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Randomized Control Trials

Glucose homeostasis, insulin resistance and inflammatory biomarkers in patients with non-alcoholic fatty liver disease: Beneficial effects of supplementation with microalgae *Chlorella vulgaris*: A double-blind placebo-controlled randomized clinical trial

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SUMMARY

Background: *Chlorella vulgaris* (*C. vulgaris*) is reported to improve dyslipidemia and hypertension; however, its effect on inflammatory biomarkers and insulin resistance has not been noticed thus far. Non-alcoholic fatty liver disease (NAFLD) as a hepatic symptom of metabolic syndrome is strongly associated with insulin resistance and inflammation.

Aim of the study: In the current interventional trial, we aimed to study the effects of *C. vulgaris* supplementation on glucose homeostasis, insulin resistance and inflammatory biomarkers in patients with NAFLD.

Methods: Seventy NAFLD patients confirmed by ultra-sonographic findings were randomly assigned into intervention group (four 300 mg tablets of *C. vulgaris*) or placebo group (four 300 mg tablets of placebos) for 8 weeks. Anthropometric measurements, liver enzymes, fasting serum glucose (FSG), insulin, high sensitive C-reactive protein (hs-CRP) and tumor necrosis factor-alpha (TNF-α) were assessed and homeostatic model assessment (HOMA) score for insulin resistance was estimated before and after the intervention.

Results: Anthropometric measurements decreased significantly in both group ($p < 0.001$). However, mean reduction in weight was significantly higher in *C. vulgaris* − treated group compared to placebo group. Serum concentrations of liver enzymes, FSG and hs-CRP also significantly decreased and serum insulin concentration and HOMA score increased significantly only in *C. vulgaris*-treated group ($P < 0.001$, $P < 0.006$ and $P < 0.025$, respectively). Mean change in serum glucose and TNF-α levels were significant between the groups even after adjusting for the serum insulin and baseline values of variables ($P = 0.014$, $P = 0.005$, $P = 0.014$, respectively); between-group differences were not significant for the other variables by the end of study.

Conclusion: To our finding, *C. vulgaris* supplementation could be considered as an adjunctive therapy to decrease weight and improve glycemic status and reducing hs-CRP as well as improving liver function in patients with NAFLD.

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Abbreviations: ALT, Alanine aminotransferase; AST, Aspartate aminotransferase; *C. vulgaris*, *Chlorella vulgaris*; HC, Hip circumference; FSG, Fasting serum glucose; HOMA-IR, Homeostatic model assessment of insulin resistance; hs-CRP, C-reactive protein; NAFLD, Nonalcoholic fatty liver disease; NASH, Nonalcoholic steatohepatitis.

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1. Introduction

Non-alcoholic fatty liver disease (NAFLD) is a cluster of metabolic abnormalities in which, fat accumulation exceeds 5–10% of hepatocytes [1]. The current worldwide prevalence of the disease is approximately 10–35% [2]. It encompasses a continuum of disease ranging from steatosis, non-alcoholic steatohepatitis (NASH), fibrosis, cirrhosis and hepatocyte carcinoma [3]. Most of NAFLD patients (90%) have at least one of the features of metabolic syndrome including insulin resistance, hypertension, dyslipidemia, diabetes and obesity [4]; Therefore, NAFLD is considered as a hepatic symptom of metabolic syndrome [5]. Nutrition, physical activity, and lifestyle modifications are initial steps in treatment of NAFLD [6]. Beside lifestyle modification, certain microalgaes and bioactive food components have received considerable scientific attention due to their beneficial effects on obesity related disorders including obesity, insulin resistance and other metabolic manifestations of NAFLD [7]. Among microalgae, *Chlorella vulgaris* (*C. vulgaris*), a single-celled freshwater green algae, is regarded as a complementary medicine [8,9]. Nutritional studies of *C. vulgaris* have revealed that this alga contains essential nutrients including amino acids and fatty acids as well as some vitamins and minerals; it is also a good source of fiber. This algae also contains intracellular phytochemicals (e.g. carotenoids, chlorophyll, tocopherols, and ubiquinone) being widely used as a nutritional supplement [9–11]. *C. vulgaris* supplements are safe and their efficacy in prevention and treatment of dyslipidemia, hyperglycemia, hypertension as well as weight loss has been revealed in several studies [12,13]. The effects of *C. vulgaris* supplementation in improving fasting serum glucose (FSG) and insulin resistance has been investigated in a scarce number of animal and human studies. In a clinical trial, consumption of chlorella tablets for 16 weeks led to activation of insulin signaling pathways in subjects who were at risk of lifestyle related diseases and therefore resulted in decreases in serum levels of fasting glucose [14]. Panahi et al. in another study reported a borderline significant reduction in insulin and FSG level with *C. vulgaris* supplementation in NAFLD patients [15]. In Aizzat study 150 mg/kg chlorella consumption on streptozocin (STZ) induced diabetic mice did not exert any remarkable changes in TNF-α level [11]. Due to the likely positive effects of *C. vulgaris* on FSG, insulin resistance and the shortage of data existing on the *C. vulgaris* effect on inflammatory biomarkers, the present clinical trial was designed to assess the effects of *C. vulgaris* supplementation on insulin resistance and inflammatory factors in NAFLD patients.

2. Materials and methods

2.1. Subjects

This double-blind, randomized controlled clinical trial was conducted among 70 obese patients with NAFLD aged 20–50 years recruited from specialized out-patients clinics of Tabriz University of Medical Sciences from 2011–December to 2012–July. All participants underwent ultrasonography for determining fatty liver by a single sonographist. Echogenicity grading was performed using Sonoace X4 Medisio (South Korea). NAFLD is defined by elevated liver enzymes (ALT and AST) and echogenicity grading of the liver based on Saverymuttu et al. [16]. Patients with liver diseases such as Wilson's disease, autoimmune liver disease, hemochromatosis, virus infection and alcoholic fatty liver as well as those with hepatotoxic, lipid lowering and antihypertensive medication, contraceptive and estrogen were excluded. Ethical approval was obtained from the Ethics Committee of Tabriz University of Medical Sciences. This study was registered in Iranian Registry of Clinical Trials (IRCT number: 201202233320N7). The patients were given a full explanation of the study procedure and signed a written informed consent before entering the clinical trial.

2.2. Study design

A computer-generated random sequence was kept to random allocation. The subjects were randomly allocated into two groups; "*C. vulgaris*" and "*placebo*" groups. For eight weeks, patients in *C. vulgaris* group received 400 mg/day vitamin E plus four tablets of 300 mg *C. vulgaris* tablets/day before breakfast (1 tablet), lunch (2 tablets) and dinner (1 tablet) while those in placebo group received 400 mg/day vitamin E and four tablets of placebos/day. The subjects were recommended to keep their usual lifestyle including diet and physical activity during the study. *C. vulgaris* and placebo tablets were given every two weeks and each subject was followed by telephone every week. To prevent selection bias, the study participants, investigators and the laboratory staff were all blinded to treatment assignment. Primary outcomes were change in Glucose homeostasis, insulin resistance, inflammatory factors indices and liver enzymes. Secondary outcomes were change in anthropometric variables.

2.3. Sample size calculation

Considering $\alpha = 0.05$ and a power of %90, sample size was calculated to be 26 per group based on the information for serum insulin concentrations, obtained from the study by Mizoguchi et al. (2010); anticipating an approximate drop-out rate of 30% during the study course, 35 subjects were recruited in each group.

2.4. C. vulgaris tablets and placebo preparation

C. vulgaris supplements contained 98% C. *vulgaris* powder, 1% separating agent (Silicic acid), 1% plant-based magnesium stearate, and were in the form of 300 mg tablets commercially available under the name of ALGOMED® (Bioprodukte Prof. Steinberg Produktions-und Vertriebs GmbH & Co KG, Germany). The placebo tablets which were identical to the *C. vulgaris* supplements in color and size, were supplied by School of Pharmacy, Tabriz University of Medical Science, Tabriz, Iran.

2.5. Anthropometric measurements

Personal characteristics including demographic and disease history were obtained from each subject. Weight and height were measured using Seca scale (Hamburg, Germany) to the nearest 100 g and 0.5 cm, respectively; participants were in light clothes with no shoes on. Waist (WC) and hip circumferences (HC) were measured by a tape measure to the nearest 0.1 cm.

2.6. Nutritional assessment

Dietary data was collected using a three-day dietary record (one weekend and two working days) and averages of 3-day energy and nutrients intakes were analyzed using *Nutritionist IV* Software.

2.7. Laboratory analysis

Blood samples were taken after 12–14 h overnight fast at the two ends of study. The serum was stored in −70 °C until biochemical analysis. Serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) concentrations were measured at baseline and at the end of study using International Federation of Clinical Chemistry (IFCC) approved method [17]. Fasting serum glucose (FSG) and serum hs-CRP (High-sensitivity C-reactive

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protein) concentrations were measured with enzymatic colorimetric assay and Immuno-turbidimetry method respectively. Serum Insulin and tumor necrosis α (TNF-α) concentrations were also determined using ELISA method. Insulin resistance was assessed by Homeostasis model assessment of insulin resistance (HOMA-IR) as calculated by the following formula:

[insulin (μU/mL) × FSG (mg/dL)]/405].

Higher HOMA-IR scores indicate lower insulin sensitivity [18].

2.8. Statistical analysis

All statistical procedures were performed using SPSS software (statistical package for social analysis, version 17, SPSS Inc., Chicago, IL, USA). All analyses were performed on an intention to treat basis, with a two sides 0.05 significant level. Normality of continuous variable was tested by Kolmogorov–Smirnov test. Data are expressed as mean ± SD for normally distributed variables. Chi-square was used for analysis of nominal or ordinal variables between groups. Differences in mean of the continuous variables between the two groups were tested using analysis of covariance (ANCOVA) with adjustment for baseline measurements of variables and covariates. Changes in biochemical parameters over the study period in each group were assessed using paired t-test. P value less than 0.05 was considered statistically significant.

3. Results

3.1. Subject characteristics

A total of seventy NAFLD patients were recruited in the present clinical trial. However, there were 6 drop-outs in intervention and 9 drop-outs in control groups. Therefore, fifty five patients completed the study (Fig. 1). No adverse effects or symptoms following *C. vulgaris* supplementation were reported by the participants. Baseline characteristics of the patients are presented in Table 1. There were no significant differences at baseline, between two groups in terms of age (37.00 ± 7.45 and 37.73 ± 8.24 years in *C. vulgaris* and placebo group, respectively), gender, marital status, education level, physical activity and NAFLD severity. There was also no significant difference in dietary intakes of energy and nutrients between two groups neither at baseline nor after 8 weeks.

Table 1
Baseline characteristics of the study participants.

Characteristic	*C. vulgaris* (N = 29) %	Placebo (N = 26) %	P[a]
Male	51.7	57.7	0.788
Married	93.1	88.5	0.659
Diploma and lower	69	46.2	0.870
Physical activity			
Inactive	37.9	42.3	0.744
Light	24.1	30.8	
Moderate	37.9	26.9	
Severity of fatty liver			
Mild	48.3	65.4	0.440
Moderate	44.8	30.8	
Severe	6.9	3.8	

[a] P values based on Chi square test.

3.2. Anthropometric measurement

As shown in Table 2 obesity indices as weight, WC, HC decreased significantly in both group and the change in the *C. vulgaris* group was markedly more than the placebo group (P < 0.001). In comparison of anthropometric variables after intervention between two groups, significant difference was only observed for weight values (P = 0.014).

3.3. Biochemical variables

Serum concentrations of ALT and AST decreased significantly after intervention in *C. vulgaris* –treated group (P < 0.001), while no change in placebo-treated group was occurred. Also, after 8 weeks supplementation with *C. vulgaris*, significant reduction in serum

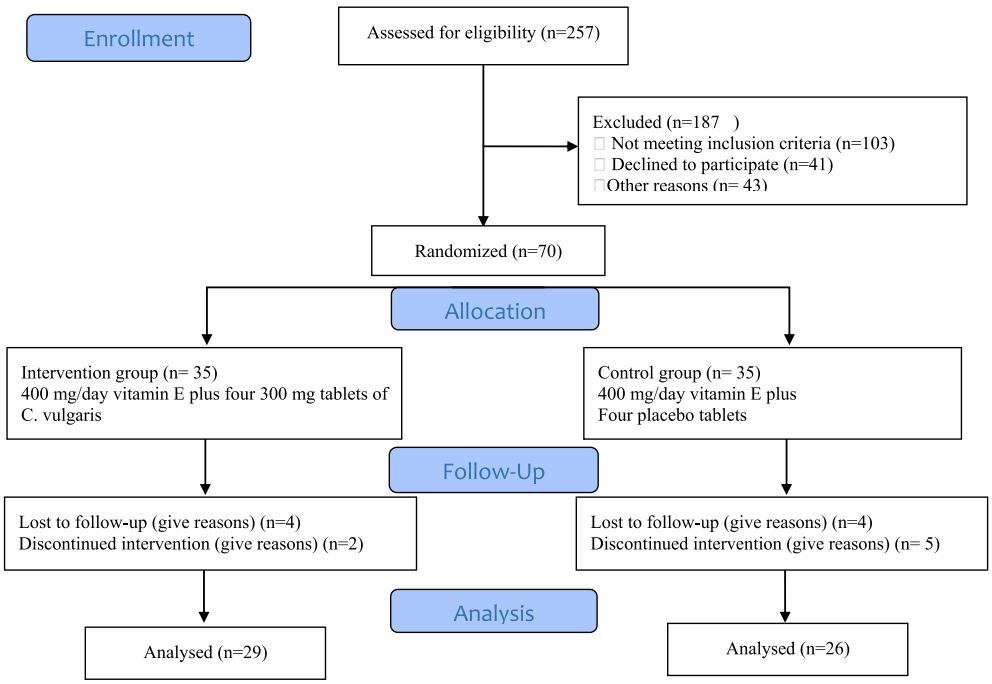

Fig. 1. Flow chart of the study.

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Table 2
Changes in anthropometric measures and liver enzymes in NAFLD patients after intervention.

		C. vulgaris (n = 29)	Placebo (n = 26)	MD (CI 95%)	P
Weight (Kg)	Before	86.21 ± 10.74	89.50 ± 13.87	3.06 (−13.67,19.81)	0.327[b]
	After	82.60 ± 11.5	87.34 ± 13.33		
	MD (CI 95%)	−3.60 (−4.23, 2.97)	−2.15 (−2.76, −1.54)	1.09 (0.236,1.96)	**0.014**[c]
	P[a]	**<0.001**	**<0.001**		
WC (cm)	Before	108.05 ± 10.01	108.92 ± 8.60	0.86 (−4.21,5.94)	0.734[b]
	After	102.90 ± 11.34	105.28 ± 8.08		
	MD (CI 95%)	−3.60 (2.97, 4.23)	−3.63 (2.14, 5.11)	1.59 (−0.957, 4.14)	0.215[c]
	P[a]	**<0.001**	**<0.001**		
HC (cm)	Before	114.41 ± 8.51	115.38 ± 10.36	0.97 (−4.13, 6.08)	0.704[b]
	After	111.08 ± 8.29	111.77 ± 8.81)		
	MD (CI 95%)	−3.32 (2.39, 4.26)	−3.61 (2.08, 5.15)	−0.16 (−1.89,1.56)	0.851[c]
	P[a]	**<0.001**	**<0.001**		
ALT (IU/L)	Before	43.59 ± 22.80	46.65 ± 37.99	3.06 (−13.67, 19.81)	0.878[b]
	After	30.38 ± 18.32	39.73 ± 22.62		
	MD (CI 95%)	−13.20 (7.73,18.68)	−6.92 (−1.55, 15.39)	7.79 (−1.26, 16.86)	0.09[c]
	P[a]	**<0.001**	0.105		
AST (IU/L)	Before	29.14 ± 12.19	32.81 ± 12.34	3.67 (−8.15, 15.49)	0.536[b]
	After	21.93 ± 9.01	28.12 ± 10.6		
	MD (CI 95%)	−7.20 (3.60, 10.8)	−4.69 (−1.13, 10.51)	3.10 (−1.72, 7.93)	0.203[c]
	P[a]	**<0.001**	0.11		

Bold indicate p-values less than 0.05 was considered statistically significant.
WC, Waist circumference; HC, Hip circumference; ALT, Alanine aminotransferase; AST, Aspartate aminotransferase; MD, Mean differences; CI, Confidence interval. Mean (SD) are presented for the measures.
[a] P value for Paired t −test.
[b] P value for Independent sample t test.
[c] P value for ANCOVA; adjusted for baseline values and serum insulin concentrations.

concentrations of FSG, insulin and consequently HOMA-IR values and hs-CRP were observed (P < 0.05) (Table 3). In comparison of biochemical variables after intervention between two groups, significant difference was only observed for FSG concentrations (P = 0.005). No significant changes was observed in serum TNF-α concentrations in both groups while intra-groups changes in TNF-α level was significant (P = 0.014).

4. Discussion

The results of the current clinical trial showed significant weight − reducing effects of eight weeks supplementation with *C. vulgaris* among patients with NAFLD. Moreover, *C. vulgaris* supplementation showed meaningful improvements in liver enzymes and reduction in CRP values. Glucose hemostasis also improved via reduction in serum FSG and increase serum insulin concentrations. By the end of the study, patients in the *C. vulgaris* group had significantly lower serum glucose and TNF-α levels compared to the placebo group; between-group differences for the other variables were not significant.

Only a limited number of studies have examined the effects of *C. vulgaris* on liver function. Lee et al. evaluated the effects of diets containing 5% and 10% w/w *C. vulgaris versus* diet without *C. vulgaris* on liver enzymes in rats reported significant changes in serum ALT and AST levels [19]. In other trial, *C. vulgaris* supplementation

Table 3
Changes in biochemical parameters in NAFLD patients after intervention.

		C. vulgaris (n = 29)	Placebo (n = 26)	MD (CI 95%)	P
FSG (mg/dl)	Before	97.28 ± 12.36	97.42 ± 15.37	0.147 (−7.36, 7.65)	0.969[b]
	After	89.31 ± 10.09	95.96 ± 14.17		
	MD (CI 95%)	−7.96 (4.71, 11.21)	−1.46 (−1.77, 4.69)	6.55 (2.04, 11.05)	**0.005**[c]
	P[a]	**<0.001**	0.361		
Insulin (μIU/ml)	Before	6.730 ± 4.98	10.72 ± 6.90	3.99 (0.66, 7.33)	0.020[b]
	After	9.68 ± 5.89	14.58 ± 2.76		
	MD (CI 95%)	2.95 (−4.98, −0.92)	3.86 (−8.44,0.724)	1.60 (−3.44, 6.64)	0.527[c]
	P[a]	**0.006**	0.095		
HOMA	Before	1.56 ± 1.04	2.52 ± 1.55	0.96 (0.23, 1.69)	0.010[b]
	After	2.07 ± 1.11	3.39 ± 2.78		
	MD (CI 95%)	0.51 (−0.96,0.06)	0.86 (−1.90, 0.17)	0.63 (−0.49, 1.76)	0.263[c]
	P[a]	**0.025**	0.098		
hs-CRP (mg/L)	Before	2.96 ± 1.99	3.29 ± 2.23	0.32 (−1.36, 2.01)	0.711[b]
	After	2.06 ± 1.21	3.41 ± 2.89		
	MD (CI 95%)	−0.89 (0.44, 1.34)	0.12 (−1.29, 1.03)	0.99 (−0.24, 2.24)	0.114[c]
	P[a]	**<0.001**	0.82		
TNF-α (ng/L)	Before	4.88 ± 3.43	3.80 ± 2.33	−1.07 (−2.95, 0.79)	0.253[b]
	After	3.76 ± 2.01	4.56 ± 3.59		
	MD (CI 95%)	−1.12 (−0.11, 2.36)	0.76 (−1.84, 0.31)	2.06 (0.30, 3.81)	**0.014**[c]
	P[a]	0.074	0.157		

Bold indicate p-values less than 0.05 was considered statistically significant
FSG, Fasting serum glucose; Hs-CRP, High-sensitivity C-reactive protein; TNF-α, Tumor necrosis factor-alpha; MD: Mean differences; CI: Confidence interval; Mean (SD) are presented for the measures.
[a] P value for Paired t -test.
[b] P value for Independent sample *t* test.
[c] P value for ANCOVA; adjusted for baseline values and serum insulin concentrations.

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(1200 mg/day) for three months in patients with NAFLD resulted to significant reductions in ALT and AST in *C. vulgaris* group *vs*. control group [15]. The possible underlying mechanism of the effects of *C. vulgaris* on liver function could be due to its weight-reducing effects [20]. To the best of our knowledge, the current study was the first trial examining the effect of *C. vulgaris* on liver enzymes and body weight.

Previous studies has reported significant reductions in serum ALT level and therefore, liver histology after weight loss in NAFLD patients [21,22]. Since weight loss in the *C. vulgaris* group was greater than the placebo group in the present study, it appears that weight loss leads to decrease in hepatic triglyceride content and gluconeogenesis which eventually results in reduction in serum ALT concentration [23].

In the present study, FSG concentrations decreased and serum insulin concentrations increased significantly in both groups; however, before-after comparisons of these parameters were only significant in *C. vulgaris*-treated group. We also found a significant increase in HOMA score in *C. vulgaris*-treated group but only the change in FSG level was significant between the two groups after adjusting for baseline measures. Studies evaluating the hypoglycemic effects of *C. vulgaris* are rare. Yuh et al. found enhanced and prolonged hypoglycemic effects of chlorella consumption on streptozocin (STZ) induced diabetic mice [24]. In a similar study by Jeong et al. [25] chlorella did not influence the glucose-stimulated insulin secretion; however, it significantly improved insulin sensitivity in type 2 diabetic and normal Wistar rats.

In normal or moderately increased body weight and normoglycemic condition, NAFLD is characterized by a feature of the metabolic syndrome i.e. hepatic insulin resistance but NAFLD cannot be regarded as a cause for insulin resistance [26]. NAFLD is highly prevalent among type 2 diabetic patients (about 70%) [26] and hepatic triglyceride accumulation in NAFLD is independent of increased BMI [27]. In insulin-resistant conditions, evidence reveals anti-lipolytic effect of insulin and an increase in fatty acids release [28] as well as promotion of hepatic triglyceride synthesis and accumulation [27]. This fat accumulation in liver consequently develops NASH and cirrhosis in NAFLD [28].

Cherng et al. reported an enhanced glucose uptake and decrease in non-estrificated fatty acid (NEFA) level after chlorella consumption in diabetic mice [29]. However, *C. vulgaris* in diabetic rats failed to show hypoglycemic effects possibly due to short duration of intervention [11]. In a human clinical trial of lifestyle-related diseases, consumption of chlorella supplement among high-risk human subjects resulted in an improvement in glucose metabolism and noticeable reduction in serum glucose concentrations [14]. It seems Chlorella enhances glucose uptake [24], decreases NEFA levels [29] and subsequently, lowers blood glucose.

Other suggested mechanisms for hypoglycemic effects of *C. vulgaris* are reduction in plasma NEFA concentration which increases glucose uptake, glucose utilization and improves hepatic glucose production suppression of [29]. Moreover *C. vulgaris* induces activation of insulin signaling pathways which may result from changes in gene expression in the peripheral blood cells [14].

Our findings showed decreased hs-CRP level in *C. vulgaris* treated group. No significant changes was observed in TNF-α level in neither of the groups while the difference in TNF-α concentration after intervention, was significantly greater in *C. vulgaris* compared to placebo group ($P = 0.014$). To the best of our knowledge, this study seems to be the first study investigating the effect of *C. vulgaris* supplementation on inflammatory markers in human. Similar to our findings, Aizzat et al. failed to show any significant change in TNF-α level after 150 mg/kg *C. vulgaris* supplementation in diabetic mice after 4 weeks [11]. However, a study examined the effectiveness of a semi − chlorella component, Val-Glu-Cys-Tyr-Gly-Pro-Asn-Arg-Pro-Gln-Phe (Chlorella-11 peptide) in rats and found a reduction in serum TNF-α level and prostaglandin E2 (PGE2) production after LPS activation. Chlorella-11 peptide was also able to suppress LPS-induced nitric oxide (NO) production and inflammation [30].

Moreover, a study showed that chlorella dichloromethane extract (CDE) reduced accumulation of thiobarbituric acid-reactive substances, enhanced glutathione level and anti-oxidative enzymes activities (superoxide dismutase, catalase, glutathione peroxidase, and glutathione reductase) in LPS-stimulated macrophages. Additionally, TNF-α also reduced through inhibition of nuclear factor (NF)-kappa B expression and increased TNF-α converting enzymes activity [31].

5. Conclusion

Our findings indicate that 1200 mg *C. vulgaris* supplementation *has potential beneficial effects in* reducing weight, serum glucose level and improving inflammatory biomarkers as well as liver function in NAFLD patients. Further studies in human subjects are needed to investigate the mechanism underlying insulin resistance and inflammation in pathogenesis of NAFLD.

Conflict of interests

The authors declare that there is no conflict of interests.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.clnu.2016.07.004.

References

[1] Paradis V, Bedossa P. Definition and natural history of metabolic steatosis: histology and cellular aspects. Diabetes Metab 2008;34:638–42.
[2] Schwimmer JB, Deutsch R, Kahen T, Lavine JE, Stanley C, Behling C. Prevalence of fatty liver in children and adolescents. Pediatrics 2006;118:1388–93.
[3] Ben MD, Polimeni L, Baratta F, Pastori D, Loffredo L, Angelico F. Modern approach to the clinical management of non-alcoholic fatty liver disease. World J Gastroenterol 2014;20(26):8341–50.
[4] Yki-Järvinen H. Non-alcoholic fatty liver disease as a cause and a consequence of metabolic syndrome. Lancet Diabetes Endocrinol 2014;2(11):901–10.
[5] Başaranoğlu M, Ormeci N. Nonalcoholic fatty liver disease: diagnosis, pathogenesis, and management. Turk J Gastroenterol 2014;25:127–32.
[6] Torres DM, Harrison SA. Diagnosis and therapy of nonalcoholic steatohepatitis. Gastroenterology 2008;134:1682–98.
[7] Park HJ, Bruno RS. Hepatoprotective activities of green tea in nonalcoholic fatty liver disease. Agro Food Ind High Tech 2010;21(1):37–40.
[8] Jo BH, Lee CS, Song HR, Lee HG, Oh HM. Development of novel microsatellite markers for strain- specific identification of *Chlorella vulgaris*. J Microbiol Biotechnol 2014;24(9):1189–95.
[9] Noguchi N, Maruyama I, Yamada A. The influence of *Chlorella* and its hot water extract supplementation on quality of life in patients with breast cancer. J Evid Based Complement Altern Med 2014;2014:1–7.
[10] Valdivia VB, Butron RO, Reynoso MP, Garcia AH, Europa EC. *Chlorella vulgaris* administration prevents HgCl2-caused oxidative stress and cellular damage in the kidney. J Appl Phycol 2011;23(1):53–8.
[11] Aizzat O, Yap SW, Sopiah H, Madiha MM, Hazreen M, Shailah A. Modulation of oxidative stress by *Chlorella vulgaris* in streptozotocin (STZ) induced diabetic Sprague-Dawley rats. Adv Med Sci 2010;55:281–8.

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[12] Shibata S, Hayakawa K, Egashira Y, Sanada H. Hypocholesterolemic mechanism of Chlorella: chlorella and its indigestible fraction enhance hepatic cholesterol catabolism through up-regulation of cholesterol 7alpha-hydroxylase in rats. Biosci Biotechnol Biochem 2007;71:916–25.
[13] Mello-Sampayo C, Luisa Corvo M, Mendes R, Duarte D, Lucas J, Pinto R, et al. Insights on the safety of carotenogenic *Chlorella vulgaris* in rodents. Algal Res 2013;2(4):409–15.
[14] Mizoguchi T, Takehara I, Masuzawa T, Saito T, Naoki Y. Nutrigenomic studies of effects of Chlorella on subjects with high-risk factors for lifestyle-related disease. J Med Food 2008;11:395–404.
[15] Panahi Y, Ghamarchehreh ME, Beiraghdar F, Zare R, Jalalian HR, Sahebkar A. Investigation of the effects of *Chlorella vulgaris* supplementation in patients with non-alcoholic fatty liver disease: a randomized clinical trial. Hepatogastroenterology 2012;59:2099–103.
[16] Saverymuttu SH, Joseph AE, Maxwell JD. Ultrasound scanning in the detection of hepatic fibrosis and steatosis. Br Med J Clin Res Ed 1986;292:13–5.
[17] Okorodudu AO, Pelletier PR, Valcour AA, Bowers Jr GN, Mc Comb RB. Evaluation of the IFCC reference method for alanine aminotransferase: spurious blank ALT activity due to contamination of D-alanine with L-alanine, and recommendations for a correction. Clin Chem 1989;35:153–6.
[18] Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia 1985;28:412–9.
[19] Lee HS, Park HJ, Kim MK. Effect of *Chlorella vulgaris* on lipid metabolism in Wistar rats fed high fat diet. Nutr Res Pract 2008;2:204–10.
[20] Petersen KF, Dufour S, Befroy D, Lehrke M, Hendler RE, Shulman GI. Reversal of nonalcoholic hepatic steatosis, hepatic insulin resistance, and hyperglycemia by moderate weight reduction in patients with type diabetes. Diabetes 2005;54:603–8.
[21] Knobler H, Schattner A, Zhornicki T, Malnick SD, Keter D, Sokolovskaya N, et al. Fatty liver–an additional and treat able feature of the insulin resistance syndrome. QJM 1999;92:73–9.
[22] Bellentani S, Dalle Grave R, Suppini A, Marchesini G. Behavior therapy for nonalcoholic fatty liver disease: the need for a multidisciplinary approach. Hepatology 2008;47:746–54.
[23] Ueno T, Sugawara H, Sujaku K, Hashimoto O, Tsuji R, Tamaki S, et al. Therapeutic effects of restricted diet and exercise in obese patients with fatty liver. J Hepatol 1997;27:103–7.
[24] Jong-Yuh C, Mei-Fen S. Potential hypoglycemic effects of Chlorella in streptozotocin-induced diabetic mice. Life Sci 2005;77:980–90.
[25] Jeong H, Kwon HJ, Kim MK. Hypoglycemic effect of *Chlorella vulgaris* intake in type 2 diabetic Goto-Kakizaki and normal Wistar rats. Nutr Res Prac 2009;3(1):23–30.
[26] Targher G, Bertolini L, Padovani R, Rodella S, Tessari R, Zenari L, et al. Prevalence of nonalcoholic fatty liver disease and its association with cardiovascular disease among type 2 diabetic patients. Diabetes Care 2007;30:1212–8.
[27] Gastaldelli A, Cusi K, Pettiti M, Hardies J, Miyazaki Y, Berria R, et al. Relationship between hepatic/visceral fat and hepatic insulin resistance in nondiabetic and type 2 diabetic subjects. Gastroenterology 2007;133:496–506.
[28] Gaggini M, Morelli M, Buzzigoli E, DeFronzo RA, Bugianesi E, Gastaldelli A. Non-alcoholic fatty liver disease (NAFLD) and its connection with insulin resistance, dyslipidemia, atherosclerosis and coronary heart disease. Nutrients 2013;5:1544–60.
[29] Cherng JY, Shih MF. Improving glycogenesis in streptozocin (STZ) diabetic mice after administration of green algae. Chlorella Life Sci 2006;78:1181–6.
[30] Cherng JY, Liu CC, Shen CR, Lin HH, Shih MF. Beneficial effects of Chlorella-11 peptide on blocking LPS-induced macrophage activation and alleviating thermal injury-induced inflammation in rats. Int J Immuno Pathol Pharmacol 2010;23(3):811–20.
[31] Park JY, Cho HY, Kim JK, Noh KH, Yang JR, Ahn JM, et al. Chlorella dichloromethane extract ameliorates NO production and iNOS expression through the down-regulation of NF kappa B activity mediated by suppressed oxidative stress in RAW 264.7 macrophages. Clin Chim Acta 2005;351(1–2):185–96.

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