Original Paper

Investigation of the Effects of *Chlorella Vulgaris* Supplementation in Patients with Non-Alcoholic Fatty Liver Disease: A Randomized Clinical Trial

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ABSTRACT

Background/Aims: To investigate the advantage of *Chlorella vulgaris* supplementation as an adjunctive therapy in patients with non-alcoholic fatty liver disease (NAFLD). **Methodology:** In a randomized, open-label clinical trial, 76 individuals with NAFLD were randomly assigned to: 1) *Chlorella* group (n=33), receiving *C. vulgaris* extract (1200mg/day) + metformin (750mg/day) + vitamin E (200mg/day) for 3 months, or 2) Metformin group (n=43), receiving metformin (1250mg/day) + vitamin E (200mg/day) for 3 months. Weight, body mass index (BMI), homeostasis model assessment of insulin resistance (HOMA-IR) index as well as serum levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), insulin, total and direct bilirubin, fasting blood sugar (FBS), glycated hemoglobin (HbA_{1c}), uric acid, albumin and lipid profile were evaluated at baseline and at the end of trial. **Results:** Weight and BMI were decreased in both groups. Serum ALT, AST, triglycerides, uric acid, HbA_{1c} and HOMA-IR index were reduced only in the *Chlorella* group whereas significant changes in total cholesterol, LDL, HDL and FBS were only observed in the metformin group. There were also borderline significant reductions in insulin and FBS in the *Chlorella* group. **Conclusions:** The findings of the present trial indicated that addition of *C. vulgaris* extract to the therapeutic regimen of NAFLD including metformin and vitamin E, is associated with favorable effects on serum levels of transaminases, triglycerides as well as insulin sensitivity. Therefore, *C. vulgaris* extract might be a promising hepatoprotective supplement for patients with NAFLD.

Key Words: *Chlorella vulgaris*; Non-alcoholic fatty liver disease; Insulin resistance; Hepatic injury; Transaminase; Lipid profile.

Abbreviations: Alkaline Phosphatase (ALP); Alanine Aminotransferase (ALT); Aspartate Aminotransferase (AST); Body Mass Index (BMI); Chlorella Vulgaris (C. vulgaris); Fasting Blood Sugar (FBS); Glycated Hemoglobin (HbA1c); High-Density Lipoprotein Cholesterol (HDL-C); Homeostasis Model Assessment of Insulin Resistance (HOMA-IR); Low-Density Lipoprotein Cholesterol (LDL-C); Non-Alcoholic Fatty Liver Disease (NAFLD); Standard Deviation (SD).

INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) is one of the most prevalent liver diseases which is histologically similar to alcoholic liver disease but occurs in patients without significant alcohol intake (1-3). It encompasses a wide histological spectrum ranging from simple hepatic steatosis to non-alcoholic steatohepatitis, hepatic cirrhosis and hepatocellular carcinoma, all of which are accompanied by fat accumulation in hepatocytes (1). The prevalence of NAFLD is about 20-40% in Western population and 5-35% in populations from Pacific and Asian countries (4,5). Owing to the worldwide increasing rate of obesity, diabetes mellitus and metabolic syndrome, the prevalence of NAFLD is expected to rise even further (6). Although the exact pathophysiology of NAFLD is not fully understood, insulin resistance and oxidative stress are regarded as key mechanisms leading to this disorder (7,8). Besides, central obesity, type 2 diabetes mellitus, dyslipidemia and metabolic syndrome have been proposed as major risk factors for NAFLD (9-11). Currently, there is no standard pharmacological treatment for NAFLD and treatments have been mainly focused on controlling the risk factors by insulin sensitizing (*e.g.* pioglitazone, rosiglitazone and metformin), lipid-lowering (*e.g.* gemfibrozil and probucol) and antioxidant (*e.g.* vitamin E, betain, probucol and N-acetylcysteine) agents.

Since weight loss may not be beneficial for all patients with NAFLD, as a considerable fraction are not obese (7), pharmacotherapy could be regarded as a plausible option for the management of the disease. However, with respect to inconsistent findings on the effectiveness of current medications and their side effects, introduction of novel hepatoprotective agents, in particular from natural sources, that could effectively inhibit liver damage would be desirable.

Chlorella vulgaris is a unicellular green algae, belonging to the phylum *Chlorophyta* which has been widely used as a food source and currently marketed as a food supplement in different forms (12,13). This microalgae is rich in choline, β-carotene, vitamins, fibers and minerals and has been shown to possess favorable anti-atherogenic, anti-inflammatory, anti-hyper-

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glycemic, antioxidant, anti-tumor, anti-bacterial and anti-viral effects (14-16). These interesting properties of *C. vulgaris*, make it a potential candidate for the prevention and treatment of NAFLD and related disorders. The present study attempted to evaluate the advantage of adding *C. vulgaris* extract to the treatment regimen of patients with NAFLD including metformin and vitamin E.

METHODOLOGY

Subjects and design

This was a randomized, open-label, clinical trial which was performed between March 2009 and December 2009 in the Baquiyatallah Hospital (Tehran, Iran). Men and women aged 35-70 years old, who satisfied all of the following inclusion criteria were eligible to participate in the study: 1) Sonographic evidence of fatty liver disease; 2) Serum low-density lipoprotein cholesterol (LDL-C) >100mg/dL (in case of diabetic patients), >130mg/dL (in case of patients with ≥2 risk factors) and >160mg/dL (in case of patients without risk factors); 3) Serum high-density lipoprotein cholesterol (HDL-C) <35; 4) Serum triglycerides > 200mg/Dl; and 5) not participating in a concomitant clinical trial.

Exclusion criteria were: 1) History of recent or past alcohol intake; 2) Any clinical, biochemical or ultrasonographic evidence of cirrhosis; 3) presence of any form of hepatitis (viral, autoimmune or iatrogenic); 4) Presence of any form of chronic liver disease other than NAFLD and any of the known causes for secondary NAFLD including jejunoileal bypass surgery, extensive small bowel resection, weight loss surgery and rapid and extensive weight loss; 5) Occurrence of any severe side effect or hypersensitivity to the administered drugs; 6) surgery; and 7) Lack of compliance to the medications.

Eighty patients met the eligibility criteria, of which 4 refused to participate in the study before taking medications. The remaining 76 participants were randomly assigned to: 1) *Chlorella* group (n=33), receiving *C. vulgaris* extract (1200mg/day; 600mg b.i.d.) + metformin (750mg/day) + vitamin E (200mg/day; 100mg b.i.d.) for 3 months, or 2) Metformin group (n=43), receiving metformin (1250mg/day) + vitamin E (200mg/day; 100mg b.i.d.) for 3 months.

C. vulgaris extract used in the study was in the form of 300mg tablets which are commercially available under trade name ALGOMED® (Bioprodukte Prof. Steinberg Produktions- und Vertriebs GmbH & Co KG, Klötze, Germany). Ultrasonographic evaluations of liver were performed at the entry of the study by a blinded expert radiologist using a Hitachi EUB 405 device with a 3.5MHz convex probe.

Anthropometric parameters (including weight, height, waist and hip circumference and BMI) together with systolic and diastolic blood pressures, serum lipid profile and levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), insulin, total and direct bilirubin, fasting blood sugar (FBS), glycated hemoglobin (HbA_{1c}), uric acid and albumin were measured for participants at baseline and at the end of study using routine laboratory tests. LDL-C was calculated using the Friedwald formula. Insulin resistance was determined using the homeostasis model assessment (HOMA) (18). HOMA-IR was calculated from fasting glucose and insulin levels using the following formula: Fasting Blood Sugar x Serum Insulin/405.

The study protocol was approved by the institutional ethics committee and written informed consent was obtained from participants.

Statistical analysis

Statistical analyses were performed using SPSS software for Windows. Values were presented as number (%) or mean ±SD. Between group comparisons were performed using independent samples *t*-test (for normally distributed data) and Mann-Whitney U test (for non-normally distributed data). Paired data were compared using paired samples *t*-test (for normally distributed data) or Wilcoxon signed ranks test (for non-normally distributed data). Categorical variables were compared using χ^2 or Fisher's exact test.

RESULTS

From the 76 patients who initially entered the trial, 54 completed the study (n=21 and 33 for the *Chlorella* and control groups, respectively). There was no significant difference in the number of drop-outs between the two groups ($p>0.05$). Reasons for dropping out included unacceptable side effects from the drug, not attending the clinic according to the study schedule and not taking the medications regularly.

Demographic data

The two groups were not significantly different at baseline regarding age, gender, educational level, systolic and diastolic blood pressures, weight and hip circumference, BMI, ALT, AST, ALP, total cholesterol, triglycerides, LDL-C, oral glucose tolerance test, FBS, insulin, HOMA-IR, HbA_{1c}, albumin, total and direct bilirubin and uric acid. HDL-C was significantly higher in the *Cholrella* group compared to the metformin group ($p<0.05$). There was also no significant difference between the groups in the prevalence of diabetes mellitus, coronary artery disease, hypertension, hypertriglyceridemia and smoking habit in the medical history ($p>0.05$). Ultrasonographic examinations at baseline showed that 45.2% of patients were in grade 1 (mild), 31.5% in grade 2 (moderate) and 23.3% in grade 3 (severe) of NAFLD. There was no significant difference in the severity of NAFLD based on ultrasonographic findings between the 2 groups at baseline ($p>0.05$, **Table 1**).

Effect of *C. vulgaris* extract on anthropometric parameters and blood pressure

There were significant reductions in weight and BMI in both *Chlorella* ($p<0.001$) and metformin groups ($p<0.05$). However, waist circumference, hip circumference and waist/hip ratio were not significantly affected in any of the groups ($p>0.05$). There were no significant changes in systolic and diastolic blood pressure in any of the groups, either ($p>0.05$, **Table 1**).

Effect of *C. vulgaris* extract on biochemical markers of liver injury (ALT, AST and ALP)

Supplementation with *C. vulgaris* extract was associated with a significant decrease in serum ALT ($p<0.05$) and AST ($p<0.05$) levels. In contrast, serum levels of these transaminases remained unchanged by the end of trial in the control group ($p>0.05$). No significant change in serum ALP was observed, neither in the *Chlorella* nor in the metformin group ($p<0.05$, **Table 1**).

Effect of *C. vulgaris* extract on biochemical markers of liver function (albumin and bilirubin)

Serum levels of total and direct bilirubin did not

change significantly in either of the groups (p>0.05). Likewise, there was no significant change in serum albumin, neither in the *Chlorella* nor in the metformin group (p>0.05, **Table 1**).

Effect of *C. vulgaris* extract on serum lipid profile and uric acid

Serum levels of total cholesterol and LDL-C were significantly decreased and HDL-C significantly increased in the metformin group (p<0.05). However, these parameters were not significantly altered in the *Chlorella* group (p>0.05). In contrast, serum triglycerides were significantly decreased in the *Chlorella* group (p<0.001) whereas there was no significant change in the metformin group (p>0.05). With respect to serum uric acid, significant reductions where observed in the *Chlorella* group (p<0.05) while there was no significant change in the metformin group (p>0.05, **Table 1**).

Effect of *C. vulgaris* extract on serum FBS, insulin, HbA1c and HOMA-IR

Serum FBS was significantly decreased in the metformin group (p<0.05). A borderline significant decrease in FBS was also observed in *Chlorella* group (p=0.06). With respect to serum insulin levels, a borderline significant decrease was observed in the *Chlorella* (p=0.06) group while there was no significant change in the metformin group (p>0.05). HOMA-IR index together with HbA_{1c} were found to be significantly decreased in the *Chlorella* (p<0.05) but not metformin group (p>0.05, **Table 1**).

TABLE 1. Clinical and biochemical characteristics of Chlorella and metformin groups.

Parameter Pre-trial Post-trial		*Chlorella* group		*p*-value Post-trial	Metformin group		*p*-value
			Pre-trial				
Age (years)		51.00±7.94	-	-	47.10±8.27	-	-
Educational level	Under diploma (%)	52.0	-	-	42.3	-	-
Diploma (%)	32.0	-	-	19.2	-	-	
Academic (%)	16.0	-	-	38.5	-	-	
Cardiovascular disease (%)		4.8	-	-	13.3	-	-
Hypertension (%)		38.1	-	-	32.3	-	-
Diabetes mellitus (%)		19.0	-	-	20.0	-	-
Hypertriglyceridemia (%)		19.0	-	-	16.1	-	-
Smoking (%)		9.5	-	-	6.7	-	-
Ultrasonographic findings	Grade 1 (%)	39.4	-	-	50.0	-	-
	Grade 2 (%)	36.4	-	-	27.5	-	-
	Grade 3 (%)	24.2	-	-	22.5	-	-
Glucose tolerance test (mg/dL)		198.00±114.54	-	-	135.77±40.17	-	-
Height (cm)		165.30±10.09	-	-	170.00±10.07	-	-
Weight (kg)		52.11±43.85	95.10±52.83	<0.001	32.15±50.87	57.13±84.84	<0.05
BMI (kg/m^2)		31.22±4.75	30.07±4.70	<0.001	31.28±5.45	30.22±4.32	<0.05
Waist circumference (cm)		106.38±11.25	107.26±10.23	>0.05	112.24±16.06	111.23±15.84	>0.05
Hip circumference (cm)		107.15±9.14	106.65±10.30	>0.05	110.36±14.53	109.97±15.26	>0.05
Waist / Hip ratio		1.25±0.45	1.25±0.63	>0.05	1.13±0.35	1.13±0.41	>0.05
Systolic blood pressure (mmHg)		126.34±12.92	125.32±15.61	>0.05	130.00±16.16	12.89±18.06	>0.05
Diastolic blood pressure (mmHg)		80.77±5.77	82.36±6.27	>0.05	85.00±7.07	84.95±8.05	>0.05
ALT (U/L)		75.37±44.54	67.17±50.35	<0.05	91.24±71.47	17.36±38.44	<0.05
AST (U/L)		88.27±37.38	75.11±42.27	<0.05	14.16±39.30	56.22±04.30	<0.05
ALP (U/L)		02.72±07.207	15.66±36.203	>0.05	63.44±00.197	36.42±57.180	>0.05
Total cholesterol (mg/dL)		60.45±00.214	19.28±32.192	>0.05	30.36±20.211	48.37±12.189	<0.05
Triglycerides (mg/dL)		75.137±47.257	64.56±32.155	<0.001	73.77±04.206	50.85±52.199	>0.05
LDL-C (mg/dL)		88.46±68.126	68.24±05.107	>0.05	68.35±37.129	65.26±61.107	<0.05
HDL-C (mg/dL)		53.12±59.51	30.13±47.51	>0.05	77.9±54.42	75.10±28.48	<0.05
FBS (mg/dL)		87.49±74.124	96.22±89.109	0.06	00.46±80.121	82.27±24.103	<0.05
HbA_{1c} (%)		64.1±18.7	24.1±23.6	<0.05	54.1±25.7	76.1±56.6	>0.05
Insulin (μIU/mL)		34.3±21.13	15.2±53.9	0.06	44.6±80.15	94.5±90.12	>0.05
HOMA-IR		05.2±13.4	63.0±57.2	<0.05	02.2±80.4	38.1±35.3	>0.05
Albumin (g/dL)		49.0±35.4	42.0±5.4	>0.05	07.0±45.4	42.0±90.4	>0.05
Total bilirubin (mg/dL)		21.0±75.0	17.0±71.0	0.05	67.0±21.1	51.0±10.1	>0.05
Direct bilirubin (mg/dL)		10.0±29.0	07.0±25.0	>0.05	37.0±43.0	21.0±36.0	>0.05
Uric acid (mg/dL)		81.1±33.7	11.1±25.5	<0.05	35.2±23.7	26.1±57.6	>0.05

BMI: Body Mass Index; ALT: Alanine Aminotransferase; AST: Aspartate Aminotransferase; ALP: Alkaline Phosphatase; LDL-C: Low-Density Lipoprotein Cholesterol; HDL-C: High-Density Lipoprotein Cholesterol; FBS: Fasting Blood Sugar; HBA_{1c}: Glycated Hemoglobin; HOMA-IR: Homeostasis Model Assessment of Insulin Resistance.

DISCUSSION

NAFLD is considered as one of the most common disorders of the liver that covers a spectrum of hepatic conditions ranging from simple steatosis to cirrhosis and hepatocellular carcinoma (1). NAFLD is also among the most frequent causes of persistently elevated liver enzymes (2). It has been frequently reported that NAFLD is associated with insulin resistance and its related metabolic abnormalities such as central obesity, hypertension, dyslipidemia, hyperglycemia and hyperuricemia. The clustering of these metabolic abnormalities is often regarded as metabolic syndrome. Hence, there is a close link between NAFLD and metabolic syndrome and emerging evidence have proposed NAFLD as the hepatic manifestation of the metabolic syndrome (11,17). In spite of its high prevalence, there is no proven treatment for NAFLD (18) except for lifestyle modification through diet changes and exercise which are among the first recommendations for patients with NAFLD (19). In the present study, the effects of *C. vulgaris* extract on biomarkers of liver injury and function as well as insulin resistance and lipid profile were investigated in patients with NAFLD who were under treatment with metformin and vitamin E. As far as we are aware, this is the first clinical trial investigating the effectiveness of *C. vulgaris* against NAFLD.

Interestingly, *C. vulgaris* was found to be associated with significant decreases in serum transaminase (ALT and AST) levels, which is indicative of reduced hepatic injury and consistent with some previous animal studies (20,21). This hepatoprotective effects could be mainly attributed to the *C. vulgaris* extract because the levels of transaminases in the metformin group which received higher dose of metformin was not significantly decreased.

Insulin resistance is a common and crucial pathomechanism of both NAFLD and metabolic syndrome. Hence, insulin sensitizers are among the therapeutic options for NAFLD. In the present study, insulin resistance was assessed using HOMA-IR index. This index was found to be significantly improved in the *Chlorella* group but not in the metformin group. Given the key role of insulin resistance in the development and progression of NAFLD (*via* mechanisms such as increased peripheral lipolysis and *de novo* lipogenesis) and its high prevalence in patients with NAFLD (which is nearly universal) (22), this observed improvement in HOMA-IR index by *C. vulgaris* containing regimen compared to the high dose metformin-containing regimen seems an intriguing finding.

Addition of *C. vulgaris* extract to metformin and vitamin E was also associated with significant reductions in serum triglycerides. This is in line with previous animal studies which reported reduction of liver and serum triglycerides following supplementation with *C. vulgaris* (14,23). Accumulation of triglycerides in hepatocytes is the hallmark of NAFLD and the first steps in its pathophysiology (24). Triglyceride levels have also been found to be correlated with hepatic insulin resistance in Caucasians (25). Another study reported a strong association between hypertriglyceridemia and elevated aminotransferase levels (26,27). Finally, triglyceride levels have been reported to be closely related to severity of NAFLD (28). All of these findings imply on the important role of hypertriglyceridemia in NAFLD. Therefore, reduction of serum triglycerides by *C. vulgaris* extract is a promising effect that, along with other favorable effects of this microalgae, could be of therapeutic importance for NAFLD. In regard to the impact of metformin on lipid profile, previous reports have been inconsistent. While some studies reported favorable effects of metformin on total cholesterol, triglycerides and HDL (29,30) there are also reports showing no change in lipid profile by metformin (31,32). In the present study, significant increases in HDL and significant decreases in LDL were observed in the metformin group, whereas triglycerides remained unaltered.

Another beneficial effect that was observed in the *Chlorella* but not metformin group was the significant reduction in the serum uric acid levels. There are previous reports indicating serum uric acid levels are significantly and independently associated with the presence of NAFLD and could be applied as a marker for the risk assessment of NAFLD (33,34). The association between serum uric acid levels and NAFLD could be due to the close link that exist between this disorder and metabolic syndrome as many studies have reported increased serum levels of uric acid in metabolic syndrome and their association with this disease (35,36). One plausible mechanism is the role of insulin resistance, an important aspect of both metabolic syndrome and NAFLD, on the metabolism of uric acid which leads to increased synthesis and decreased excretion of uric acid (37,38). Therefore, decrease in serum uric acid levels could be at least partly attributed to the observed decrease in HOMA-IR index in the *Chlorella* group. This hypothesis is somewhat corroborated by the observation that uric acid levels remained statistically unchanged in the metformin group, for which the HOMA-IR index was not significantly altered, either.

In previous studies elevated levels of insulin and HbA_{1c} have been reported in patients with NAFLD which could be both the consequences of insulin resistance (39,40). In the present study, no significant change in these parameters as well as HOMA-IR was observed in the metformin group which might be due to the relatively short duration of the trial. Nevertheless, these parameters were improved in the *Chlorella* group who received even lower doses of metformin, in the same period.

In summary, the findings of the present trial indicated that addition of *C. vulgaris* extract to the therapeutic regimen of NAFLD including metformin and vitamin E, is associated with favorable effects on serum levels of transaminases, triglycerides as well as insulin sensitivity. Aside from these effects, *C. vulgar*is is a rich source of dietary antioxidants such as lutein, α-tocopherol, ascorbic acid, α- and β-carotene and selenium (41-43). Given the role of oxidative stress and lipid peroxidation in the pathophysiology of NAFLD, the antioxidant properties of *C. vulgaris* should be also taken into consideration for the observed effects. Therefore, supplementation with *C. vulgaris* might be beneficial to improve insulin sensitivity and hepatoprotection in patients with NAFLD who are under treatment with metformin and vitamin E. Weaker effects on serum LDL, HDL and ALP that was observed in the *Chlorella* group compared to the metformin group could be mainly because of lower metformin dose in the former group, though the short duration of the trial may be also partially responsible. Future blinded and controlled trials with larger number of patients and extended follow-up periods are warranted for better evaluation of *C. vulgaris* effects in NAFLD.

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