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A randomized controlled trial of 6-week *Chlorella vulgaris* supplementation in patients with major depressive disorder

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ABSTRACT

Background: Major depressive disorder (MDD) is a widespread psychiatric disorder with incapacitating symptoms. Oxidative stress has been identified to play a role in the pathophysiology of MDD.
Objective: To evaluate the therapeutic effectiveness of a chemically defined and antioxidant-rich *Chlorella vulgaris* extract (CVE) as adjunct to standard treatment in patients suffering from MDD.
Methods: Subjects with MDD diagnosis according to DSM-IV criteria who were receiving standard antidepressant therapy were assigned to add-on therapy with CVE (1800 mg/day; $n = 42$), or continued standard antidepressant therapy alone ($n = 50$) for a period of 6 weeks. Changes in the frequency of depressive symptoms were assessed using the Hospital Anxiety and Depression Scale (HADS) and Beck Depression Inventory II (BDI-II) scale.
Results: There were significant reductions in total and subscale BDI-II and HADS scores in both CVE and control groups by the end of trial. The magnitude of reductions in total BDI-II score [−4.14 (−5.30 to −2.97)] as well as physical [−2.34 (−2.84 to −1.84)] and cognitive [−1.12 (−1.62 to −0.61)] subscales were significantly greater in the CVE versus control group, however, reduction of the affective symptoms was greater in the control compared with the CVE group [0.95 (0.18–0.72)]. Total HADS [−3.71 (−4.44 to −2.98)] as well as individual subscales of depression [−1.46 (−2.02 to −0.90)] and anxiety [−2.25 (−2.74 to −1.76)] were reduced to a greater degree in the CVE group. CVE was well tolerated and no serious adverse event was reported.
Conclusion: This pilot exploratory trial provides the first clinical evidence on the efficacy and safety of adjunctive therapy with CVE in improving physical and cognitive symptoms of depression as well as anxiety symptoms in patients who are receiving standard antidepressant therapy.

1. Introduction

Depression is a debilitating mental disorder with a severe impairment to quality of life, and has been predicted to be the second leading cause of global disability by 2020.[1] The prevalence of depression in the global population is 4.3%,[2] and around 8–12% of people experience at least one episode of depression during their life.[3] Although different types of antidepressant agents are available for the treatment of depression and related disorders, still a considerable proportion of patients are not treatment-responsive and require additional options to control their symptoms.[4] Moreover, common antidepressants, such as selective serotonin reuptake inhibitors (SSRIs), tricyclic antidepressants (TCAs), and monoamine oxidase inhibitors (MAOIs) have different side effects and drug–drug/drug–food interactions.[5] For these reasons, searching for alternative antidepressant agents with proper efficacy and safety is necessary.[6–8]

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Oxidative stress is an important pathophysiological mechanism for several psychological disorders including major depressive disorder (MDD). Brain tissue is particularly vulnerable to the damaging effects of free radicals owing to its high oxygen utilization, modest content of antioxidants, rich content of lipids, and presence of oxidation-driving metals and neurotoxic excitatory mediators e.g., glutamate.[9,10] Several lines of evidence have shown depleted levels of antioxidants in plasma and brain tissue of patients suffering from MDD.[11–13] Notably, evidence from animal and human studies implies that such redox imbalances in MDD are effectively reversed following antioxidant therapy.[14]

Chlorella vulgaris is a unicellular green microalgae with many pharmacological activities including antioxidant, anti-inflammatory, anti-hypertensive, detoxifying, anti-atherosclerotic, anti-hyperglycemic, and anti-microbial effects.[15–20] This algae has been used as a dietary supplement and alternative medicine in Far East countries for hundreds of years. It has antioxidant and anti-inflammatory capacities and contains several micro- and macro-nutrients, such as carbohydrates, proteins, nucleic acid, essential amino acids, fatty acid (omega 3 and 6), vitamins, dietary fiber, and growth factors, which have all been shown to reduce depressive symptoms through multiple mechanisms.[21,22] In particular, *C. vulgaris* is a rich and diverse source of several antioxidants which have been shown to be protective against depression individually. The antioxidant and anti-inflammatory capacities of has also been shown in previous studies.[16–18,21,22] In spite of numerous health benefits of *C. vulgaris* in experimental models, and the unique antioxidant content of this algae, there has been no randomized controlled trial investigating its efficacy in psychological disorders.

The present study aimed to investigate the impact of short-term supplementation with chemically-defined *C. vulgaris* extract (CVE) as adjunctive to standard antidepressant therapy in patients suffering from MDD.

2. Methods

2.1. Subjects

Included subjects were male and females aged 18–65 years for whom a diagnosis of MDD was made according to the DSM-IV criteria, and were receiving standard antidepressant medications. Exclusion criteria were presence of epilepsy, mental retardation, bipolar disorder, obsessive-compulsive disorder, uncontrolled thyroid disease, or hypersensitivity to algal preparations. Patients who received any psychological intervention or psychotherapy were also excluded from the study. Study participants were recruited from the psychiatry clinics of the Baqiyatallah and Rofeideh Hospitals, both in Tehran, Iran.

2.2. Design

This study was designed as a randomized open-label controlled trial conducted between October 2012 and August 2013. One hundred and twenty-five subjects fulfilled the inclusion criteria and were randomly assigned to receive CVE as adjunct to their standard antidepressant therapy (CVE group; $n = 60$) or continue their standard antidepressant therapy alone (control group; $n = 65$) for a period of 6 weeks. CVE was administered at a daily dose of 1800 mg/day for 6 weeks. CVE was administered in the form of 300 mg tablets. Subjects were asked to take two CVE capsules, three times a day, after each meal with sufficient water. The study was approved by the institutional Ethics Committee, and written informed consent was obtained from participants.

Table 1
Ingredients of *C. vulgaris* extract tablets and their respective quantity according to the manufacturer's* leaflet.

Ingredient	Quantity
Fat (g/100 g)	8.65
Protein (g/100 g)	52.0
Carbohydrates (g/100 g)	13.6
Ash (g/100 g)	6.56
Water (g/100 g)	3.63
Dietary fiber (g/100 g)	15.6
Energy (Kcal/100 g)	340
Fatty acids	
Saturated fatty acid (g/100 g)	2.16
Monounsaturated fatty acid (g/100 g)	1.69
Poly unsaturated fatty acid (g/100 g)	3.34
Trans fatty acid (g/100 g)	0.06
n-3 Fatty acids	
Linoleic acid (g/100 g)	1.282
α-Linolenic acid (g/100 g)	1.964
n-6 Fatty acids	
Octodecatetaenoic acid (g/100 g)	0.003
Eicosadienoic acid (g/100 g)	0.011
Arachidonc acid (g/100 g)	0.009
Docosatetraenoic acid (g/100 g)	0.020
Vitamins	
β-Carotene (mg/100 g)	180.8
Vitamin B1 (mg/100 g)	1.5
Vitamin B2 (mg/100 g)	4.8
Vitamin B3 (mg/100 g)	23.8
Vitamin B5 (mg/100 g)	1.3
Vitamin B6 (mg/100 g)	1.7
Vitamin B12 (μg/100 g)	125.9
Vitamin C (mg/100 g)	15.6
Folic acid (μg/100 g)	26.9
Biotin (μg/100 g)	191.6
Para-amino-benzoic acid (mg/100 g)	0.6
Minerals	
Phosphorus (mg/100 g)	959
Potassium (mg/kg)	21450
Magnesium (mg/kg)	4425
Calcium (mg/kg)	2710
Iron (mg/kg)	680
Copper (mg/kg)	19.0
Zinc (mg/kg)	54.5
Manganese (mg/kg)	39.5
Iodine (mg/kg)	12.9
Chromium (mg/kg)	0.575
Miscellaneous	
Lutein (mg/100 g)	84.3
Lycopin (mg/100 g)	0.307
Zeaxanthin (mg/100 g)	0.679
Chlorophyll (g/kg)	15.21

* Administered *C. vulgais* extract tablets were from Bioprodukte Prof. Steinberg (Produktions- und Vertriebs GmbH & Co., KG, Klötze, Germany).

Administered CVE tablets were from a commercial source (ALGOMED®; Bioprodukte Prof. Steinberg Produktions- und Vertriebs GmbH & Co., KG, Klötze, Germany). The tablets contained 98% *C. vulgaris* powder, 1% separating agent (silicic acid), and 1% plant-based magnesium stearate. The tablets were ~9 mm in diameter and ~300 mg in weight. Chemical composition of CVE is summarized in Table 1.

2.3. Efficacy measures

Efficacy measures in the present study were changes in the psychological status based on the Beck Depression Inventory II (BDI-II) and Hospital Anxiety and Depression Scale (HADS). The HADS is a self-administered rating tool that consists of two subscales addressing anxiety (HADS-A) and depression (HADS-D).[23] Each subscale of HADS consists of seven questions on a four-point

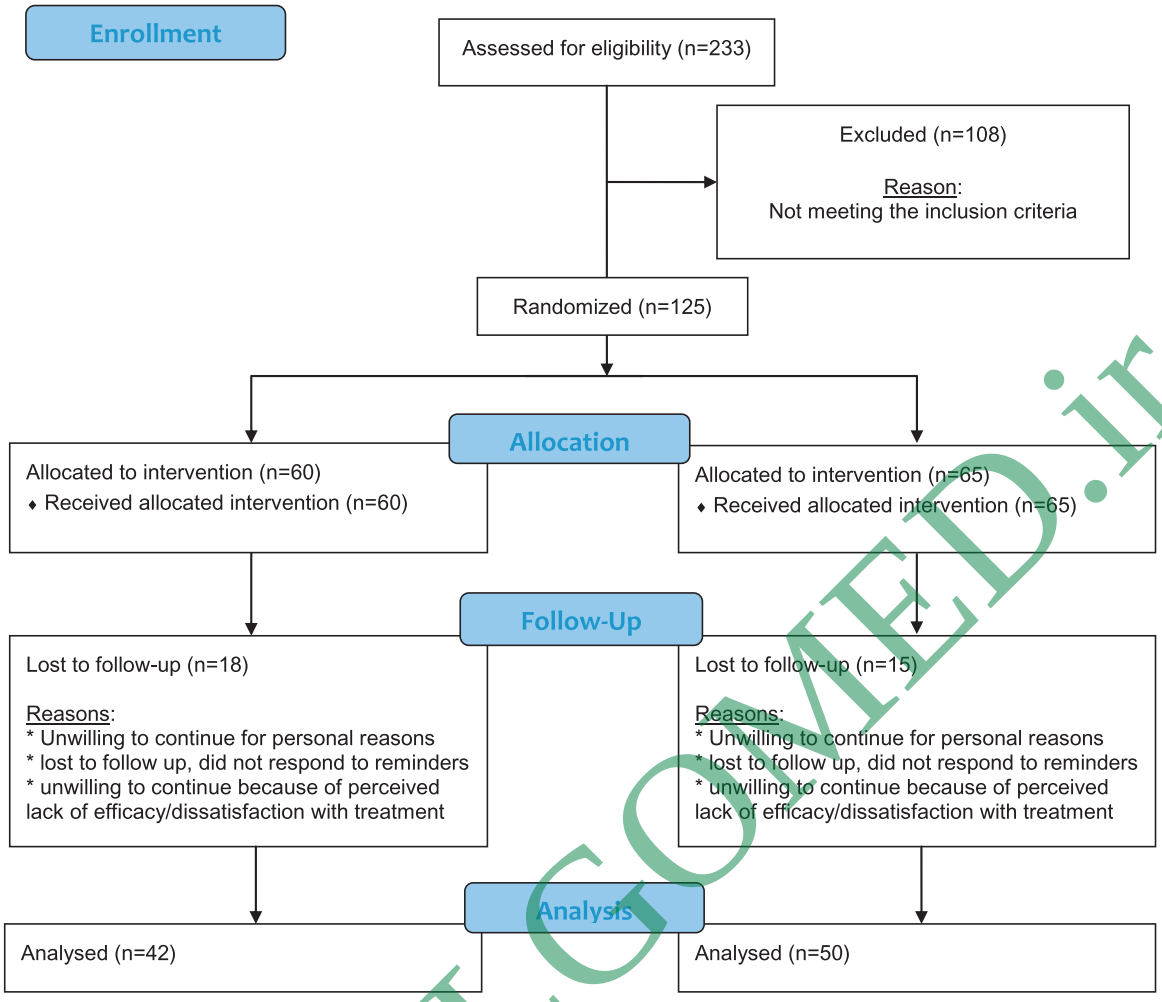

Fig. 1. Flowchart of the trial.

Likert scale, ranging from 0 (no distress) to 3 (maximum distress). Thus, the maximum score of each HADS subscale is 21. The reliability and validity study of the Iranian version of HADS, in accordance with the original study, has identified the optimum cutoff score of each HADS subscale to be 6.[24]

BDI-II is a widely used index for the assessment of depression severity. This self-reporting rating scale has three subscales addressing affective, physical (somatic) and cognitive symptoms. BDI-II evaluates 21 common symptoms of depression, each on a four-point Likert scale ranging from 0 (no distress) to 3 (maximum distress). The maximum BDI-II score is 63 which reflects the most severe symptoms.[25] The optimum cut-off score has been reported to be 15 based on the reliability and validity study of the Iranian version, in accordance with the original study.[24]

2.4. Statistical analysis

Statistical analyses were performed using the SPSS software version 11.5 (SPSS Inc., Chicago, Illinois, USA). Data were expressed as mean (95% confidence interval [CI]). Between–group comparison of changes in the evaluated parameters was considered statistically significant if the if the 95% CI of the mean difference between changes excluded the value zero. Categorical variables were compared using Chi-square test. Repeated measures analysis of covariance (ANCOVA) was used to adjust for the effect of potential confounders on the association between CVE supplementation and changes in psychological indices used in this study. A two-sided p-value of <0.05 was considered to be statistically significant. The power of analysis to detect statistically significant difference between CVE and control groups was computed using the PS software.[26]

3. Results

Ninety-two subjects completed the trial; 42 in the CVE and 50 in the control group. The number of subjects who were lost to follow-up in the CVE and control group was 18 and 15, respectively (Fig. 1). The number of drop-outs was not significantly different between the study groups ($p > 0.05$). CVE and control groups were comparable regarding age, gender, weight, and marital status. Likewise, the use of different classes of antidepressants including selective serotonin reuptake inhibitors (SSRIs), tricyclic antidepressants (TCAs), benzodiazepines (BZDs), serotonin norepinephrine reuptake inhibitors (SNRIs), and β-blockers was not different between the study groups (Table 2). All patients were on polytherapy with antidepressants. With respect to psychological indices, baseline total HADS as well as HADS-D scores were lower whilst physical BDI-II score was higher in the CVE versus control group. Other indices were comparable between the groups.

Between–group comparisons revealed significantly greater reductions in total BDI-II score [−4.14 (−5.30 to −2.97)] as well as physical [−2.34 (−2.84 to −1.84)] and cognitive [−1.12 (−1.62 to −0.61)] subscale scores in the CVE versus control group. However, there was a greater reduction in affective subscale score in the con-

Table 2
Demographic and baseline characteristics of the study groups.

	CVE	Control
n	42	50
Age (y)	40.79 ± 10.87	40.40 ± 9.56
Weight (kg)	77.50 ± 10.24	74.26 ± 10.76
Female	57.1%	58.0%
Married	73.8%	70%
TCAs	42.9%	46.0%
BZDs	78.6%	70.0%
SSRIs	66.7%	70.0%
SNRIs	57.1%	42.0%
β-Blockers	45.2%	54.0%

CVE: *Chlorella vulgaris* extract; TCA: tricyclic antidepressants; BZD: benzodiazepines; SSRI: selective serotonin reuptake inhibitors; SNRI: serotonin-norepinephrine reuptake inhibitors.

trol versus CVE group [0.95 (0.18–1.72)] (Table 3). With respect to HADS, reductions in total score [−3.71 (−4.44 to −2.98)] as well as subscale scores of anxiety (HADS-A) [−2.25 (−2.74 to −1.76)] and depression (HADS-D) [−1.46 (−2.02 to −0.90)] were significantly greater in the CVE versus control group (Table 3). Power analysis indicated a sufficient power of >90% for all assessed indices, except BDI-II affective subscale for which the power was found to be 61.5%.

Baseline differences in total HADS, HADS-D, and physical BDI-II scores between the study groups were adjusted by univariate ANCOVA. All three mentioned indices remained statistically significant after adjustment for respective baseline values.

When the analysis was restricted to the subgroup of patients taking fluoxetine a similar pattern of changes were found, i.e., greater reductions in BDI-II total and physical and cognitive subscale scores, as well as HADS total and subscale scores in the CVE versus control group. The same as total population, there was a weaker effect of CVE versus control on BDI-II affective score in fluoxetine users.

CVE was safe and well-tolerated during the course of trial. Reported adverse events were one case of nausea and one case of diarrhea, none of them causing withdrawal from the trial. Weight change in the CVE group during the study was greater from that in the control group [control-adjusted change: −1.51 kg, 95% CI(−2.44 to −0.58)].

4. Discussion

The present pilot controlled trial set out to determine the therapeutic efficacy of adjunctive therapy with CVE, a natural product rich in antioxidants useful for the treatment of depression, in patients with MDD. The results showed a promising impact of CVE in the alleviation of somatic and cognitive symptoms of depression, and anxiety, as reflected by a significant reduction in BDI-II and HADS scores. However, CVE was not found to have a beneficial effect on the BDI-II affective symptoms subscale, which can be attributed to the insufficient statistical power. This calls for additional larger scale studies with longer term durations of follow-up to enable a more precise assessment of the impact of CVE on affective symptoms of depression.

Reduction of oxidative stress by CVE could be regarded as a plausible mechanism contributing to the observed antidepressant properties. Depression is accompanied by a depletion in total antioxidant status[11] and deregulated activity of antioxidant enzymes, such as glutathione peroxidase[27] and superoxide dismutase.[28] Such deficiencies can impair protection against reactive oxygen species (ROS), causing damage to essential cellular components e.g., membrane lipids, proteins and DNA.[27] CVE contains a complex mixture of antioxidants, such as chlorophyll, α-carotene, β-carotene, ascorbic acid, α-tocopherol, lutein, lycopene, zeaxanthin, and also some trace elements including zinc, copper, and magnesium that are required for the action of antioxidant metalloenzymes. The antioxidant activities of *Chlorella* have been previously shown in both in vitro and different experimental models.[19,29–33] There has been also two clinical reports showing increased serum concentrations of antioxidants following short-term supplementation with CVE.[16–18]

Neuroanatomical and neurochemical investigations have revealed that MDD is associated with impaired neurogenesis, neuronal plasticity and neurodegeneration. Depression is also associated with a decrease in proliferation of neural stem cells.[34] *C. vulgaris* contains sporopollein, which has been shown to be as effective as cholestyramine in binding neurotoxins. Moreover, alpha- and gamma-linoleic acid present in CVE have several neuroprotective functions, including enhancement of peroxisomes formation.[35] Another neuroprotective component of CVE is methyl cobalamine which is the most absorbable form of vitamin B12.[35] In a previous investigation, addition of vitamin B12 to antidepressant regimen of patients with low normal B12 levels was shown to significantly improve depressive symptoms.[36]

Malfunctioning of the mitochondrial electron transport chain is implicated in the pathogenesis of a range of neuropsychiatric disorders such as depression, bipolar disorders and schizophrenia. A lower ATP production has been reported in biopsied muscles from depressed individuals with somatic complaints. Moreover, a higher prevalence of depression has been demonstrated in patients with mitochondrial disorders.[37] CVE contains several components that aid energy production and promote the biogenesis of mitochondria.[38,39] Acceleration of energy production by CVE can enhance physical functioning,[35] as also shown by improvement of physical BDI-II scores in the present study. CVE has also anti-obesity and laxative properties[40] and could potentially prevent weight gain and constipation as common complaints of patients with MDD.[34]

To the authors' knowledge, this is the first study to assess the impact of CVE as a known marketed nutritional supplement in patients with MDD. The present results showed positive effects of short-term CVE supplementation on anxiety as well as somatic

Table 3
Comparison of magnitude of changes in the evaluated efficacy measures between the study groups.

	CVE			Control			
	Baseline	End-trial	Change	Baseline	End-trial	Change	Between–group difference
BDI-II	40.40 ± 8.04	33.43 + 7.76	−6.98 (−7.97 to −5.98)	40.44 + 9.45	37.60 + 9.07	−2.84 (−3.46 to −2.22)	−4.14 (−5.30 to −2.97)
HADS	36.14 + 5.60	29.71 + 5.46	−6.43 (−7.01 to −5.85)	38.82 + 6.41	36.10 + 6.71	−2.72 (−3.18 to −2.26)	−3.71 (−4.44 to −2.98)
BECK-affective	13.24 + 3.07	10.92 + 2.83	−2.31 (−2.70 to −1.92)	13.80 + 2.64	10.54 + 2.77	−3.26 (−3.93 to −2.59)	0.95 (0.18–1.72)
BECK-somatic	12.69 + 2.83	9.69 + 2.94	−3.00 (−3.36 to −2.64)	10.54 + 2.77	9.88 + 2.72	−0.66 (−1.01 to −0.31)	−2.34 (−2.84 to −1.84)
BECK-cognitive	14.48 + 3.31	12.88 + 3.32	−1.60 (−1.98 to −1.21)	16.10 + 5.18	15.62 + 5.03	−0.48 (−0.82 to −0.14)	−1.12 (−1.62 to −0.61)
HADS-anxiety	18.62 + 2.85	15.19 + 2.80	−3.43 (−3.81 to −3.05)	18.56 + 3.27	17.38 + 3.28	−1.18 (−1.50 to −0.86)	−2.25 (−2.74 to −1.76)
HADS-depression	17.52 + 3.06	14.52 + 2.97	−3.00 (−3.37 to −2.63)	20.26 + 3.64	18.72 + 3.81	−1.54 (−1.95 to −1.13)	−1.46 (−2.02 to −0.90)

Values are shown as mean ± SD (for baseline and end-trial values) and mean (95% confidence interval) (for change values). If the 95% Confidence Interval of the mean difference between changes excludes the value zero, the difference is significant. CVE: *Chlorella vulgaris* extract; BECK: Beck Depression Inventory II; HADS: Hospital Anxiety and Depression Scale.

and cognitive symptoms of depression, with no serious adverse event during the course of trial. Despite these positive findings, the present study was limited by the short duration of follow-up, and its exploratory pilot nature. Since MDD is a chronic disorder, it would be ideal to assess the impact of treatment on long-term complications and comorbidities of MDD as well as the quality of life of patients. In addition, participants of the present trial represented a heterogeneous population with respect to the consumption of anti-depressive medications. Hence, future studies are encouraged to assess the differential effects of CVE in combination with each specific class of antidepressants. Lack of blinding was another limitation of the present open-label trial that necessitates future double-blind confirmatory trials using CVE placebo tablets matched in color, taste and aroma.

5. Conclusion

Findings of the present exploratory pilot trial, being the first of its kind, showed that addition of CVE to the routine antidepressant treatment of MDD is associated with an improved control of somatic and cognitive symptoms of depression, and anxiety, but not affective symptoms of depression. Whilst these findings represent the first clinical evidence on the efficacy of CVE in treating MDD, future double-blind studies are required to validate the present results with larger populations and longer durations follow-up. The benefit of adjunctive therapy with CVE in other psychological disorders such as bipolar disorder, post-traumatic stress disorder and schizophrenia also merits further investigation. Finally, future studies are recommended to assess the changes in plasma antioxidant status and correlate these changes with improvements in the symptoms of depression and anxiety.

Conflict of interest

The authors have no competing interest to declare.

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