Density Lipoprotein-cholesterol, HDL-cholesterol: High Density Lipoprotein-cholesterol, Malondialdehyde = MDA
**DISCUSSION**

In the present study we the effect of spirulina on lipid profile, glucose, and malondialdehyde levels in new cases of type 2 diabetes were assessed. After eight weeks of spirulina supplementation, significant differences were shown in the serum levels of total cholesterol, low-density lipoprotein (LDL)-cholesterol, triglyceride, and malondialdehyde. Spirulina is thought to improve blood lipid profile (Torres-Duran et al., 1998) and decrease oxidative stress (Shklar, Schwartz, 1988). Dyslipidemia and oxidative stress are observed in most diabetic patients. Spirulina may be a good supplementation for type 2 diabetic patients.

**TABLE IV** - The correlation coefficients between fasting blood glucose, blood profile and MDA at baseline and biochemical parameters changes (spirulina group)

| Changes after intervention | FBS (mg/dl) | Insul (ng/dl) | TG (mg/dl) | TCHOL (mg/dl) | HDL-CHOL (mg/dl) | LDL-CHOL (mg/dl) | MDA (nm/l) | FBS (mg/dl) | Insul (ng/dl) | TG (mg/dl) | TCHOL (mg/dl) | HDL-CHOL (mg/dl) | LDL-CHOL (mg/dl) | MDA (nm/l) |
|---------------------------|-------------|---------------|------------|---------------|-----------------|-----------------|------------|-------------|---------------|------------|---------------|-----------------|-----------------|------------|
| Spirulina                 | -0.964**    | -0.504        | -0.532     | -0.235        | -0.051          | -0.029          | -0.014     | -0.045      | -0.045        | 0.449      | -0.252         | -0.140          | 0.621          | -0.383     |
| Control                   | -0.504      | -0.252        | -0.094     | -0.345        | -0.022          | -0.008          | -0.006     | -0.008      | -0.008        | 0.008      | -0.025         | -0.126          | -0.065         | 0.295      |

FBS: Fasting blood glucose, Insulin: insul., TG: Triglyceride, TCHOL.: Total-cholesterol, HDL-CHOL.: High density lipoprotein-cholesterol, LDL-CHOL.: Low density lipoprotein-cholesterol, MDA: Malondialdehyde.
patients. Study of (Park, Ahn, 2007) on healthy elderly people with normal fasting blood glucose level showed that there is an association between the effects of spirulina supplementation and a decrease in fasting blood glucose level, while another study indicated no decrease in fasting blood glucose among type 2 diabetic patients (Lee, Park, 2008). Similar to the results of the present study, a study indicated that spirulina may have no effects on blood glucose levels in diabetic patients (Lee et al., 2008). A study is reported the anti-diabetic property of spirulina (Layam, Reddy, 2006). In the present study, oral administration of spirulina did not change blood glucose level and had no hypoglycemic effect, while other studies have shown that administration of spirulina affects blood glucose levels and helps control blood glucose levels in streptozotocin-induced diabetic animals (Pandey et al., 2011; Sharoud, 2015). Thus, spirulina may not be used as a functional food for the regulation of blood glucose in diabetic patients. The results of the present study are consistent with some other studies (Layam, Reddy, 2006; Lee et al., 2008). Study of Park and Kim (Park, Kim, 2003) indicated that triglyceride, total cholesterol, and LDL-cholesterol serum levels significantly reduced in Korean elderly people after spirulina intervention for 24 weeks. Our results are in agreement with the findings of other studies (Parikh, Mani, Iyer, 2001; Park, Kim, 2003; Torres-Duran et al., 1998). Abnormal glucose metabolism may be accompanied by abnormal lipid metabolism. This may be considered that there is an association between abnormal metabolism of lipid and metabolic disorders in diabetic patients. Some studies reported increased lipid profiles in diabetic rats (Parikh, Mani, Iyer, 2001; Sethi et al., 2004). This may be associated with insulin deficiency and elevated cortisol levels, which has a significant role in the accumulation of fat (Jurgonski, Juskiewicz, Zdunczyk, 2008). Naturally, lipoprotein lipase hydrolyzes triglycerides activates by insulin. Hypertriglyceridemia may occur when the enzyme is inactivated due to insulin deficiency (Hristova, Aloe, 2006). Spirulina may reduce the endogenous synthesis of lipids. The reduced glucose-6-phosphatase activity through the pentose phosphate pathway may decrease glutathione/oxidized glutathione ratio, which can convert NADPH to NADP+ (Shirwaikar, Rajendran, 2004). Spirulina may also have an important role in the production of high NADP+ that regulates lipogenesis, reduces tissue damage (because of oxidative stress), and causes high resistance for diabetes (Bopanna et al., 1997). Spirulina may also have therapeutic effects such as preventing and decreasing the damages caused by hyperlipidemia and antioxidant activity (Bertolin et al., 2009). Studies on different animal models revealed that spirulina reduces plasma and hepatic total cholesterol, LDL-cholesterol, and triglycerides, while studies on humans have shown a significant decrease in the total cholesterol, LDL-cholesterol, and triglycerides levels (Ramamoorthy, Premakumari, 1996). These findings are in agreement with our results (Ramamoorthy, Premakumari, 1996; Shklar, Schwartz, 1988; Torres-Duran et al., 1998). Some other studies have reported that spirulina lowering effect on triglycerides can be because of spirulina’s influence on lipoprotein metabolism (Iwata, Inayama, Kato, 1990). Their studies on spirulina-treated rats showed a significant elevation in lipoprotein lipase activity when compared to rats with a high fructose diet. Free radicals may play an important role in the pathogenesis of diabetes (Gürler et al., 2000). Free radicals are associated with many biochemical pathways such as glucose antioxidation, polyol pathway, and protein glycation (Aly, Mantawy, 2012). Lipid peroxidation can cause injury proteins, lipids, carbohydrates, and nucleic acids and also mediators of tissue injury in cardiovascular pathology and cell membrane or internal cellular components destruction (Burton et al., 1990). There is an important association between oxidative stress and the etiology of diabetic complications (Giugliano, Ceriello, Paolisso, 1996). Studies of Kim and Kim (Kim, Kim, 2005) on healthy elderly subjects with spirulina supplementation for eight weeks indicated elevated total antioxidant status and reduced thiobarbituric acid reactive substance. Similar to the present study, the study on spirulina extract has shown that spirulina decreases the oxidative process (Pinero Estrada et al., 2001). The correlation analysis showed that baseline serum triglycerides, total cholesterol, LDL-cholesterol, and
MDA levels indicated a higher decrease in this lipid profile and MDA levels. Although significant elevation in the serum MDA level of diabetic subjects was observed in the present study, it significantly decreased after spirulina supplementation (from 6.0±1.43 nm/l to 4.88±1.16 nm/l). Oral administration of spirulina may prevent the pathogenic effects of diabetes. This may be because of the secretion of insulin from pancreatic islet β-cells or blood glucose transport to the peripheral tissue (Quoc and Pascaud, 1996). The study of (Nagaoka et al., 2005) also showed the mechanism by which spirulina decreases hypercholesterolemia (Ramamoorthy, Premakumari, 1996). Different hypotheses have been suggested to identify possible mechanisms for the hypolipidemic effect of Spirulina. Gamma-linolenic acid (GLA) content of spirulina is necessary to synthesis Prostaglandin (PG) in our body. Prostaglandin (Especially PG1) can affect and regulate different biochemical functions such as the regulation of cholesterol synthesis (Mani et al., 2007). It was reported that Spirulina inhibited lipid peroxidation (Expressed as malondialdehyde) (Miranda et al., 1998). Phycocyanin (a pigment of spirulina) induces the formation of free radicals. It has been shown that the effect of Phycocyanin was attributed to the inhibition of reaction involved in the formation of the reactive metabolite and possibly because of its radical scavenging activity (Kuriakose, Kurup, 2010).

In conclusion, our study concluded that spirulina supplementation may have a beneficial effect on lipid profile and MDA levels through an interventional 8 weeks. This effect may protect subjects against free radicals and the development of some diseases such as atherosclerosis. The study also indicated that the spirulina supplementation showed a potential lipid-lowering effect on new case type 2 diabetic patients which may help the diabetics to have control on lipid levels. Further studies are required to assess the exact mechanism of spirulina actions on lipid profiles, FBS, and MDA levels.

**ETHICS APPROVAL AND CONSENT TO PARTICIPATE**

The ethnic committee of Golestan University of Medical Sciences approved the study (With ethics number: IR.GOUMS.1396.41)

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**CONFLICT OF INTEREST**

The authors declared no conflicts of interest.

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