

Assessment of anti-depressant activity of omega 3 fatty acids in rodents

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ABSTRACT

Background: Depression contributes to significant disease burden at national and global levels. At the personal and domestic level too, depression leads to poor quality of life, causing a huge socioeconomic impact. In the world, over 300 million people are estimated to have depression and the numbers of depressed persons are only projected to go up.

Methods: The forced swim test (FST) is one of the most commonly used animal models for assessment of antidepressant effects in rodents. In the modified version of this test, the rats are forced to swim in a glass tank with no means of escape, inducing a behaviour of immobility, which resembles a state of despair, akin to depression in humans. The rats were divided into 6 groups: 1. control group: treated with distilled water; 2. standard group treated with fluoxetine Hcl (10mg/kg); 3.test-1 group treated with omega-3 FAs (300mg/kg); 4.test-2 group treated with a higher dose of omega-3 FAs (500 mg/kg); 5.test-3 group treated with omega-3 FAs (300mg/kg) and fluoxetine (10mg/kg); and 6.test-4 group treated with omega-3 FAs (500 mg/kg) and fluoxetine (10mg/kg).

Results: The independent between-groups ANOVA yielded a statistically highly significant result, $F(5, 30) = 9.47, P < 0.001$. Thus, the null hypothesis of no difference between the means was rejected. To further evaluate the nature of the differences between the means of the six groups, the statically significant ANOVA result was followed by Tukey's honest significant difference post-hoc tests.

Conclusions: This study finds that omega 3 fatty acids have intrinsic antidepressant activity, and the combination of fluoxetine and omega 3 fatty acids has significantly more antidepressant effect than fluoxetine alone in the forced swim test done on Wistar rats.

Keywords: α -linolenic acid, Eicosapentaenoic acid, Forced swim test

INTRODUCTION

Depression is a very common illness, affecting individuals of all ages, genders and different socioeconomic groups in India and the world. Depression contributes to significant disease burden at national and global levels. At the personal and domestic level too, depression leads to poor quality of life, causing a huge socioeconomic impact. In the world, over 300 million people are estimated to have depression and the number of depressed persons, is only projected to go up. Depression is ranked by World Health Organization as the single largest contributor to disability, globally. The burden of depression in India is high. According to the data released by WHO in 2017, 4.5 % of

all Indians suffer from depression.¹ Many studies done in India show that, 17- 46% of patients attending primary health centres (PHCs), suffer from common mental disorders.² Among the patients diagnosed with CMDs at PHCs, the commonest mental disorder was depression (63.6%).³

Since age-old times, it's known that consuming certain types of food, commonly called "brain foods" today, such as fish and nuts results in better mental health and staved off cognitive decline in people. It was found that these brain foods contain essential fatty acids called omega-3 fatty acids, particularly α -linolenic acid (ALA) (plant oils), eicosapentaenoic acid (EPA) and docosahexaenoic acid

(DHA) (later both commonly found in fish oils). This study was carried out to assess the possible anti-depressant effect of these fatty acids in albino wistar rats using forced swim test and also if omega-3 fatty acids possibly work in cohesion with anti-depressant drugs.

METHODS

Drugs

Fluoxetine hydrochloride was given at a dose of 10mg/kg, administered orally by dissolving in water. The standard solution of fluoxetine was prepared by dissolving 20mg in 10ml of distilled water. The solution had a concentration of 2mg/ml. The omega-3 fatty acids were given at graded doses of 300mg/kg and 500mg/kg. The omega-3 FAs were administered in pure form, without the use of any vehicle. The omega-3 fatty acids preparation in given form, contained EPA (eicosapentaenoic acid) and DHA (docosahexaenoic acid) in the ratio of 3:2. Distilled water was given at dose of 10ml/kg (Table 1).

Animals

Total of 36 wistar rats, weighing between 180-280grams of either sex were selected for the study. The animals were from the Institutional Animal House, Department of Pharmacology, Gandhi Medical College.

Forced swim test

The forced swim test (FST) is one of the most commonly used animal models for assessment of antidepressant effects in rodents. It was described for the first time by Porsolt et al, in the year 1977 and works on the principles of behavioural despair.⁴ The modified version was released by Lucki et al, to increase reliable detection of SSRIs, with alterations consisting of increasing the water depth.⁵ The rats are forced to swim in a glass tank of dimensions 60cm height, 30cm width and 30cm breadth, with no means of escape. It is done in two stages for rats: the pre-test (24hrs before) and the test. During both the stages, after an initial struggle, the rodents enter a state of despair and become immobile. The total duration of this immobility is measured study and is equated to state the of behavioural despair.⁶

Experimental procedure

Habituation

The wistar rats were housed at controlled temperature, and a standard light/dark cycle was maintained. The rats were handled daily for a few minutes for 4 days, before initiating the behavioural testing.

Preparation for pre-test

The rats were moved into the pre-test room, which also served as the test room. The pre-test (and test) was

performed in a separate room from the storage room. They were left for about 1 hr after moving, to minimize the arousal caused by transportation. The pre-test was performed at the same stage of the light/dark cycle as other experiments in the series.

Table 1: Total number of groups.

| Group | No. of rats | Drugs/Dose |
|----------|-------------|---|
| Control | 6 rats | Distilled water (10ml/kg) |
| Standard | 6 rats | Fluoxetine Hcl (10mg/kg) |
| Test 1 | 6 rats | Omega-3 FAs (300mg/kg) |
| Test 2 | 6 rats | Omega-3 FAs (500mg/kg) |
| Test 3 | 6 rats | Omega-3 FAs (300mg/kg) + Fluoxetine (10mg/kg) |
| Test 4 | 6 rats | Omega-3 FAs (500mg/kg) + Fluoxetine (10mg/kg) |

Pre-test

The glass tank was filled to depth of 30cm (modified FST) with water of temperature 23-25°C. The pre-test (not recorded) was done for a total duration of 15mins on each rat, after which the rats were removed from water, towel dried and shifted back to their home cages.

Drug regime

All group specific drugs were given orally, 24hr (immediately after pre-test), 5 hr and 60 minutes before the commencement of the test.

Swim test

After 24 hours of the pre-test, the same experimental setup as the one for the pre-test was used. The test was performed for a total duration of 5mins, after which the rats were removed from the water and dried with towels.

Video recording of each rat was done during the testing phase of the forced swim test. A cumulative stopwatch was used to obtain the onset of immobility and the total time spent in immobile posture, by observing the videos.

RESULTS

Latency to first immobility period

The descriptive statistics associated with the latency to first immobility period are reported in Table 2. It shows that the latency to immobility, was the least in the Control group, with the mean time of 122.83 seconds. This shows that the rats from this group had the least time taken among all the groups to first enter the state of behavioural despair.

The rats of the two groups, test 1 and test 2, have means of 131.83 seconds and 139.33 seconds respectively, indicating that omega-3 fatty receiving rats, swam for longer duration before entering the state of behavioural

despair for the first time, compared to those from control. Table 2 further shows that standard groups, test-3 and test-4 also had longer average swimming time before the first entry into the state of behavioural despair (immobility), compared to the control group.

Table 2: Latency to first immobility period (in seconds).

| Group | Mean | ±SD | ±SEM |
|----------|--------|-------|------|
| Control | 122.83 | 9.86 | 4.03 |
| Standard | 143.67 | 8.89 | 3.63 |
| Test 1 | 131.83 | 10.89 | 4.45 |
| Test 2 | 139.33 | 10.76 | 4.39 |
| Test 3 | 151.67 | 9.54 | 3.90 |
| Test 4 | 156.67 | 9.67 | 3.95 |

Mean = Expressed in seconds; SD = Standard deviation; SEM = Standard error of the mean

One-way ANOVA and Tukey's HSD multiple comparisons results

To further evaluate the statically significant ANOVA result was followed by Tukey's Honest Significant Difference post-hoc tests. The results from Tukey's honest significant Difference post-hoc tests have been reported in Table 3.

Table 3: Results associated with Tukey's HSD multiple-comparisons.

| Groups | Vs | Group | P-value | Inference |
|----------|----|----------|---------|-----------------|
| Control | Vs | Standard | 0.012 | Significant |
| Control | Vs | Test1 | 0.627 | Non-significant |
| Control | Vs | Test 2 | 0.073 | Non-significant |
| Control | Vs | Test 3 | <0.001 | Significant |
| Control | Vs | Test 4 | <0.001 | Significant |
| Standard | Vs | Test 1 | 0.336 | Non-significant |
| Standard | Vs | Test 2 | 0.973 | Non-significant |
| Standard | Vs | Test 3 | 0.732 | Non-significant |
| Standard | Vs | Test 4 | 0.241 | Non-significant |
| Test 1 | Vs | Test 2 | 0.781 | Non-significant |
| Test 1 | Vs | Test 3 | 0.019 | Significant |
| Test 1 | Vs | Test 4 | 0.002 | Significant |
| Test 2 | Vs | Test 3 | 0.293 | Non-significant |
| Test 2 | Vs | Test 4 | 0.053 | Non-significant |
| Test 3 | Vs | Test 4 | 0.951 | Non-significant |

Further statistical analysis by Post hoc Tuckey's HSD test revealed, that the two test groups containing omega-3 fatty acids alone (test 1 - 300mg/kg; test 2 - 500mg/kg) did not significantly differ from onset time of the control group. Furthermore, there was no significant difference between the means of standard fluoxetine-alone group, and the two test groups - test 3 (Omega 3 FA 300mg/kg + Fluoxetine 10mg/kg) and test 4 (Omega 3 FA 500mg/kg + Fluoxetine 10mg/kg). This indicates that the add-on effect of omega-3 fatty acids on fluoxetine, didn't significantly enhance the effects seen with fluoxetine-alone, for this parameter.

Total duration of immobility

Like with the previous parameter, to further evaluate the nature of the differences between the means of the six groups, similar procedure was followed.

The descriptive statistics of this important parameter are presented in the Figure 1.

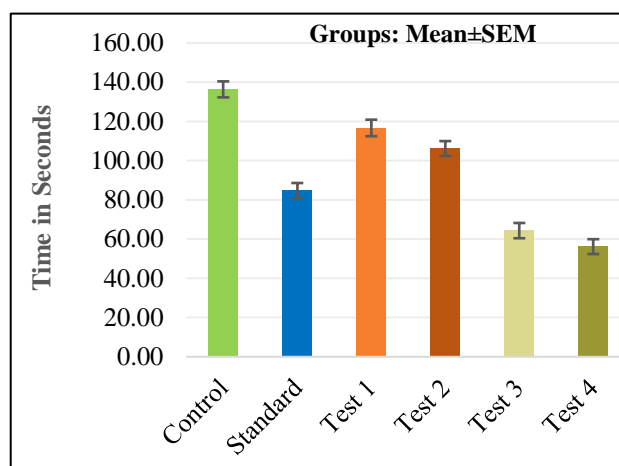


Figure1: Total duration of immobility.

Figure 1 shows, that the total duration of immobility was the longest in the control group, having the highest mean value of 136.33seconds. Thus, indicating that the rats from this group, had spent the longest average cumulative duration in the state of immobility or behavioural despair, among all the rats.

The rats of the two groups, group test 1 and group test 2, indicating that omega-3 fatty receiving rats swam for longer total duration, compare to those from control and spent less lime in the state of immobility or behavioural despair. The rats from the test groups, test 3 and test 4 have the mean values of 64.33seconds and 56.17seconds, for the total duration of immobility also indicating that the rats from the two test groups which received the combination dose of omega-3 fatty acids and fluoxetine, had swam for the longest average duration and, spent the least amount of time being in the state of immobility or behavioural despair.

One-way ANOVA and Tukey's HSD multiple comparisons results

Before the ANOVA test was performed, the assumption of homogeneity of variances was tested using Levene's Test and found tenable, $F(5, 30) = 0.072, P = 0.99$.

The one-way ANOVA indicated that there was high significant difference between the means of the groups ($p < 0.001$).

On further evaluation by doing Post hoc Tukey's HSD multiple-comparisons (Table 4), it was noted that the fluoxetine-alone containing standard group, omega-3 fatty acids alone test groups and the test groups having omega-3 fatty acids in combination with fluoxetine, all differed statistically significant compared to the control group. There was also no statistical difference between the two test groups -test 1 and test-2. There was significant difference in total duration of immobility between fluoxetine alone standard group and omega 3 fatty acid alone test groups, indicating the anti-depressant effect of omega-3 alone, was modest compared to fluoxetine. Based on the Table 4, it can also be observed that the anti-depressant effect of the test groups having combinations of fluoxetine with omega-3 fatty acids, was statistically better than fluoxetine-alone standard group. This indicates that the add-on effect of omega-3 fatty acids on fluoxetine, was profound, significantly potentiating the anti-depressant effects of fluoxetine.

Table 4: Results associated with Tukey's HSD multiple-comparisons.

| Groups | Vs | Group | P-Value | Inference |
|----------|----|----------|---------|-----------------|
| Control | Vs | Standard | <0.001 | Significant |
| Control | Vs | Test1 | 0.016 | Significant |
| Control | Vs | Test 2 | <0.001 | Significant |
| Control | Vs | Test 3 | <0.001 | Significant |
| Control | Vs | Test 4 | <0.001 | Significant |
| Standard | Vs | Test 1 | <0.001 | Significant |
| Standard | Vs | Test 2 | 0.007 | Significant |
| Standard | Vs | Test 3 | 0.012 | Significant |
| Standard | Vs | Test 4 | <0.001 | Significant |
| Test 1 | Vs | Test 2 | 0.435 | Non-significant |
| Test 1 | Vs | Test 3 | <0.001 | Significant |
| Test 1 | Vs | Test 4 | <0.001 | Significant |
| Test 2 | Vs | Test 3 | <0.001 | Significant |
| Test 2 | Vs | Test 4 | <0.001 | Significant |
| Test 3 | Vs | Test 4 | 0.691 | Non-significant |

DISCUSSION

Today, many studies are being done to check if naturally available remedies and supplements can be used as aids or replacements to the existing anti-depressant drugs. Omega-

3 fatty acids are among various such supplements, being evaluated for a possible role in this regard.

In the present study, forced swim test, a despair-based depression model was used to evaluate the anti-depressant activity of omega-3 fatty acids. The two most widely used for testing drugs, the tail suspension test and forced swim test (which are based on behavioural-despair), are more economical and allow for a simple and fast screening for potential anti-depressant drugs. However, tail suspension test cannot be used in rats and it is only intended to be used in mice. All the drugs (including vehicle in control group) and their combinations were given three times orally, 24 hr (immediately after pre-test), 5 hr and 60 minutes before the commencement of the test. The final oral dose was given 60 mins before the swim test as studies have found for it was found to penetrate brain better, in contrast to 30 mins-before dosing for other routes.⁷

To assess the anti-depressant effect, the parameters used in this study for Forced Swim Test (FST), were latency to immobility and total duration of immobility. The primary parameter in FST is the measure of total duration of immobility. It is measured by summing the total time spent by the rats, exhibiting immobile behaviour and minor movements strictly necessary to keep the rodent's head above water. It excludes the time spent actively exploring the water tank and the time spent in trying to escape.⁷ Anti-depressant drugs significantly reduce the total duration of immobility.

Latency to immobility is the measure of delay between the start of the test and the appearance of the first instance of immobility. This parameter was added as an additional measure in the test, as it increases the sensitivity to the anti-depressant agents.⁸ Hence, these two parameters were chosen in the present study.

The analysis of the results of the two test parameters and their significance is as follows:

Latency to immobility

The observations of this parameter which were presented in the results section earlier, showed that even though the One-way ANOVA showed significant difference between the groups, the groups which received omega-3 fatty acids alone, didn't significantly differ from the control. The groups which did which received combination of omega-3 and fluoxetine, also did not differ significantly from fluoxetine alone standard group.

These observations are probably because, this parameter is known to be more sensitive for detecting the effects of tricyclic antidepressants (imipramine, desipramine) and selective serotonin/norepinephrine reuptake inhibitors (SNRIs: duloxetine and venlafaxine) than for serotonin reuptake inhibitors (SSRIs: fluoxetine and escitalopram).⁸ Since omega-3 fatty acids alone didn't seem to affect the outcome for this parameter, its mechanism may perhaps be

different when compared to tricyclic anti-depressants and SNRIs, or that the dosages may be insufficient to induce any changes.

Total duration of immobility

Like with previous parameter, the one-way ANOVA, indicated that there was high significant difference between the means of the groups for this parameter. The omega-3 fatty alone groups were significantly different than the control group, while the groups which received combination of omega-3 fatty acids and fluoxetine were significantly better than standard fluoxetine alone group.

The net beneficial value of this add-on effect due to omega-3 fatty acids may be in that, an exploration for a reduction in anti-depressant dose of fluoxetine can be done in the future, thereby potentially decreasing the side-effects due to fluoxetine. The immobility can also be decreased by the compounds which can increase the locomotor activity, such as stimulants, convulsant and anti-cholinergics and thereby, producing a false positive result in FST. However, previous studies have reported that the omega-3 enriched diet produced no significant effects on locomotor activity in experimental animals.^{9,10} Hence, in the present, the reduction of immobility seen in FST, following omega-3 administration, was specifically due to its anti-depressant effects and not due to any locomotion affects.

There are very few studies done in the past to explore the role of omega-3 fatty acids as potential anti-depressants. One of the earliest such similar studies was done by Naliwaiko et al, showed a significant anti-depressant effect of omega-3 fatty acids, which conforms with present study.¹¹ While the study done by Naliwaiko et al, fed the rats with a high dose of 3g/kg of omega- FAs for their entire lifetimes, present study only gave very low dosages of 300mg/kg and 500mg/kg (almost 10 times smaller), and for a very short duration (3 times within 24 hrs) and yet, the results obtained by both the studies were the same. This may be because for this animal paradigm (FST), such chronic administration of the anti-depressants at very high doses may not be required to elicit their effects.

The beneficial effects of omega- fatty acids in depression may be due to various mechanisms. One of the proposed mechanisms may include neuroendocrine modulation due the induced membrane changes, potentially altering the regulation of dopaminergic and serotonergic neurotransmission, which are dysfunctional in depressed individuals.¹² Alteration of serotonergic neurotransmission may also have a synergistic effect with fluoxetine.

Studies indicate that in depression, there is a release of inflammatory mediators such as interleukin-1 β (IL-1 β) and tumor necrosis factor- α (TNF- α).¹³ Hence, Omega-3 fatty acids which are well-documented inhibitors of TNF- α and IL-1, may also exhibit their anti-depressant through suppression of these pro-inflammatory mediators.¹⁴ Newer studies have shown that, fluoxetine reversed the depression

induced changes in T cell proliferation and, IL -2, INF- γ and TNF- α production.¹⁵

The Brain derived neurotrophic factor (BDNF) has been a focus lately, with studies showing that there is a decrease of BDNF in depressed individuals.¹⁶ Chronic administration of omega-3 fatty acids has been found to increase the levels of BDNF, thus reversing the effects of low BDNF, seen in depression.¹⁷ Chronic fluoxetine treatment was also found to induce brain region-specific upregulation of genes associated with BDNF induced long term potentiation.¹⁸ This mechanism may not explain the observations of enhanced anti-depressant action of the omega-3 fatty acid and fluoxetine combination seen in the present study, as it requires a chronic administration. However, it may provide a rationale for a long-term clinical use of fluoxetine and omega-3 FAs combination.

The findings of the present study, done to assess the anti-depressant effects of omega-3 fatty acids, have yielded very positive results. This study has not only shown that omega-3 fatty acids alone can produce anti-depressant effects, but also shown that they can significantly enhance the effects of fluoxetine, when used in combination with it. Thus, when used in combination, omega-3 fatty acids may potentially facilitate the decrease in the clinically active doses of SSRIs like fluoxetine, required for treating depression. Furthermore, in susceptible individuals when used for long term as nutritional supplements, they may also prove to be beneficial in prevention of depression.

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Ethical approval: The study was approved by the Institutional Ethics Committee (428/01C/CPCSEA/18-GMC-IAEC)

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