ORIGINAL ARTICLE

Nigella sativa extract in the treatment of depression and serum Brain-Derived Neurotrophic Factor (BDNF) levels

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Background: Here, we aimed to investigate the therapeutic effects of *Nigella sativa* extract on serum brain-derived neurotrophic factor (BDNF) and depression score in patients with depression. **Materials and Methods:** This clinical trial was performed in 2021 in the hospitals of military forces in Tehran on 52 male patients with major depressive disorder treated with sertraline. We used the Depression, Anxiety, and Stress Scale-21 Items (DASS-21) questionnaire to assess the patients. Serum BDNF levels were measured by the enzyme-linked immunosorbent assay. Patients were then divided into two groups receiving 1000 mg *N. sativa* oil extract, daily, and placebo. Both groups received sertraline for at least 3 months. DASS-21 questionnaire and serum BDNF levels were measured after 10 weeks. **Results:** After treatments, we observed significantly decreased DASS-21 score (−11.24 ± 5.69) in the intervention group ($P < 0.001$) and placebo (−2.72 ± 6.19, $P = 0.032$), but patients in the intervention group had significantly lower scores (50.1 ± 6.8 vs. 58.2 ± 5.6, respectively, $P < 0.001$). Furthermore, patients in the intervention group had significantly decreased depression score (−5.5 ± 2.47, $P < 0.001$) and lower scores compared to the placebo ($P < 0.001$) (18.6 ± 2.7 vs. 23.4 ± 2.1 in intervention and placebo, respectively). We also observed significantly increased BDNF levels in the intervention group after the treatments (6.08 ± 3.76, $P < 0.001$) compared to the placebo group (29.4 ± 3.6 vs. 24.9 ± 2.1, $P < 0.001$). Serum BDNF levels had also significant reverse correlations with DASS-21 score ($r = -0.35$, $P = 0.011$) and depression score ($r = -0.45$, $P = 0.001$). **Conclusion:** The use of *N. sativa* resulted in decreased depression score and increase in serum BDNF levels that indicate the importance and efficacy of this drug.

Key words: Brain-derived neurotrophic factor, depressive disorder, *Nigella sativa*

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INTRODUCTION

Depression is one of the most common types of psychiatric disorders. Patients with depression need treatment by psychiatrists, psychologists, and mental health professionals.[1] Depression is a complex disease and is common in developing and developed societies, especially in the military personnel.[2] An estimated 17.3 million adults in the United States had at least one major depressive episode. This number represented 7.1% of all US. adults.[3] Epidemiologic studies in Iran indicated that depression is a public issue and constitutes 35%–45% of mental illnesses in Iran and generally covers about 8%–20% of the population.[4] The prevalence of depression among military personnel has been assessed between 20% and 70% based on different situations,[5] and based on

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a recent meta-analysis, the prevalence of depression was 50% among Iranian soldiers.[6]

N. sativa has antioxidant properties and research has shown that *N. sativa* inhibits the effects of ischemia caused by oxidative tension and protects erythrocytes from lipid peroxidation and increased osmotic properties by the means of H_2O_2.[7] The effects of this extract on CNS diseases such as epilepsy and psychiatric disorders such as bipolar disorder have been studied.[8] It has been reported that *N. sativa* could have significant influences on mood disorders.[9,10]

Brain-derived neurotrophic factor (BDNF) is a protein encoded by BDNF gene and belongs to the family of neurotrophins, which causes the expansion of the neural network. BDNF is one of the most important factors in the brain that promotes the growth and development of the CNS and PNS and is most active in the hippocampus and cortical part of the brain.[11] It has been indicated that patients with different psychiatric diseases including depression have lower serum levels of BDNF and therapeutic strategies could increase the levels.[12]

Regarding the importance of depression and its prevalence and the possible roles of *N. sativa* in the treatment of depression, in the present study, we aimed to investigate the roles of *N. sativa* on the severity of depression and also compare the serum levels of BDNF as possible indicator and justifying factor for changes in patient's moods.

MATERIALS AND METHODS

This is a double-blinded clinical trial that was performed in 2021 in the hospitals of military forces in Tehran affiliated to AJA University of Medical Science. The current study was conducted on patients with depression that referred to the psychiatry clinic of our medical center as outpatient. The study protocol was approved by Research Committee of AJA University of Medical Sciences and the Ethics committee has confirmed it (Ethics code: IR.AJAUMS.REC.1399.220, Iranian Registry of Clinical Trials (IRCT) code: IRCT20200217046523N17).

The inclusion criteria were age over 18 years, diagnosis of major depressive disorder by expert psychiatrists based on Diagnostic and Statistical Manual of Mental Disorders-5th Edition, being controlled with sertraline for at least 3 months, having a depression score between 14 and 28 based on Depression, Anxiety, and Stress Scale-21 Items (DASS-21), and signing the written informed consent to participate in this study. Patients with other systemic diseases (including cardiovascular disease, renal, hepatic, genetic, or autoimmune disorders) or those that receive anti-depressants other than sertraline did not enter the study. The exclusion criteria were intolerance to the drug, improper use of the drugs, sensitivity to *N. sativa*, worsening the patient's condition, requiring other anti-depressants, lack of proper follow-up, and patient's will to exit the study.

The blinding method was so that none of the patients and the clinical assessor and data collectors had knowledge about the types of drugs and grouping of the patients. We administered the drugs using different codes to the patients. The physicians and the patients and data analyst were blinded, and data were decoded after the data analysis.

Based on the formula for estimating the sample size and 90% reliability equal to 1.96 and 0.84 for the test power, and the effects size of 0.62 S, we considered 54 patients as the study population. Patients were recruited based on the mentioned criteria. At the beginning of the study, the demographic data of patients including age, gender, educational level, and duration of the disease were collected by a checklist.

For all patients, the DASS-21 questionnaire was completed assessing the depression, anxiety, and stress of cases. This questionnaire has 21 questions that have general, low, medium, and high scales and the final score of the questionnaire is based on the total score of three domains from 21 to 84. The three domains include depression, anxiety, and stress each having seven questions. Higher scores on this scale indicate higher anxiety, depression, and stress. In each domain, the scores more between 7 and 14, 14 and 21 and 21 and 28 were considered as mild, moderate, and severe disorder, respectively. The validity and reliability of this questionnaire in Iranian population have been examined by Maroufizadeh *et al*.[13] The validity of the retest for the DASS was 0.81, 0.89, and 0.78, respectively, using Cronbach's alpha, and the validity of this scale has been proven.

Those patients with depression score between 14 and 28 entered the study. The range of 14–28 is determined as moderate to severe depression. For the next step, we collected 5 ml blood from all patients using venipuncture. We should note that no special preparations were required and all of the samplings were performed between 10 and 12 am. Blood samples were poured into separate jars and clotted after 30 min at the room temperature. They were then centrifuged at 15,000 rpm for 15 min. The patients' serum was immediately removed and stored at a temperature of −20°. Prior to the measurements, the serums were brought to the room temperature and gently liquefied. Serum BDNF levels were measured by laboratory methods and by Bio-Rad 680 analyzer for BDNF by the enzyme-linked immunosorbent assay in all patients.

The patients were then divided into two groups using Random Allocation software each containing 27 patients. The first group received capsules containing 1000 mg *N. sativa* oil extract generated under provision of the pharmaceutical and Iranian medicine professors of AJA University of Medical Sciences according to the protocol used in the study by Khoddami *et al*.[14] The second group received placebo capsules containing starch with the exact appearance similar to the intervention group. The capsules were administered daily for 10 weeks. One capsule was taken each day and the patients were recommended to take them in the afternoon to increase the absorption. The patients were visited once during the interventions and possible side effects were evacuated.

After 10 weeks form the beginning of the study, the patients were visited and DASS-21 questionnaire and serum BDNF levels were measured again. Data were collected and compared to the beginning of the study. We also visited the patients 2 weeks after the study termination for evaluating any possible complications.

The obtained data were entered into the Statistical Package for the Social Sciences (IBM® SPSS® Statistics, Chicago, IL, USA) software version 24. Quantitative data were reported as mean ± standard deviation and qualitative data as frequency distribution (percentage). Independent *t*-test and Chi-square test were used to analyze the data. $P < 0.05$ was considered statistical significance threshold.

RESULTS

A total number of 54 patients were recruited based on the criteria. We should note that before the study initiation, 4 patients did not enter due to low depression scores (lower than 14). During the study, 2 patients (one in each group) were excluded due to improper follow-up. At the end, data of 52 patients were analyzed. The study population was consisted of 52 men, with a mean age of 23.96 ± 2.8 years. Analysis of educational level among patients indicated that 23 cases (44.2%) had bachelor, 10 patients (19.2%) had master's degree, and 19 patients (36.6%) had diploma. There were no significant differences between two groups of intervention and placebo regarding age and educational levels ($P = 0.689$ and $P = 0.654$, respectively). The demographic data of patients are summarized in Table 1.

We also observed no significant differences between two groups regarding duration of disease (2 ± 1 years for both groups, $P = 0.707$).

Evaluations of DASS-21 score indicated no significant differences between two groups at the beginning of the study regarding total DASS-21 score and the domains especially depression ($P = 0.748$ for DASS-21 score and $P = 0.603$ for depression). After treatments, we observed significantly decreased DASS-21 score (-11.24 ± 5.69) in the intervention group ($P < 0.001$) and placebo (−2.72 ± 6.19, $P = 0.032$), but patients in the intervention group had significantly lower scores (50.1 ± 6.8 vs. 58.2 ± 5.6, respectively, $P < 0.001$). We assume that the mild reduction in the DASS-21 score in the control group could be due to the placebo effects. Furthermore, patients in the intervention group had significantly decreased depression score (−5.5 ± 2.47, $P < 0.001$) and lower scores compared to the placebo ($P < 0.001$). No significant differences could be observed between two groups regarding the anxiety and stress scores ($P > 0.05$). It was also indicated that patients in the intervention group had significantly reduced anxiety ($P = 0.031$) and stress ($P = 0.011$) scores [Table 2].

We also observed significantly increased BDNF levels in the intervention group after the treatments (6.08 ± 3.76, $P < 0.001$) and the values were significantly higher compared

Table 1: Demographic data of cases

	Intervention (*n*=26)	Placebo (*n*=26)	Total	*P**
Age (years) (mean±SD)	24.2±3.6	24.5±3.1	23.96±2.8	0.689
Education, *n* (%)				
Diploma	9 (36.1)	10 (38.4)	19 (36.6)	0.654
Bachelor	13 (50)	10 (38.4)	23 (44.2)	
Master's degree	4 (13.9)	6 (23.2)	10 (19.2)	

*Using *t*-test. SD=Standard deviation

Table 2: Comparison of Depression, Anxiety, and Stress Scale-21 score and the domains among patients

Variable*	Intervention (*n*=26)	Placebo (*n*=26)	*P*[a]
DASS			
Before treatment	61.2±7.1	61.1±6.4	0.748
After treatment	50.1±6.8	58.2±5.6	<0.001
Difference	−11.24±5.69	−2.72±6.19	<0.001
P[b]	<0.001	0.032	
Depression			
Before treatment	23.1±2.1	23.4±2.5	0.603
After treatment	18.6±2.7	23.4±2.1	<0.001
Difference	−5.5±2.47	−0.24±2.17	<0.001
P[b]	<0.001	0.947	
Anxiety			
Before treatment	23.3±2	23.6±2	0.814
After treatment	21.7±3	21.7±3	0.545
Difference	−1.96±4.11	−1.32±4.66	0.572
P[b]	0.031	0.204	
Stress			
Before treatment	16.2±7.7	15.3±7.4	0.810
After treatment	6.4±6.6	14.5±7.5	0.207
Difference	−4.04±7.29	−1.16±7.22	0.167
P[b]	0.011	0.430	

*Data are represented by mean±SD; [a]*P*: Between group analysis; [b]*P*: Within group analysis. SD=Standard deviation; DASS=Depression, Anxiety, and Stress Scale

Table 3: Comparison of serum BDNF levels in patients

BDNF*	Intervention (*n*=26)	Placebo (*n*=26)	*P*[a]
Before treatment	23.4±3.1	23.1±3.7	0.754
After treatment	29.4±3.6	24.9±2.1	<0.001
Difference	6.08±3.76	1.1±2.86	<0.001
P[b]	<0.001	0.153	

*Data is represented by mean±SD; [a]*P*: Between group analysis; [b]*P*: Within group analysis. SD=Standard deviation; BDNF=Brain-derived neurotrophic factor

to the placebo group (29.4 ± 3.6 ng/ml vs. 24.9 ± 2.1 ng/ml, $P < 0.001$). These data are shown in Table 3.

Based on Pearson correlation, we observed a significant reverse correlation between duration of disease and DASS-21 score ($r = -0.30$, $P = 0.032$). Serum BDNF levels had also significant reverse correlations with DASS-21 score ($r = -0.35$, $P = 0.011$) and depression score ($r = -0.45$, $P = 0.001$). No further correlations were observed between the variables. We should mention that no complication was reported during the study and also within 2 weeks after the end of the study.

DISCUSSION

Based on our data, the usage of *N. sativa* extract was associated with significant decrease in the depression score and also increase in the serum BDNF levels. These data show the clinical potentials of *N. sativa* extract. Developing supportive therapeutic strategies in patients with psychiatric issues have high clinical importance. In the present study, we assessed the use of *N. sativa* extract in patients with depression and indicated significant developments in patients. Furthermore, we evaluated the amounts of serum BDNF to confirm the changes in the psychiatric condition of patients. Based on these data, we observed significant reverse correlations between serum BDNF levels and DASS-21 score ($r = -0.35$) and depression score ($r = -0.45$). We must note that to the best of our knowledge, such correlations between BDNF levels and psychiatric conditions have not been reported previously.

Our data also indicated significantly decreased DASS-21 score in patients that received placebo which could be justified by the use of sertraline as the main treatment for depression in patients. We also indicated significant correlations between the duration of disease and DASS-21 score and also between serum BDNF levels and DASS and depression scores.

Previous studies on the possible effect of BDNF in depression have shown that BDND is one of the most important brain factors and by binding to specific tyrosine kinase receptors, it triggers intracellular cascades and eventually neuronal differentiation. The normal ranges of BDNF have been reported 18–26 ng/ml and also up to 20 ng/ml in some studies[15] and higher levels of serum BDNF could be observed in athletes.[16] As we selected our study population among male adults attending military service, this could justify a higher mean BDNF levels compared to some previous studies.[17] Various studies have been performed on the relationship between BDNF levels in the brain and neurological diseases. These studies have shown that lowering BDNF levels increases the risk of Alzheimer's disease, stress, anxiety and depression. Therefore, many efforts have been made to use BDNF as a prognostic factor as well as treatment in these diseases.[18] A 2017 study by Phillips found that BDNF levels are decreased in depression, and treatments that increased this factor could also help improve depression. In this study, it was shown that increasing physical activity in depressed patients helps to improve their condition by increasing BDNF levels.[12] Another study conducted in 2020 by Yang *et al*. showed that increased levels of BDNF could improve the effects of antidepressant drugs by increasing the expression of receptors in the brain, but more studies are needed in this regard.[19] These studies indicate the importance and reliability of BDNF for depression. The results of our study supported this theory as we found significantly increased levels of BDNF associated with improvements in depression scores in patients that were treated with *N. sativa*.

In the present study, we indicated that the use of *N. sativa* could improve the DASS-21 score and especially depression score in patients. An important point is that we used *N. sativa* as supplementary treatment for depression and this drug was used beside sertraline. It should also be noted that the use of *N. sativa* was not associated with major reduction in the depression scores. These improvements were also associated with increased BDNF levels. There have also been some studies on the use of *N. sativa* on mood disorders and psychological problems. Based on the studies, *N. sativa* has strong antioxidant properties that have been evaluated in various diseases. Recent studies have shown that this extract can be effective in mental illness and CNS disorders,[20,21] but the exact mechanism of these effects as well as the extent of these effects in depression is still under investigation. In 2021, Rahmani *et al*. showed that *N. sativa* oil supplementation had significant improving effects on kidney function tests, glycemic parameters, oxidative stress, inflammation, quality of life, and depression status in diabetic hemodialysis patients. Furthermore, in 2013, Bin Sayeed *et al*. studied the effectiveness of *N. sativa* extract on 48 patients in terms of mood control and anxiety. In this study, it was reported that this drug acts effectively in patients with mood disorders, and in addition, no side effects were reported in this study.[10] They also reported that more studies were needed, especially on depression. Another study was performed in 2020 by Ahirwar *et al*. The

results of our study were in line with these previous reports indicating the effectiveness of *N. sativa* on depression.

In another study by Bin Sayeed *et al*. in 2013, *N. sativa* extract was used in 40 human subjects to determine its effect on attention, cognition, and memory. This study also showed positive effect of *N. sativa* on these factors.[22] As indicated, human studies on *N. sativa* have shown the effectiveness of this drug on mood disorders and depression and these studies have also investigated the side effects of this drug. Almost all studies that have examined the side effects of black seed at regular doses have found no side effects.[23-25] Our results were also consistent with these reports. We also had no reported complications during 10 weeks of study and even within 2 weeks of follow-ups.

Investigation of the mechanism of *N. sativa* has shown that this drug increases 5-HT metabolism in the brain and therefore with the help of antioxidant mechanisms can be effective in improving depression in rats.[26] Among the human studies of this drug, we can mention the study of Akhondian *et al*., which showed that *N. sativa* prevents resistant epilepsy in children, and the properties of this drug can be used in neurological diseases.[27] Khazdair stated that the use of *N. sativa* prevents neurotoxicity induced by other drugs.[28] The effects of this drug on improving memory and slowing down the process of Alzheimer's disease have also been studied, which shows the effectiveness of this drug.[29] These researches show the efficacy of *N. sativa* on various psychological and neurological disorders.

An important point of the current study was that we used serum levels of BDNF as an indicator for improvements in depression score. Our data prove the effectiveness of *Nigella sativa* as a supplementary drug in the treatments of depression that could also alter the levels of BDNF. We believe that these results have high clinical importance and psychiatrists should pay more attention to the possible roles of *N. sativa* in depression. The limitation of our study was that we evaluated these effects on restricted population of male adults because this study was performed in a military hospital. We recommend that further studies should be conducted on both genders and larger populations.

CONCLUSION

The use of *N. sativa* as a supplementary treatment resulted in decreased depression score and increase in serum BDNF levels that indicate the importance and efficacy of this drug.

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Conflicts of interest

There are no conflicts of interest.

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