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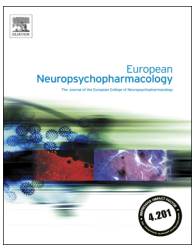

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Eicosapentaenoic acid versus docosahexaenoic acid in mild-to-moderate depression: A randomized, double-blind, placebo-controlled trial

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Abstract
Controversy exists as to whether eicosapentaenoic acid (EPA) or docosahexaenoic acid (DHA) or both are responsible for the efficacy of n-3 polyunsaturated fatty acids in depression. We conducted a single-center, randomized, double-blind, placebo-controlled, multi-arm, parallel-group trial, comparing the efficacy of EPA versus DHA as adjuvants to maintenance medication treatments for mild-to-moderate depression. Eighty-one mild-to-moderately depressed out-patients were randomly assigned to receive either 1 g/d of EPA or DHA or placebo (coconut oil) for 12 weeks. The primary outcome measure was the 17-item Hamilton Depression Rating Scale (HDRS) final score in the modified intention-to-treat population, which comprised of all randomized patients with at least 1 post-randomization observation (n=62; 61.3% female; mean age 35.1±1.2 years). Allocated treatments were well tolerated. Although there was no significant difference between groups at baseline, patients in the EPA group showed a significantly lower mean HDRS score at study endpoint compared with those in the DHA ($p<0.001$) or placebo ($p=0.002$) groups. Furthermore, response to treatment (defined as a ≥50% decrease from the baseline HDRS score) was only observed in 6 patients receiving EPA, while no one in any of DHA or placebo groups responded to treatment. Overall, these data suggest greater efficacy of EPA compared to DHA or placebo as an adjunctive treatment in mild-to-moderate depression. However, further, randomized controlled trials are needed to support these findings.

1. Introduction

Depression is a serious public health concern with a lifetime prevalence of about 16% (Kessler et al., 2003), and is associated with substantial morbidity, mortality, and health

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care costs (Andrade et al., 2003). In Iran, the prevalence of depression is also considerable and it is more common among females than males (Sadeghirad et al., 2010). Although, at the individual level, disability from subclinical or mild-to-moderate depression is lower than for major depressive disorder (MDD), it is of major public health significance due to its greater prevalence (Judd et al., 2002) and associated increased risk of mortality (Cuijpers and Smit, 2002), coronary heart disease (Rugulies, 2002), and developing subsequent MDD (Cuijpers and Smit, 2004). Generally, existing treatments for depression have limited efficacy (Bech et al., 2000) and despite the development of new antidepressant medications with improved side-effect profiles, approximately, 60% of patients treated with antidepressants do not achieve remission (Fagiolini and Kupfer, 2003). Therefore, novel approaches to the management of depression need to be found.

The n-3 polyunsaturated fatty acids (n-3 PUFAs) found in fish and marine derivates, particularly eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), have been suggested to provide an alternative to antidepressant medications with fewer side effects. Lower dietary intakes of fish or n-3 PUFAs have been significantly correlated with world-wide prevalence of depression (Hibbeln, 1998; Hibbeln and Salem, 1995). Moreover, there is increasing evidence from epidemiological and clinical studies that EPA and/or DHA have beneficial effects in those suffering from mood disorders, especially depression (Sontrop and Campbell, 2006).

Thus far, a large number of studies have examined the antidepressant efficacy of n-3 PUFAs (mostly as an adjunctive treatment) in patients with unipolar MDD, resulting in positive (da Silva et al., 2008; Jazayeri et al., 2008; Mischoulon et al., 2008; Nemets et al., 2002; Peet and Horrobin, 2002; Rondanelli et al., 2010; Su et al., 2003) and negative (Bot et al., 2010; Carney et al., 2009; Chiu et al., 2008; Grenyer et al., 2007; Lucas et al., 2009; Marangell et al., 2003; Mischoulon et al., 2009; Silvers et al., 2005) findings. However, the potential antidepressant effects of n-3 PUFAs on mild-to-moderately depressed patients are not yet well studied and findings from the rare previous trials conducted on these patients are contradicting, yielding in positive (Frangou et al., 2006; Tajalizadekhoob et al., 2011) and negative (Rogers et al., 2008) results. These mixed findings may be due to methodological differences including the use of different combinations (i.e. EPA, DHA, or EPA+DHA) or doses (ranging from 0.2 to 9.6 g/d) of n-3 PUFAs. Furthermore, to our knowledge, no previous study has compared the efficacy of EPA versus DHA for the treatment of depression and controversy exists as to whether EPA or DHA or both are responsible for the observed beneficial effects of n-3 PUFAs in depressed patients. Therefore, we conducted a randomized, double-blind, placebo-controlled trial to compare the efficacy of EPA versus DHA as adjuvants to maintenance antidepressant medications for the treatment of mild-to-moderate depression.

2. Experimental procedures

2.1. Participants

Participants were recruited from outpatients who were referred to Bahman neuropsychiatry clinic in Yazd, Iran. Eligible participants required to have following conditions: a diagnose of mild-to-moderate depression, verified with the structured clinical interview according to Diagnostic and Statistical Manual of Mental Disorders, 4th Edition (DSM-IV) (American Psychiatric Association (APA), 1994); ages between 18 and 75 years; a Beck Depression Inventory (BDI) score between 10 and 28; a 17-item Hamilton Depression Rating Scale (HDRS) score between 8 and 18; and ability to understand the study and provide written informed consent. Exclusion criteria included: any change in type or dose of antidepressant medications during the study period or within the 4 weeks before enrollment; taking fish oil or n-3 PUFA supplements in the preceding 6 months; consuming more than 3 servings of fish per week; any diagnose of mental disorders according to DSM-IV other than mild-to-moderate depression; significant risk of suicide or homicide; current use of anticoagulants, mood stabilizers or anticonvulsants; history of cupping (a traditional Chinese medicine therapy) in the preceding 3 months; history of multiple adverse drug reactions or allergy to marine foods or the study drugs; history of electroconvulsive therapy; severe or uncontrolled medical illness including cardiovascular, hepatic, renal, respiratory, endocrine, neurologic, hematologic or gastrointestinal disorders; alcohol or substance dependence as defined by DSM-IV criteria; smoking; pregnancy, breastfeeding, and in case of fertile women, not using acceptable methods of contraception; development of serious adverse events during the study period; and lack of adherence to the study protocol.

The study protocol was approved by the ethics board of the Shahid Sadoughi University of Medical Sciences, Yazd, Iran. After being informed of the purpose, risks and benefits of the study, all participants provided written informed consents at baseline. All eligible subjects were living in the city of Yazd, Iran, and were recruited from September 23, 2010 to December 21, 2010. The last participant completed his final visit in March 15, 2011.

2.2. Study design

This study was a single-center, randomized, double-blind, placebo-controlled, multi-arm, parallel-group trial. A total of 81 mild-to-moderately depressed outpatients were assigned in a 1:1:1 fashion to 3 groups, using simple randomization (Beller et al., 2002), to receive either 2 oral soft gelatin capsules per day (1 g/d) of EPA or DHA or placebo (pure coconut oil) as adjuvants to maintenance antidepressant medications for 12 weeks. The allocation sequence was generated by an independent statistician at the Shahid Sadoughi University of Medical Sciences and was given to an independent clinician who dispensed allocated treatments. All participants were assessed and enrolled at the Bahman neuropsychiatry clinic by trial clinicians who were blinded to treatment allocation. The randomization sequence was concealed until the last subject completed his final visit. The decision to choose the daily dose of 1 g/d of EPA or DHA was based on previous dose-finding studies (Mischoulon et al., 2008; Peet and Horrobin, 2002) in which this particular dose has been associated with the most significant improvement in depression severity and fewest side effects. Each of the DHA and EPA capsules (purchased from Minami Nutrition, Belgium) contained approximately 500 mg DHA or ethyl-eicosapentaenoate, respectively, along with small amounts of mixed tocopherols (4–11 mg) as antioxidant to increase product shelf life. Placebo capsules were manufactured by Viva Pharmaceutical, Inc., Richmond, British Columbia, Canada. All capsules were similar in look, and Subjects were asked to take them separately with food. Before randomization, the capsules were placed in identical, opaque, sealed containers by a person other than researchers and were coded with the letters A, B, or C. Each participant was given three containers, one for each month, and was asked to return them at next visits. In addition to weekly follow-ups by phone, subjects underwent detailed follow-up assessments at week 6 and

12 of the study. Adherence to the study protocol and adverse events were recorded at each visit. Compliance was defined as consumption of $\geq$90% of attributed capsules and was assessed by capsule count. In order to assess the blinding of the study, at final visit, all participants were asked to guess their treatment group. Subjects, researchers, and clinicians were blinded to the allocated treatments until the last participant completed his final visit.

An interviewer-administered general questionnaire was used to collect some sociodemographic, lifestyle, and clinical information of study participants at baseline. Additionally, at baseline and end of the study, participant's weight and height were measured and their body mass index (BMI) was then calculated by dividing weight by square of height (kg/m^2). In order to assess potential changes in dietary intake over the study period, three 24-h dietary recalls were completed for each participant through interview, both at baseline and end of the study. Nutritionist IV software (First Databank, Hearst Corp, San Bruno, CA, USA) was then used to compute dietary intakes of EPA (g/d), DHA (g/d), and energy (kj/d). Anthropometric measurements and completing of dietary recalls for all participants were performed by a trained nutritionist who was unaware of patient's allocated treatments.

2.3. Outcome measures

In this study, depression severity was assessed by BDI and 17-item HDRS that both have established validity and reliability (Beck et al., 1961; Hamilton, 1960). However, we used BDI only as a screening tool at baseline because it is recommended as an acceptable screening instrument for trial inclusion. 17-Item HDRS on the other hand is considered the most reliable instrument to assess changes in depression severity during randomized controlled trials (Vieweg et al., 2011). Depression severity according to HDRS score is as follows: 8–13 mild depression, 14–18 moderate depression, 19–22 severe depression, and $\geq$23 very severe depression. HDRS was completed at baseline, week 6, and week 12 of the study. Our primary outcome measure was the HDRS total score at study endpoint. Additional analyses were done on response to treatment (defined as a $\geq$50% decrease from the baseline HDRS score) and remission (defined as a final HDRS score of $\leq$7).

2.4. Statistical analysis

The sample size was computed on the basis of the primary outcome measure (HDRS total score). Using the standard deviations obtained from previous studies (Frangou et al., 2006; Mischoulon et al., 2008), a minimum of 21 participants per group would be required to detect a minimum difference of 6 points in mean HDRS score between groups with a power of 80% and a type I error of 5%. To allow for drop-out rate of 30%, it was finally decided that 27 participants per group would be recruited.

All analyses were performed on the modified intention-to-treat (ITT) population, which comprised of all randomized patients with at least one post-randomization assessment, using the SPSS statistical software, version 16.0 (SPSS Inc., Chicago, IL, USA). Chi square test or Fisher's exact test were used to compare categorical variables between groups, as appropriate. In case of continuous variables, Kolmogrov–Smirnov test was initially used to assess the normality assumption and one-way analysis of variance (ANOVA) or Kruskal–Wallis test were then used for comparison between groups, where appropriate. We additionally used paired *t*-test or Wilcoxon's signed-rank test for comparison of continuous variables between baseline and week 12 of the study within each group, as appropriate. HDRS total scores at study end-point and 12-week changes in HDRS total score were compared between the 3 treatment groups using an analysis of covariance (ANCOVA) model with treatment (EPA or DHA or placebo) as main effect and type of antidepressant medications and baseline HDRS score as covariates. Pair-wise differences between groups were then examined by performing a Bonferroni post-hoc test to adequately adjust for multiple comparisons. A p value of <0.05 was considered statistically significant.

3. Results

3.1. Participant flow and baseline characteristics

Figure 1 shows the participant flow throughout the study. A total of 115 patients were assessed for eligibility. Of these, 24 did not meet inclusion criteria, and 10 refused to participate. The remaining 81 patients were randomly assigned and allocated to 3 equal treatment groups. Of these patients, 19 (23.5%) discontinued intervention prior to week 2 (EPA: n=6, DHA: n=7, placebo: n=6), such that no post-randomization HDRS scores were available for them, resulting in a population of 62 subjects (61.3% female; mean age 35.1$\pm$1.2 years) eligible for modified-ITT analysis. The reasons for these dropouts were as follow: adverse events including gastrointestinal disturbances and headache (EPA: n=2, DHA: n=3, placebo: n=4), change in dose of antidepressant medications (in each group: n=1), and patient decision (EPA: n=3, DHA: n=3, placebo: n=1). There was no significant difference in drop-out rate between groups. Finally, 62 (76.5%) patients (EPA: n=21, DHA: n=20, placebo: n=21) completed the 12-week intervention.

Table 1 shows baseline sociodemographic, lifestyle, and clinical characteristics of the study participants. Participant's baseline characteristics were similar in all groups. Furthermore, there were no significant differences in any baseline variables between modified-ITT population and those who discontinued intervention prematurely.

A summary of anthropometric and dietary intake data at baseline and study endpoint is shown in Table 2. There were no significant differences in BMI or dietary intakes of energy,

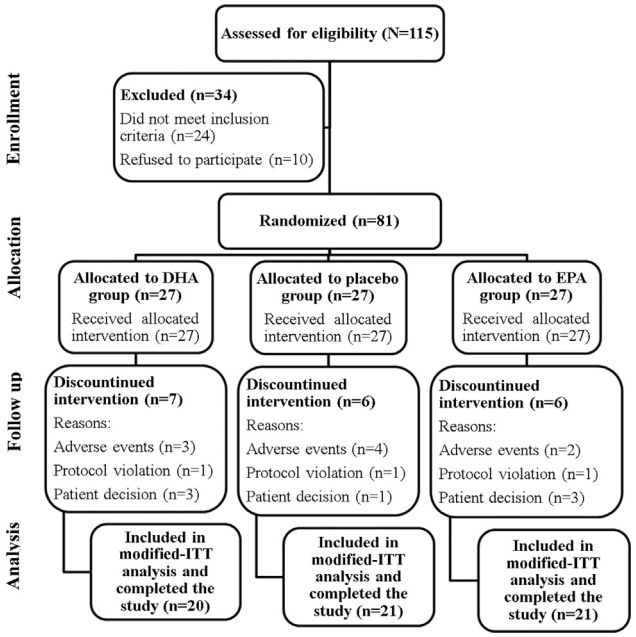

Figure 1. Participant flow diagram throughout the study. DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; modified-ITT, modified intention-to-treat.

Table 1 Baseline sociodemographic, lifestyle, and clinical characteristics (modified intention-to-treat population, $n=62$)[a].

	EPA ($n=21$)	DHA ($n=20$)	Placebo ($n=21$)
Age (years)	37.5±12.4	34.0±13.1	33.8±9.9
Female	13 (61.9)	12 (60.0)	13 (61.9)
Married	16 (76.2)	14 (70.0)	13 (61.9)
Education >12 years	8 (38.1)	6 (30.0)	6 (28.6)
Employed	8 (38.1)	7 (35.0)	5 (23.8)
Nutritional supplements intake[b]	15 (71.4)	10 (50.0)	14 (66.7)
Sunlight exposure	6 (28.6)	6 (30.0)	5 (23.8)
Duration of antidepressant treatment before enrollment (months)	4.0±0.9	3.7±0.8	3.9±0.8
Type of antidepressants			
Tricyclics/Bupropion/MAOIs	2 (9.5)	3 (15.0)	7 (33.3)
SSRIs	8 (38.1)	11 (55.0)	7 (33.3)
Combination of 2 types of antidepressants	11 (52.4)	6 (30.0)	7 (33.3)
BDI score	13.8±3.7	14.5±3.9	15.7±4.8
HDRS score	15.9±2.0	15.7±2.4	15.5±2.3

BDI, Beck Depression Inventory; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; HDRS, Hamilton Depression Rating Scale; MAOIs, monoamine oxidase inhibitors; SSRIs, selective serotonin reuptake inhibitors.
[a]Values are mean±standard deviation or *n* (%).
[b]Nutritional supplements included vitamins and/or minerals.

Table 2 Summary of anthropometric and dietary intake data at baseline and study endpoint (modified intention-to-treat population, $n=62$)[a].

		EPA ($n=21$)	DHA ($n=20$)	Placebo ($n=21$)	*P* value[b]
BMI (kg/m^2)	Baseline	25.1±5.5	24.3±3.7	26.0±3.6	
	Week 12	25.2±5.4	24.6±4.0	25.8±3.6	0.68
	P value[c]	0.28	0.15	0.47	
Energy (kj/d)	Baseline	9068±4200	8496±2193	7899±2137	
	Week 12	9364±4538	8512±2093	7670±2141	0.23
	P value[c]	0.82	0.98	0.77	
EPA (g/d)	Baseline	0.03±0.05	0.04±0.10	0.04±0.07	
	Week 12	0.04±0.07	0.04±0.10	0.04±0.08	0.59
	P value[c]	0.83	0.48	0.77	
DHA (g/d)	Baseline	0.08±0.13	0.11±0.27	0.07±0.09	
	Week 12	0.07±0.10	0.10±0.27	0.06±0.09	0.74
	P value[c]	0.70	0.78	0.26	

BMI, body mass index; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid.
[a]Values are mean±standard deviation.
[b]Obtained by one-way analysis of variance or Kruskal–Wallis test for between group comparisons.
[c]Obtained by paired *t*-test or Wilcoxon's signed-rank test for within group comparisons.

EPA, and DHA between groups at baseline or at end of the study. In addition, no significant differences were observed in any of above mentioned variables between baseline and end of the study within each group.

3.2. Depressive outcomes

Depressive outcomes of modified-ITT population are shown in Table 3. Although there was no significant difference between groups in mean HDRS total score at baseline, we observed significant differences at study endpoint ($p<0.001$). As shown in Table 3 and Figure 2, patients receiving EPA showed a significantly lower mean HDRS total score at study endpoint compared with those who received DHA ($p<0.001$) or placebo ($p=0.002$). Additionally, we found significant differences between groups in terms of 12-week changes in HDRS score ($p<0.001$). Pair-wise comparisons revealed that patients in the EPA group had a significantly greater reduction in HDRS score after 12 weeks of the study compared to patients in the DHA or placebo groups (both $p=0.001$). However, no significant differences were observed in terms of final HDRS score or 12-week changes in HDRS score between DHA and placebo groups. Response to treatment was only observed in 6 (28.6%)

Table 3 Depressive outcomes of modified intention-to-treat population (n=62)[a].

	EPA (n=21)	DHA (n=20)	Placebo (n=21)	P value[b]	Unadjusted pairwise between group difference[c]					
					EPA-DHA	P value	EPA-Placebo	P value	DHA-Placebo	P value
Unadjusted final HDRS score	10.3 (0.7)	13.7 (0.6)	13.7 (0.6)	<0.001	−3.4 (0.9) [−5.6; −1.2]	0.001	−3.4 (0.9) [−5.6; −1.2]	0.001	0.0 (0.9) [−2.2; 2.2]	1.000
Unadjusted 12-week change in HDRS score	−5.6 (0.8)	−2.0 (0.4)	−1.9 (0.5)	<0.001	−3.6 (0.9) [−5.8; −1.5]	<0.001	−3.8 (0.9) [−5.9; −1.6]	<0.001	−0.1 (0.9) [−2.3; 2.0]	1.000
					Adjusted pairwise between group difference[c]					
					EPA-DHA	P value	EPA-Placebo	P value	DHA-Placebo	P value
Adjusted final HDRS score	10.5 (0.6)	13.8 (0.6)	13.6 (0.7)	<0.001	−3.3 (0.8) [−5.3; −1.3]	<0.001	−3.1 (0.9) [−5.2; −1.0]	0.002	0.2 (0.9) [−2.0; 2.4]	1.000
Adjusted 12-week change in HDRS score	−5.1 (0.5)	−2.1 (0.6)	−2.0 (0.6)	<0.001	−3.1 (0.8) [−5.0; −1.2]	0.001	−3.2 (0.8) [−5.2; −1.2]	0.001	−0.1 (0.9) [−2.2; 2.0]	1.000

DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; HDRS, Hamilton Depression Rating Scale.
[a]Values are mean (standard error) and [95% confidence intervals].
[b]Obtained by one-way analysis of variance for unadjusted values and by analysis of covariance for adjusted values (covariates included: type of antidepressant medications and baseline HDRS score).
[c]All p values for pairwise between group differences were obtained by Bonferroni post-hoc test.

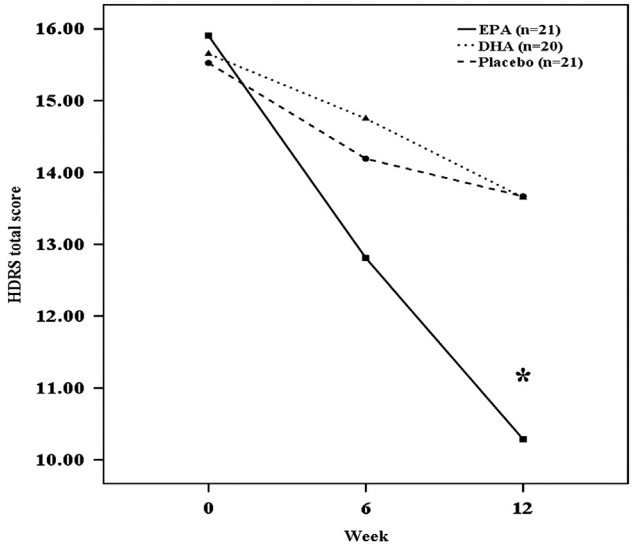

Figure 2. HDRS total scores over time in the modified intention-to-treat population (adjusted on type of antidepressant medication and baseline HDRS score). DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; HDRS, Hamilton Depression Rating Scale. *p<0.001.

patients in the EPA group at study endpoint. Of these, 5 (23.8%) achieved remission. No one in any of DHA or placebo groups responded to treatment or experienced remission.

3.3. Compliance, blinding, and adverse events

Data on compliance, blinding, and reported adverse events are presented in Table 4. Capsule count showed a compliance of more than 90% in all groups. Only 16 (25.8%) patients guessed right about their treatment group. A total of 17 (27.4%) patients reported mild and self-limited adverse events. No significant differences were observed in terms of compliance, blinding, and reported adverse events between groups.

4. Discussion

To our knowledge, this is the first study to compare the efficacy of EPA versus DHA as adjunctive treatments in depression. The findings showed greater efficacy of 1 g/d EPA compared to 1 g/d DHA or placebo as an adjuvant to antidepressant medications for the treatment of mild-to-moderate depression during 12 weeks.

Overall, allocated treatments were well tolerated by participants as indicated by the reported adverse events and moderately low drop-out rate. Only 23.5% of participants discontinued the 12-week intervention. This is comparable to the withdrawal rates of about 30% in 6-week trials of antidepressant medications (Barbui et al., 2000). Approximately, a half of drop-outs in this study were attributed to adverse events, which is surprising, given the good tolerability of medium-chain triglycerides (MCTs), the major type of fat in coconut oil, and n-3 PUFAs. However, it is noteworthy that in the previous studies some mild and self-limited side effects have been attributed to the use of n-3 PUFAs (e.g. increased risk of bleeding, nausea, diarrhea, constipation, headache, and dizziness) (Carney et al., 2009; Lucas et al., 2009; Mischoulon et al., 2008) or MCTs (e.g. diarrhea, bloating, reflux, stomachache, and dizziness) (Tajalizadekhoob et al., 2011; Bot et al., 2010). Furthermore, as an adjunctive trial, we cannot entirely rule out the potential contribution of medication-related side effects to these drop-outs.

Table 4 Data on compliance, blinding, and reported adverse events (modified intention-to-treat population, $n=62$).

	EPA ($n=21$)	DHA ($n=20$)	Placebo ($n=21$)	P value[a]
	n (%)	n (%)	n (%)	
Compliance				1.00
Patients who had taken ≥90% of attributed capsules	20 (95.2)	19 (95.0)	19 (90.5)	
Blinding				0.94
Patients who guessed right about their treatment group	5 (23.8)	5 (25.0)	6 (28.6)	
All reported adverse events combined	6 (28.6)	6 (30.0)	5 (23.8)	0.90
Gastrointestinal disturbance	4 (19.0)	5 (25.0)	3 (14.3)	0.67
Headache	3 (14.3)	1 (5.0)	2 (9.5)	0.86
Dizziness	1 (4.8)	2 (10.0)	1 (4.8)	0.68

DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid.
[a]Obtained by Chi square test or Fisher's exact test.

In the present study, all the modified-ITT population also completed the 12-week intervention. Although similar findings have been reported in some trials of n-3 PUFAs in depression (Rondanelli et al., 2010; Bot et al., 2010), this is an unusual finding compared to the whole literature. Since, as shown in Figure 2, a reduction in mean HDRS total scores was observed within all study groups over time, a potential explanation for this completer rate could be the presence of an encouraging feeling of treatment-related improvement in depressive symptoms among modified-ITT population of our study.

In line with previous trials of n-3 PUFAs in mild-to-moderate depression (Frangou et al., 2006; Rogers et al., 2008; Tajalizadekhoob et al., 2011), a large proportion of participants in our study were female. However, the randomization resulted in an appropriate distribution of baseline key variables between the study groups including gender, type of antidepressant medications, and HDRS total score. Therefore, our results could not be influenced by inappropriate distribution of baseline variables between the study groups. Furthermore, our findings could not be affected by inadequacy of blinding because 74.2% of patients could not guess right about their treatment group. The present study was conducted during fall and winter. Therefore, the significant improvement of depressive symptoms in the EPA group, could not be attributed to seasonal-related changes in mood because the major characteristic of these changes is the incidence of depressive episodes in fall/winter with remissions in spring/summer (Lam and Levitan, 2000; Sohn and Lam, 2005).

An interesting finding of this research was the low response and remission rates among study participants. This may be explained in part by a floor effect because our entry HDRS scores were lower than in most studies (i.e. the lower the entry HDRS scores, the less likely treatment will result in a significant reduction of these scores).

Overall, the results of this study are in line with existing evidence of greater efficacy of n-3 PUFAs as an adjunctive therapy compared to monotherapy in depression (Martins, 2009). However, we cannot generalize these results to patients who simultaneously start receiving the antidepressant medications and n-3 PUFAs because the previous consumption of these drugs by participants of our study may have sensitized their brain to the beneficial effects of n-3 PUFAs.

Thus far, only 2 randomized controlled trials have examined the efficacy of n-3 PUFAs in unipolar mild-to-moderate depression. In the first study, Rogers et al. (2008) failed to show any beneficial effect of 1.5 g/d of n-3 PUFAs (containing 630 mg EPA and 850 mg DHA) on mild-to-moderately depressed individuals. In contrast, findings from the second study conducted by Tajalizadekhoob et al. (2011) were in line with present study and showed the efficacy of 6 months supplementation with 1 g/d of n-3 PUFAs (containing 180 mg EPA and 120 mg DHA) in mild-to-moderate depression among Iranian outpatients. According to our results with respect to greater efficacy of EPA compared to DHA in depressed mood, we believe that the use of a higher DHA/EPA ratio can be the potential explanation why Rogers et al. (2008) could not show the efficacy of n-3 PUFAs for the treatment of mild-to-moderate depression.

Regarding the controversy over whether EPA or DHA or both are responsible for the reported therapeutic effects of n-3 PUFAs supplementation in depression, the results of a recent meta-analysis of randomized controlled trials by Martins (2009) provide evidence that EPA may be more efficacious than DHA in the treatment of depression. According to this meta-analysis, significant reduction in symptoms of depression were observed in 8 trials using pure ethyl-EPA and in 13 trials using supplements containing more than 50% EPA, while no such reduction were observed in 3 trials using pure DHA or in 4 trials using supplements containing more than 50% DHA. Moreover, the results of meta-analysis of the effects of EPA in clinical trials in depression by Sublette et al. (2011) were also in line with our findings, showing the efficacy of Supplements containing EPA≥60% of total EPA+DHA versus supplements with EPA<60% of total EPA+DHA in the treatment of primary depression.

Although we cannot entirely rule out the possibility of modulation of background drug pharmacokinetics by EPA in the present study but there are some reasons regarding why EPA may be more effective than DHA in the treatment of depression. There is growing evidence from experimental studies that DHA supplementation may have some damaging effects on the nervous system (Leonardi et al., 2007, 2005; Yang et al., 2007), while EPA supplementation on the other hand has been reported to have neuroprotective effects (Lonergan et al., 2004; Lynch et al., 2007; Minogue et al.,

2007). Additionally, with respect to the inflammatory hypothesis of depression, various studies have reported elevated production of pro-inflammatory eicosanoids from arachidonic acid by the cyclooxygenase system (Ohishi et al., 1988; Piccirillo et al., 1994), and it is noteworthy that EPA but not DHA is an important substrate for cyclooxygenase and can compete with arachidonic acid at this point (Simopoulos, 2002). Furthermore, incorporation of EPA but not DHA into cell membranes inhibits the action of phospholipase A_2 and therefore decreases the production of second messenger molecules such as arachidonic acid (Finnen and Lovell, 1991). These effects may play important roles in psychophysiology of depression.

The present study had several strengths including the moderately low drop-out rate, successful blinding, capturing data on dietary intake of relevant n-3 PUFAs, and its double-blind, placebo-controlled design. In addition, the 12-week duration of intervention in this study was in line with previous trials in depression (Bot et al., 2010; da Silva et al., 2008; Frangou et al., 2006; Mischoulon et al., 2008; Peet and Horrobin, 2002; Rogers et al., 2008; Silvers et al., 2005) and enabled us to detect relatively long-term effects of n-3 PUFAs on the treatment of depression. Moreover, this study included mild-to-moderately depressed outpatients and its results are therefore more relevant to general population compared to findings of previous studies that included individuals with more severe depressive symptoms from clinical settings. The use of low doses of n-3 PUFAs that are easily achievable through increased intake of oily fishes or through the use of widely available n-3 PUFAs supplements was another strength of this research.

Several limitations are also inherent in our study. Firstly, a large proportion of participants in this trial were female so it is not possible to draw conclusions about males. Furthermore, because our study was based on a convenient sample, the possibility of voluntary bias cannot be entirely ruled out. The main limitation of present study was the lack of evaluating plasma fatty acid profile in order to evaluate participant's adherence to treatments. However, this problem was limited, to some extent, by repeated follow-up visits and a capsule count that showed a compliance rate of over 90% in all of the study groups.

In summary, findings from this study suggest that EPA may be more efficacious than DHA or placebo as an adjuvant to antidepressant medications for the treatment of mild-to-moderate depression. However, larger, randomized controlled trials of sufficient methodological quality and duration, are needed to further confirm these findings.

Trial registration

This trial was registered at http://www.irct.ir as IRCT201010054873N1.

Role of the funding source

Shahid Sadoughi University of Medical Sciences, Yazd, Iran, funded the present study but had no further role in study design; in the collection, analysis and interpretation of data; in the writing of the report; and in the decision to submit the paper for publication.

Contributors

H. Mozaffari-Khosravi, S-E. Shariati-Bafghi, and M. Yassini-Ardakani designed the study, wrote the protocol, and managed the literature searches, acquisition of data, and analyses. M. Karamati undertook the statistical analysis. S-E. Shariati-Bafghi and M. Karamati wrote the first draft of the manuscript and H. Mozaffari-Khosravi revised it critically for important intellectual content. All authors contributed to and have approved the final manuscript.

Conflict of interest

H. Mozaffari-Khosravi received research funding and is the vice-chancellor for research in the Shahid Sadoughi University of Medical Sciences, Yazd, Iran. M. Yassini-Ardakani is the president of Bahman neuropsychiatry clinic in Yazd, Iran. S-E. Shariati-Bafghi and M. Karamati declare that they have no conflicts of interest.

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