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The Effect of *Chlorella vulgaris* Supplementation on Liver Enzymes, Serum Glucose and Lipid Profile in Patients with Non-Alcoholic Fatty Liver Disease

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ABSTRACT

Background: Non-alcoholic fatty liver disease (NAFLD) is becoming a public health problem worldwide and using microalgae is a new approach on its treatment. The aim of this study was to investigate the effect of Chlorella vulgaris supplementation on liver enzymes, serum glucose and lipid profile in patients with NAFLD.

Methods: This double-blind randomized placebo-controlled clinical trial was conducted on 60 NAFLD patients from specialized clinics of Tabriz University of Medical Sciences from December 2011 to July 2012. The subjects were randomly allocated into 2 groups: 1) "intervention" (n=30) received 400 mg/day vitamin E plus four 300 mg tablets of *Chlorella vulgaris* and, 2) "placebo" (n=30) received 400 mg/day vitamin E and four placebo tablets per day for 8 weeks. Weight, liver enzymes and metabolic factors were assessed in fasting serum and dietary data was collected at baseline and end of the study.

Results: Weight, liver enzymes, fasting blood sugar (FBS) and lipid profile decreased significantly in both groups (P<0.05). The differences in weight, ALP and FBS between the two groups were statistically significant (P=0.01, P=0.04 and P=0.02, respectively).

Conclusion: *C. vulgaris* seems to improve FBS and lipid profile and therefore could be considered as an effective complementary treatment in NAFLD.

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Introduction

Obesity is a complex disorder that becoming a public health problem in the worldwide.[1] The increase of obesity has played an important role in the increasing prevalence of nonalcoholic fatty liver disease (NAFLD) as well.[2] It is characterized by fat accumulation in exceeding 5-10% hepatocyte.[3] NAFLD is considered as an emerging epidemic in many countries.[4] The prevalence of NAFLD is estimated 10-35% in general population[5] and 5-30% in Asia.[6] It encompasses a spectrum of disease ranging from steatosis, non-alcoholic steatohepatitis (NASH), fibrosis, cirrhosis and hepatocyte

carcinoma.[7] Although health promotion strategies regarding lifestyle factors such as diet therapy, increasing physical activity, surgical intervention and pharmacotherapy are recommended for NAFLD treatment,[8] there is no consensus.[9] However, sometimes weight loss is not achievable without using supplements for controlling food intake.[10] One of the weight loss supplements is chlorella vulgaris *(C. vulgaris)* as a functional food supplements.[11]

Chlorella – a unicellular green microalgae- is popular as functional food and alternative medicine in worldwide, particularly in Asia.[12,13] It is used as a dietary supplement because of providing high amount of amino acids (apart from methionine and tyrosine), minerals, vitamins, fiber and bioactive compounds.[11,14] Many studies have reported certain beneficial physiological effects against oxidation, cataract, bacterial and viral infection, and inflammation as well as weight loss, hypoglycemic and hypocholesterolemic effects in rats, mice and other laboratory animals.[15,16] However, there are limited clinical trials particularly in human.[17] Lee et al. reported that chlorella containing diets increased total amount of excreted feces and lipids and subsequently, lowered liver and serum total lipids, triglycerides (TG) and total cholesterol (TC) without any significant reduction in alanin aminotransferase (ALT) and aspartate aminotransferase (AST) concentrations in animals.[18] 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 (FBS) and TC.[17]

Chlorella vulgaris, a strain of chlorella, has been recently studied.[18] There is evidence reporting hypoglycemic and hypolipidemic effects of *C. vulgaris*.[19,20] For example, *C. vulgaris* significantly decreases serum glucose and improve insulin sensitivity in diabetic rats.[14] Indeed, *C. vulgaris* may be effective for the prevention of dyslipidemia.[20] Supplementation with *C. vulgaris* decreased weight, ALT, AST and TG levels in NAFLD patients.[21]

There is no convincing evidence for hypoglycemic and hypolipidemic effects of *C. vulgaris* in NAFLD patients. As there are limited evidence, e.g. RCTs and also human studies indicating the health beneficial effects of *C. vulgaris* in NAFLD, therefore, this study was aimed to investigate the effect of *C. vulgaris* supplementation on Liver enzymes, serum glucose and lipid profile in patients with NAFLD.

Materials and Methods

This double-blind, randomized controlled clinical trial was carried out on 70 obese adult patients with NAFLD recruited from clinics of Tabriz University of Medical Sciences from December 2011 to July 2012. Sample size was determined based on primary information obtained from the study by Mizoguchi et al. (2008)[17] for high-density lipoprotein cholesterol (HDL-c). The study protocol was approved by the Ethics Committee of Tabriz University of Medical Sciences and was registered in the Iranian Registry of Clinical Trials website (IRCT201202233320N7).

Sample Size was computed 26 per group by considering α=0.05 and a power of 90%. This number was increased to 30 per group to accommodate the anticipated dropout rate.

All participants underwent ultrasonography for determining fatty liver by a single sonographist. Echogenisity grading was performed using Sonoace X4 Medisio (South Korea). NAFLD is defined by elevated liver enzymes (ALT greater than 31 mg/dl and 41 mg/dl and AST greater than 31 mg/dl and 47 g/dl in women and men , respectively) and echogenicity grading of the liver was based on Saverymuttu et al.[22] i.e. "mild steatosis" as a slight increase in liver echogenicity and exaggeration of liver and kidney echo discrepancy, and relative preservation of echoes from the walls of the portal vein., "moderate steatosis" as a loss of echoes from the walls of the portal veins, particu-

larly from the peripheral branches, resulting in a feature less appearance of the liver as well as a greater posterior beam attenuation and a greater discrepancy between hepatic and renal echoes, and "severe steatosis" as a greater reduction in beam penetration, loss of echoes from most of the portal vein wall, inc. the main branches and a large discrepancy between hepatic and renal echoes.

Of totally 247 recruited patients, only 60 confirmed NAFLD patients met the inclusion criteria inc. aged 20-50 years from both genders, and BMI equal or over 30 kg/m^2. 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, metformin consumption and antihypertensive medication, contraceptive and estrogen were excluded.

The aim of the study was carefully explained to the patients and their written informed consent was obtained. Personal characteristics inc. demographic and disease history were obtained. Weight and height were measured with Seca scale (Hamburg, Germany) to the nearest 100 g and 0.5 cm, respectively and BMI was estimated. Dietary data was collected using a three-day dietary record and averages of 3-day energy and macro-nutrients intakes were analyzed using Nutritionist IV software. A self-reported questionnaire was used to estimate physical activity level.[23-25]

Patients were randomly allocated using a computer-generated random sequence into two groups; "intervention" and "placebo". The subjects were randomly allocated into two groups "*C. vulgaris*" and "placebo" groups. As vitamin E is commonly prescribed for NAFLD patients,[25] therefore, all patients in both groups received vitamin E 400 mg/d plus four 300 mg tablets of *C. vulgaris* (commercially available under the name of ALGOMED® (Bioprodukte Prof. Steinberg Produktions- und Vertriebs GmbH & Co KG,Germany) before breakfast (1 tablet), lunch (2 tablets) and dinner (1 tablet) was considered as *C. vulgaris* group while those in placebo group received 400 mg/d vitamin E and four placebos per day for 8 weeks (Fig.1). The dose of *C. vulgaris* used in this study was based on the trial conducted already.[21]

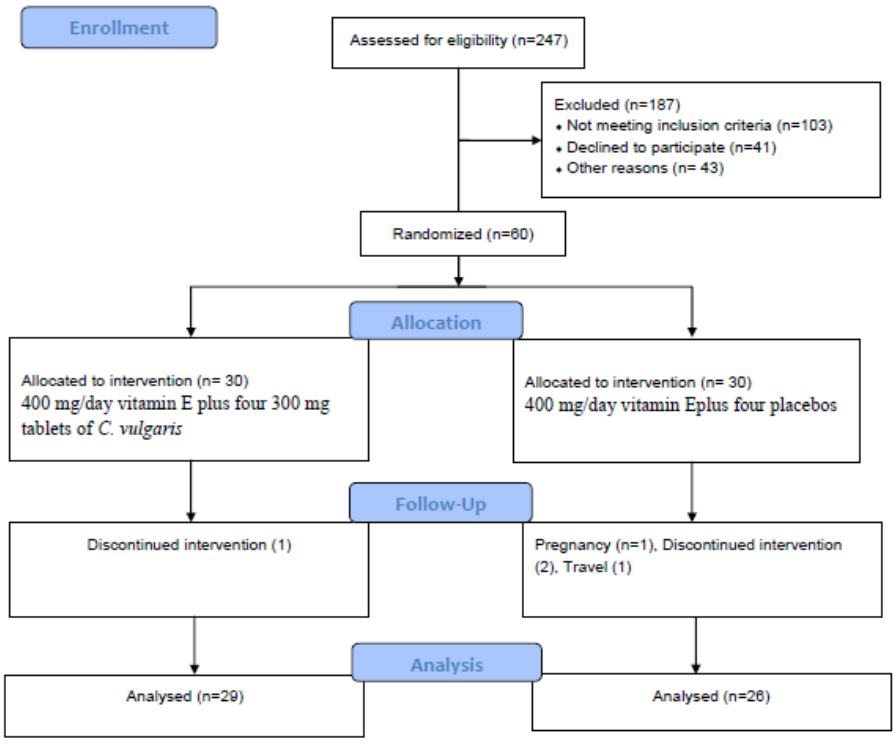

Fig. 1: Flow chart of the study

The placebo tablets supplied by School of Pharmacy, Tabriz University of Medical Sciences, Iran were similar to *C. vulgaris* tablets in color and size. All participants were asked to maintain their usual lifestyle. Ten ml fasting blood samples was taken from each subject and after serum separation was used for assessing serum ALT, AST, ALP, FBS and lipid profile (inc. TC, LDL-C, HDL-C and TG). Liver enzymes and metabolic factors were measured by International Federation of Clinical Chemistry and Laboratory Medicine (IFCC)[23] and enzymatic colorimetric assay respectively.

All statistical procedures were performed using SPSS software (ver. 17.0). Normality of continuous variable was tested by Kolmogorov-Smirnov test. Data are expressed as mean ± SD for normally distributed variables. Differences in mean of the continuous variables between the two groups were tested using analysis of covariance (ANCOVA) for adjusting for baseline measurements and covariates. Changes in biochemical parameters over the study period were estimated by after intervention minus baseline amount was done using paired *t*-test. *P* value less than 0.05 was considered statistically significant.

Results

Of totally 60 patients, 55 patients completed the study (n=29 and 26 for *C. vulgaris* and placebo groups, respectively) (Fig.1). General characteristics of the study subjects are listed in Table 1.

There were no significant differences at baseline between the two groups in terms of age (37.00± 7.45 yr and 37.73±8.24 yr in intervention and placebo group, respectively), gender, marrital status, education level, physical activity and the severity of fatty liver. The mean daily macronutrient and dietary fiber intakes were shown in Table 2. Dietary data assessment over the study showed no significant changes in total energy intake, fat, protein and dietary fiber over the study in both group. There was significant reduction in carbohydrate intake in *C. vulgaris* group (P=0.02). There were no significant differences in the mean nutrient intakes between the two groups at baseline and also after 8 weeks.

The mean ± SD of biochemical factors before and after intervention are shown in Table 3. At the beginning of the study, the groups were similar based upon weight, liver enzymes, serum FBS and lipid profile.

Weight decreased significantly in both groups (P<0.001). The mean weight change in the *C. vulgaris* group was significantly greater than the placebo group (P=0.001).

Both group showed significant reductions in ALT and ALP levels after 8 week period (P<0.001). AST declined in *C. vulgaris* group (P<0.001) while AST level remained unchanged in the placebo group (P>0.05). After 8 weeks of supplementation, no significant changes were observed in liver enzymes concentrations between the two groups (P>0.05).

Table 1: Baseline characteristics of the NAFLD patients in each group

Characteristic	*C. vulgaris*(n= 29) N (%)	Placebo(n= 26) N (%)	Sig. (Chi square)
Male	15 (51.7)	15 (57.7)	0.788
Married	27 (93.1)	23 (88.5)	0.659
Less than high school	9(31)	14(53.8)	
Completed high school	14(48.2)	8(30.76)	0.870
University certificate	6(20.68)	49(15.38)	
Physical Activity†			
Inactive	11 (37.9)	11 (42.3)	
Light activity	7 (24.1)	8 (30.8)	0.744
Moderate activity	11 (37.9)	7 (26.9)	
Severity of fatty liver‡			
Mild	14(48.3)	17(65.4)	
Moderate	13 (44.8)	8(30.8)	0.440
Severe	2(6.9)	1(3.8)	

† PAL assessed based on *Johansson et al*/‡ Fatty liver grading was based on *Saverymuttu* et al.

Table 2: Mean ± SD daily total energy and macronutrient intakes before and after intervention

		C. vulgaris (n=29)	Placebo(n=26)	*P***
Energy (Kcal)	Before	1975±424	2008±584	0.81
	After	1944±370	1969±550	0.84
	MD (CI 95%)	-30(-72.69 to 11.24)	-39(-81.08 to 2.49)	
	*P**	0.145	0.064	
Carbohydrate(g)	Before	291.1±83	248±94.9	0.079
	After	264±70.3	248±70.3	0.409
	MD (CI 95%)	50.8(108.38 to 210.17)	0.12(-48.3 to -21.02)	
	*P**	0.02	0.996	
Dietary fiber (g)	Before	15.2±5.2±5.2	16.6 ±16.3	0.225
	After	14.16±4.42	20.3±16.6	0.201
	MD (CI 95%)	-1(-2.4 to .30)	3.6 (-3 to -10.3)	
	*P**	0.642	0.272	
Protein (g)	Before	72.2±17.4	67.3±23.5	0.388
	After	71.6±15.9	67.1±23.7	0.979
	MD (CI 95%)	-0.5(-2.8 to 1.8)	-2 (-1.4 to 0.9)	
	*P**	0.642	0.621	
Fat (g)	Before	54.7±16.5	58,3±23.4	0.516
	After	51.80±18.80	58±22.5	0.06
	MD (CI 95%)	-2.9(-5 to 0.8)	-.0.32(-2.1 to 1.5)	
	*P**	0.07	0.723	

***Paired *t*-test/**Independent *t* test**

Table 3: Mean ± SD biochemical factors before and after intervention

		C. vulgaris (n=29)	Placebo(n=26)	*P*
Weight (Kg)	Before	86.21±10.74	89.50±13.87	0.327[b]
	After	82.60±11.5	87.34±13.33	0.01[c]
	MD (CI 95%)	-3.60(-4.23 to -2.97)	-2.15 (-2.76 to -1.54)	
	P[a]	<0.001	<0.001	
BMI (kg.m^2)	Before	32.44±3.61	33.19±4.26	0.489[b]
	After	31.09±3.82	32.41±4.24	0.01[c]
	MD (CI 95%)	-1.35(-1.59 to 1.11)	-0.77(-1.59 to -1.11)	
	P[a]	<0.001	<0.001	
ALT (IU/L)	Before	43.59±22.80	42.62±23.71	0.878[b]
	After	30.38±18.32	36.88±22.83	0.154[c]
	MD (CI 95%)	-13.20 (-18.68 to -7.73)	-5.73 (-47 to -2.42)	
	P[a]	<0.001	0.01	
AST (IU/L)	Before	29.14±12.19	28.69±12.34	0.894[b]
	After	21.93±9.01	25.62±10.6	0.188[c]
	MD (CI 95%)	-10.80 (-29.04 to -12.75)	-7.68 (-21.33 to 14.58)	
	P[a]	<0.001	0.07	
ALP (IU/L)	Before	188.59±55.31	194.15±70.21	0.744[b]
	After	158.79±52.72	191.50±63.13	0.04c
	MD (CI 95%)	-29.79 (-43.28 to -16.30)	-2.65(-8.44 to-6.83)	
	P[a]	<0.001	<0.001	
FBS (mg/dl)	Before	97.28±12.36	97.42±15.37	0.565[b]
	After	89.31±10.93	95.96±14.17	0.02[c]
	MD (CI 95%)	-7.96 (-11.21 to -4.71)	-1.46 (-4.69 to -1.77)	
	P[a]	<0.001	<0.001	
TC (mg/dl)	Before	212.03±40.60	204.88±37.24	0.501[b]
	After	186.59±3.99	192.35±33.81	0.821[c]
	MD (CI 95%)	-25.44 (-35.10 to -15.79)	-12.53(-20.22 to -4.84)	
	P[a]	<0.001	<0.001	
LDL-c (mg/dl)	Before	131.08±35.63	122.51±31.59	0.561[b]
	After	110.92±28.37	112.51±31.59	0.825[c]
	MD (CI 95%)	-20.16 (-30.40 to -9.92)	-10.17 (-19.31 to -1.02)	
	P[a]	<0.001	<0.001	
HDL-c (mg/dl)	Before	41.59±8.36	43.58±10.65	0.442[b]
	After	45.93±8.54	43.81±10.39	0.845[c]
	MD (CI 95%)	4.34(1.19 to 7.49)	0.23(-2.94 to 3.40)	
	P[a]	0.04	<0.001	
TG (mg/dl)	Before	178.86±8.36	169.73±81.14	0.694[b]
	After	156.36±66.27	160.81±70.61	0.318[c]
	MD (CI 95%)	-22.50 (-43.83 to -1.16)	-8.92(-26.15 to 8.30)	
	P[a]	<0.001	<0.001	

ALT: -Alanin aminotransferase; AST: Aspartate aminotransferase ; ALP:Alkalin phosphatase ; FBS: Fasting blood sugar; TC: Total cholesterol; LDL-c: Low-Density lipoprotein; HDL: High- Density lipoprotein; TG: Triglycerides; MD: Mean differences; CI: Confidence Interval/[a] : Paired *t*-test;[b]: Independent *t* test ;[c]: Based on Ancova adjusted for baseline values and carbohydrate intake

Both groups showed significant reduction in the serum glucose level after 8 week period (P<0.001) and the mean change in glucose level between the two group reached statistically significant level (P=0.02) i.e. the *C. vulgaris* group had more reduction than the placebo group. Serum TC, LDL-c, TG levels decreased significantly (P<0.001) while HDL-c level increased (P=0.04) in the both groups. No significant changes in serum lipid profile were observed between the two groups (P>0.05).

Discussion

This study has provided insights into the weight loss effect of *C. vulgaris* supplementation among NAFLD patients. In the present study, significant reductions were found in ALT and ALP levels in both groups and AST declined in *C. vulgaris* group. Although there are limited studies examining the effect of chlorella on liver function, Lee et al.[18] had tested different diets containing 5% and 10% w/w chlorella compared with diet without chlorella on liver enzymes in rats and found that ALT and AST levels did not change after consumption of chlorella-containing diets. Indeed, Panahi et al.[21] in a clinical trial with *C. vulgaris* consumption (1200 mg/day) for three months among NAFLD patients showed significant reductions in ALT and AST in *C. vulgaris* group *vs.* control group. The probable effect of chlorella on liver function could be due to its effect on weight.[26] To the best of our knowledge, the only study found to investigate the effect of chlorella (i.e. *C. vulgaris*) on liver enzymes as well as weight was Panahi et al. study.[21] They assessed the effect of metformin 750 mg and vitamin E (200mg/day) plus *C. vulgaris* in NAFLD patients as a supplement (1200 mg/day) on weight and serum ALT and AST levels in compared with control group (only metformin and vitamin E). They found statistically significant reduction in weight parallel to decreases in ALT and AST levels in NAFLD patients after consuming *C.vulgaris.*

Mechanism underlying the improvement of liver enzymes could be explained by weight loss effects of *C. vulgaris*. Among adults with NAFLD showed significant changes in ALT and liver histology after weight loss.[27,28] Since weight loss in the *C. vulgaris* group was greater than the placebo group in the present study, it seems weight loss leads to decrease in hepatic triglyceride content and glyconeogenesis which eventually results in reduction in ALT concentration.[29] On the other hand, decline in liver enzymes correlated with improvement in insulin resistance.[30,31]

In the present study, FBS level decreased significantly in both group after at the end of the study. The change in FBS level was statistically significant between the two groups. Yuh et al. examined the effect of 100 mg/kg chlorella consumption on streptozocin (STZ) induced diabetic mice and found hypoglycemic effect, enhanced and prolonged the hypoglycemic effects of exogenous insulin at a dose which does not produce hypoglycemia in STZ mice.[12] Cherng et al. in another study reported a decrease in Non-esterificated fatty acid (NEFA) level and enhanced glucose uptake after chlorella consumption in diabetic mice.[19] Results of a study on diabetic rats for the effect of *C. vulgaris* on serum glucose level did not support the hypoglycemic effects of *C. vulgaris*,[11] possibly due to short period of time. In a human clinical trial, consumption of chlorella supplement among high- risk human subjects for lifestyle-related diseases resulted in an improvement in glucose metabolism and noticeable reduction in serum glucose level.[17] Chlorella enhances glucose uptake,[12] decreases NEFA level[19] and subsequently, lowers blood glucose. However, studies testing the hypoglycemic effects of *C. vulgaris* are rare. Several mechanisms for hypoglycemic effects of *C. vulgaris* have been proposed, i.e. reduction in plasma NEFA concentration which improves glucose uptake, glucose utilization, enhances the suppression of hepatic glucose production[19] as well as activation of insulin signaling pathways results from changes in gene expression in the peripheral

blood cells[17] are the possible mechanisms. Chlorella consumption attenuated apoptosis and increased cell survival in pancreatic beta cells mediated by p53 down regulating.[32]

There were reductions in TC, TG and LDL-C concentrations not only in the *C. vulgaris* but also in placebo group with no difference between the groups. Shibata et al. tested three different chlorella containing diets (0%, 5%, and 10% w/w) on hyperlipidemic rats and found lower TG and TC levels in 5% and 10% groups *vs.* 0% group.[15] Consumption of chlorella powder resulted in a significant decrease in serum and liver cholesterol and TG in rats.[18] Finally, in a recent study by Panahi et al.[33] it was reported that, TC, LDL-c and TG are reduced in dyslipidemic subjects with supplementation of 600 mg/day *C. vulgaris*. The hypolipidemic effect of *C. vulgaris* appears to be largely attributable to its fiber content (i.e.13 g/100). Fiber causes high fecal steroid exertion as a consequence of the reduction in intestinal absorption Indeed, *C. vulgaris* consumption has reported to enhance the hepatic degradation of cholesterol by up-regulation the expression of Cholesterol 7α hydroxylase(the enzyme converts cholesterol to bile acids).[19] TG lowering effects of *C. vulgaris* is possibly related to the lowering effect in plasma NEFA level because plasma NEFA is stored as TG in the liver.[22] *C. vulgaris* supplement contains niacin (23.8 mg/100g *C. vulgaris* or 0.28 mg in 4 tablets) as a reputed medication for hyperlipidemia in each day as well as Omega-3 fatty acids which may be responsible for the hypotriglyceridemic effects of this microalgae.[33]

As we tested the metabolic effects of *C. vulgaris* on relatively low sample size, with low dose and during short follow-up period, further long-term clinical trials are suggested.

Conclusion

Overall, 1200 mg *C. vulgaris* supplementation compared with placebo could better decrease weight, serum glucose level and improve lipid profile as well as liver functionin NAFLD patients. Further studies in human subjects are needed to investigate the mechanism underlying insulin resistance, inflammation or oxidative stress in pathogenesis of NAFLD.

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Competing Interests

The authors declare that there is no conflict of interests.

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