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Stress-Induced Behavioral Deficits in Rats Treated with Omega-3 Polyunsaturated Fatty Acids

Faisal Khan[1,2]*, Khurshid Jalal[1,2], Shazia Nawaz[1], Ishtiaq Ahmad Khan[1] and Darakhshan Jabeen Haleem[1]

[1]Dr. Panjwani Center for Molecular Medicine and Drug Research (PCMD), International Center for Chemical and Biological Sciences (ICCBS), University of Karachi (UOK), Karachi, Pakistan

[2]H.E.J. Research Institute of Chemistry, International Center for Chemical and Biological Sciences (ICCBS), University of Karachi (UOK), Karachi, Pakistan

***Corresponding Author:** Faisal Khan, Dr. Panjwani Center for Molecular Medicine and Drug Research (PCMD), International Center for Chemical and Biological Sciences (ICCBS) and H.E.J. Research Institute of Chemistry, International Center for Chemical and Biological Sciences (ICCBS), University of Karachi (UOK), Karachi, Pakistan.

Received: January 16, 2023; **Published:** January 24, 2023

Abstract

Omega-3 polyunsaturated fatty acids (omg-3 PUFAs) complex contains docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) a source of fish oils and is widely prescribed in the treatment of many cerebral and cardiovascular diseases. They play a significant role in the myelination of neurons of the brain, ultimately enhancing cerebral activities. Although behavioral studies reported variable reports with the use of omg-3 PUFAs. Episodic forced immobilization stress for 2-h reduces body weight and cumulative food intake. Such immobilization behaviors cause behavioral deficits in stressed induced anxiety. In the current study, it was found that administration of omg-3 in doses of 0.5 g/kg and 1 g/kg for 20 days altered the body weight at 0.5 g/kg but did not alter food intake after repeated immobilization stress for 2-h for five days. The treatment reduced locomotor activity in an open field arena and enhanced anxiety-like behavior in an elevated plus-maze test after single and repeated immobilization stress. The present study implied that the unnecessary use of omg-3 supplements can cause anxiety.

Keywords: *Anxiety; Omega-3; Stress; Open Field; Elevated Plus Maze Test*

Introduction

The relationship between stress and anxiety/depression is well established [1,2]. Together with this, an increase in the prevalence of anxiety and depression is becoming a noteworthy health concern across the globe [3]. Conventional drugs for treating anxiety and depression are not very effective and relapse is common [4,5]. These treatments are associated with substantial negative effects such as headaches, seizures, sexual dysfunction, addiction, and suicide [6]. Therefore, there has been a noteworthy rise in using natural remedies which can reduce stress effects on behavior. In this context, there is an escalating rise in research interest on the biological effects of omega-3 polyunsaturated fatty acids (omg-3 PUFAs) because of the reports showing their health benefits.

One of the most well-investigated forms of stress in rats is the forced immobilization model. There are reports that single and repeated immobilization stress for 2 hrs lowers the cumulative food intake and body weight gain in rats [7]. The animals developed symptoms of

anxiety and depression-like behavior [8]. Lowering food intake and other behavior no longer remains upon repeated immobilization [8]. Repeated exposure to a predictable stressor produces adaptive changes that led to behavioral tolerance [9].

Aim of the Study

The current study aims to investigate the effects of omg-3 on stressed-induced behavioral deficits particularly intake of food and body weight, and motor-like and anxiety-like behaviors. Effects of single and repeated exposure to immobilization stress are monitored to understand if the treatment can also improve behavioral tolerance to predictable stress.

Materials and Methods

Experimental protocol

Male albino Wistar rats (n = 36) of body weight (200 ± 20 grams) were used in this study. On the first day of the experiment, 36 animals were randomly divided into 3 groups (n = 12/in each group). In the first step, body weight and food intake were measured between 7:00 a.m. to 8:00 a.m. In the second step, these animals were pre-treated orally for 14 days with water (control group) or omg-3 (treatment groups) in doses of 500 mg/kg and 1000 mg/kg between 10:00 a.m. to 11:00 a.m. daily. On day 15, the animals of each group were divided into a stressed and unstressed group (n = 6 animals in each group). One-hour after treatment with water or omg-3, animals of each stressed group were subjected to 2-h immobilization stress from 12:00 a.m. to 2:00 p.m. and then placed back into their home cages. During this period unstressed group animals remained in their home cages. On the following day (day 16), body weights and food intake were monitored between 7:00 a.m. to 8:00 a.m. Following this, activities in an elevated plus maze (EPM) and open field (OF) were monitored between 9:00 a.m. to 10:00 a.m. to find the effects of a single exposure to immobilization stress on anxiety-like behavior and motor behavior, respectively. After that, animals were treated with water or omg-3. After a gap of 1 hour, animals of the stressed group were exposed to second immobilization stress for 2-h from 12:00 a.m. to 2:00 p.m. keeping animals of unstressed groups in their cages during this period. The animals were subjected to repeated immobilization stress, 2 hours/day for five days. Effects on body weight and food intake before the treatment of omg-3 were measured daily. Effects of 6th immobilization stress on anxiety on anxiety-like behavior and motor activity were monitored daily on day 20.

Days	Bodyweight and food intake (7:00 a.m. to 8:00 a.m.)	EPM and OF (9:00 a.m. to 10:00 a.m.)	Drug administration (10:00 a.m. to 11:00 a.m.)	Single and repeated immobilization stress (12:00 a.m. to 2:00 p.m.)
1-14				
15				
16				
17-19				
20				

Animals

Male Albino-Wistar rats of weight range from 200 ± 20 grams and age of 4 to 5 weeks were obtained from the animal house facility of Dr. Panjwani Center for Molecular Medicine and Drug Research (PCMD), International Center for Chemical and Biological Center (ICCBS), University of Karachi-Pakistan were used in the present study. Animals were accommodated in polyacrylic opaque cages (two animals/cage) with free access to a standard rat chow diet and tap water. The light and dark cycles (12:12 hrs) were maintained from 6:00 a.m. to 6:00 p.m. with a controlled room temperature (25 ± 2°C) and humidity (40 ± 5%). Rats were kept in this novel environment for 5 days for

acclimatization and to reduce handling stress. All the experimental protocols were approved by the institutional ethical and animal care committee and were executed with the concurrence of the National Institute of Health Guide for Care and the use of laboratory animals (ASP No. 2020-016).

Omg-3 supplements and treatment

Locally purchased soft gel fish oil capsules containing 500 mg omg-3 from "The Vitamin Company, USA" were used. Each capsule contained EPA and DHA in a ratio of 3:2 (300 mg: 200 mg).

Oral gavage connected to a 1 ml syringe was used to administer omg-3 in doses of 500 mg/kg and 1000 mg/kg to the experimental groups and tap water (1 ml/kg) to the control group.

Monitoring body weight and food intake

All the animals weighed 200 ± 20 grams on starting day. Body weights were monitored from 7:00 a.m. to 8:00 a.m. after single and repeated immobilization stress. Percentage changes in body weight were calculated as: % age change in body weight = {(Body weight after the treatment)/(body weight before starting treatment)} x 100.

150 gm of food was placed in the hopper on starting day. Food left in the hopper was measured on the next day and every day between 7:00 a.m. to 8:00 a.m. More food was added to the hopper every day to fill the hopper and weighed it. Cumulative food intake for 20 days was determined by adding 20 days of intake.

Open field test

The purpose of monitoring the OF activity was to determine the motor-like behavior of rats treated with omg-3 or water. The OF apparatus comprised a plain square arena with a dimension of 76 cm x 76 cm, surrounded by boundary walls. These boundary walls comprised an opaque polyacrylic plastic sheet with a height of 42 cm. The open arena of the OF was divided by black lines into 25 (5 x 5) equal squares. A camera was installed above the apparatus to record the exploration of rats in the apparatus.

To measure activity, a rat was placed in the central square of the OF. The number of squares crossed with all four paws was counted immediately for 5 minutes. Afterward, the rat was picked up and housed back in its cage. The apparatus was cleaned after placement for the exposure of the next rat.

Elevated plus maze test

The purpose to monitor the EPM test was to measure the anxiety-like behavior of rats treated with omg-3 or water. The EPM apparatus was comprised of four equal arms made up of an opaque polyacrylic plastic sheet, extending from a central open area space of 10 cm x 10 cm. Each arm had a length of 50 cm and a width of 10 cm. Two of these arms were open called "open arms" but the other two arms were surrounded by three-sided walls with a height of 15 cm while one side was open to the central space called "close arms". The apparatus was elevated from the floor at a height of 60 cm. A camera was placed above the apparatus to record the behavior of rats in the apparatus.

To measure the activity in EPM, a rat was placed in the central open area. In which the head of an animal faces one of the closed arms. The animal was allowed freely to explore the open and closed arms for 5 minutes. During this time several entries and time spent with open arms were measured. After which the rat was picked and placed back in its home cage and the apparatus was cleaned for the next rat exposure.

Immobilization stress

The purpose is to monitor the effects of omg-3 on stressed-induced anxiety. The stressed group animals were immobilized in steel wire grids. These grids of 10 in. × 9 in. were fitted with a Perspex plate of 9 in. × 6.5 in. at the head end were used. The Perspex plats had an area of 3 in. x 3 in. cut area the center of the grid for the rat to rest effortlessly, Immobilization was effected by pressing the forelimbs of the rat through the adjacent gaps in the metal grid and taping them together with zinc oxide plaster. Also, hind limbs in between two gaps were taped, and the head of the animal rested on the Perspex plate. At the end of the 2-h immobilization, their limbs were detached from grids to avoid internal and external injury and placed back into their respective cages.

Statistical analysis

IBM-SPSS (Version 21.0) software was used to analyze the data of percentage change in body weight, cumulative food intake, and stress data were statistically tested by ANOVA. Data are represented as mean ± SD. Dose-related effects (factor 1) in open field activity and elevated plus maze test scored on days 16^{th} and 20^{th} (factor 2; repeated measure), and repeated stress for five days (factor 3) were analyzed by three-way ANOVA repeated measure design. Post-hoc analysis was done by Tukey's test and p-values < 0.05 were regarded as significant.

Results

Figure 1: Effects of single and repeated immobilization stress on percentage changes in body weight in rats pre-treated with omg-3 for 15 days

Data on body weight changes were analyzed by two-way ANOVA (repeated measure design) and showed a significant effect of repeated measure (days) ($F = 374.080$ df1, 30 $p < 0.01$), stress ($F = 356.666$ df1, 30 $p < 0.01$), the interaction between repeated measure (days) x omg-3 ($F = 9.137$ df2, 30 $p < 0.01$), repeated measure (days) x stress ($F = 31.177$ df1, 30 $p < 0.01$), repeated measure (days) x omg-3 x stress ($F = 15.894$ df2, 30 $p < 0.01$), omg-3 ($F = 18.040$ df2, 30 $p < 0.01$), and omg-3 x stress ($F = 106.097$ df2, 30 $p < 0.01$). Post-hoc analysis showed that single immobilization stress for 2 hrs decreased body weight in water-treated stress animals compared to water-treated unstressed animals and increased the body weight in low-dose treated stressed animals compared to low-dose treated unstressed animals and water treated stressed animals, high-dose treated stressed animals increased body weight compared to water treated stressed animals. Repeated (2 hrs/day for 5 days) immobilization stress decreased body weight in water treated stressed animals compared to water unstressed group. Omg-3 (0.5 g/kg) treated stressed animals decreased the body weight compared to low-dose treated unstressed animals but increased the body weight after repeated stress compared to water treated stressed animals. At a high dose of omg-3 (1 g/kg) repeated exposure to stress showed that high-dose treated stress animals increased body weight changes as compared to water treated stressed group. Results suggested that a low dose of omg-3 single repeated stress increased the body weight but after repeated immobilization stress decreased body weight.

Figure 2: Effects of single and repeated immobilization stress on changes in daily food intake on rats pre-treated with omg-3 for 15 days

Data on food intake changes were analyzed by three-way ANOVA (repeated measure design) and showed significant effects of omg-3 ($F = 8.621$ df2, 30 $p < 0.01$, stress ($F = 156.295$ df1, 30 $p < 0.01$). Effects of repeated measure (days) ($F = 18.679$ df 5,150 $p < 0.01$), repeated measure (days) x omg-3 interaction ($F = 3.439$ df 10, 150 $p < 0.01$), repeated measure (days) x stress interaction ($F = 14.307$ df 5,150 $p < 0.01$) were also significant. Effects on the interaction between stress x omg-3 ($F = 1.417$ df 2, 30 $p > 0.05$) and interaction of repeated measure (days) x omg-3 x stress ($F = 0.653$ df 10,150 $p > 0.05$) were not significant. Post-hoc analysis showed that single and repeated (2hr/for 5 days) immobilization stress decreased daily food intake in water-treated animals as compared to respective day-unstressed animals.

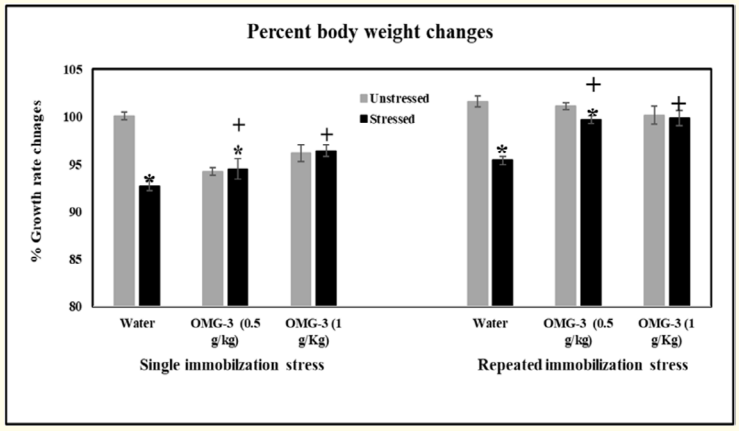
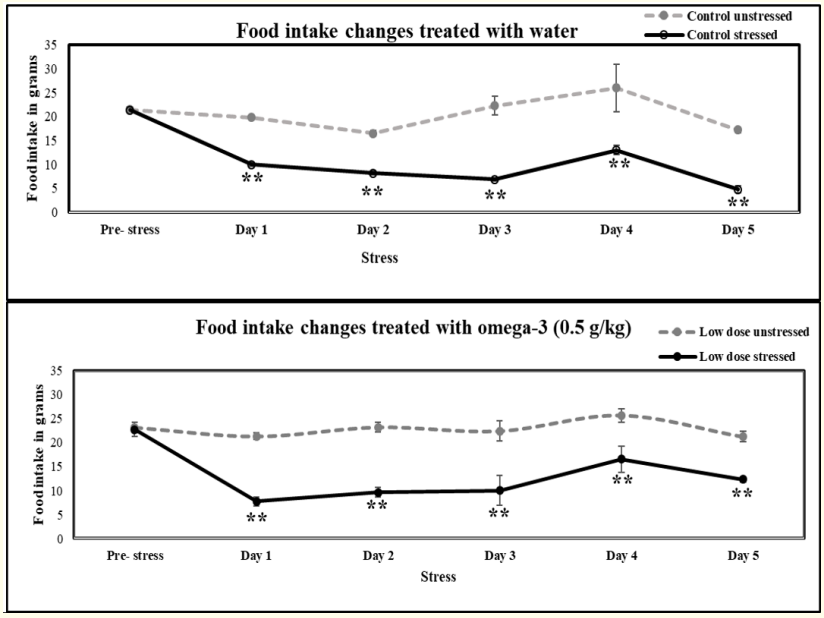


Figure 1: *Effects of single (2 hours) and repeated (2hrs/day for 5 days) immobilization stress with and without omg-3 (0.5 g/kg and 1 g/kg) administration on body weight changes with prior 15 days omg-3 treatment. Data represented as mean ± SD (n = 6). Significant difference by Tukey's test *p < 0.01) from respective unstressed animals and +p < 0.01 from respective water-treated animals following three-way ANOVA repeated measure design.*

Daily food intake was gradually increased in omg-3 (0.5 g/kg) treated stress animals as compared to the respective day unstressed group from day 1 to day 5. In stressed animals of omg-3 (1 g/kg) treated animals' food intake was decreased on day 3 and day 5. Results suggested that a high dose of omg-3 tended to attenuate the stressed induced decrease in food intake.



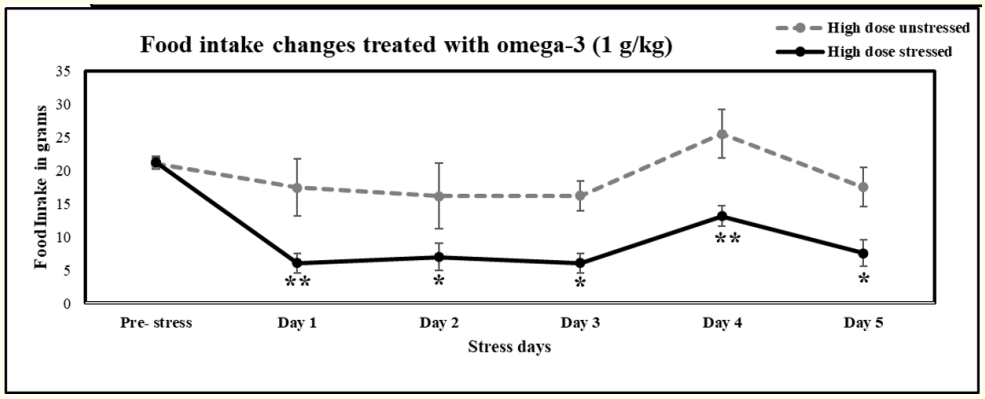


Figure 2: *Effects of single (2 hours) and repeated (2hrs/day for 5 days) immobilization stress with and without omg-3 (0.5 g/kg and 1 g/kg) administration on food intake with prior 15 days omg-3 treatment. Data represents as mean ± SD (n = 6). Significant difference by Tukey's test *p < 0.05 and **p < 0.01) from respective day unstressed animals following three-way ANOVA repeated measure design.*

Figure 3: Effects of single and repeated immobilization stress on behavior in EPM on rats pre-treated with omg-3 for 15 days

Data on time spent in the open arm of EPM (Figure 3A) after single and repeated immobilization stress was analyzed by a three-way ANOVA (repeated measure design). Three-way ANOVA revealed significant effects of omg-3 ($F = 7.185$ df2, 30 $p < 0.05$), stress ($F = 6.428$ df1, 30 $p < 0.05$) and interaction between omg-3 x stress ($F = 243.905$ df2, 30 $p < 0.01$). Effects of repeated measure (days) ($F = 244.955$ df1, 30 $p < 0.01$), repeated measure (days) x stress interaction ($F = 77.453$ df1,30 $p < 0.01$) and interaction of repeated measure (days) x omg-3 x stress ($F = 87.926$ df 2,30 $p < 0.01$) were also significant. The effect on the interaction between repeated measure (days) x omg-3 ($F = 0.197$ df 2,30 $p > 0.05$) was not significant.

Data on several entries in the open arm of EPM (Figure 3B) were analyzed by three-way ANOVA (repeated measure design) and showed a significant effect on the interaction between omg-3 x stress ($F = 277.732$ df 2,30 $p < 0.01$). Significant effects were also observed on the repeated measure (days) ($F = 26.110$ df 1, 30 $p < 0.01$), the interaction between repeated measure (days) x omg-3 ($F = 29.485$ df 2, 30 $p < 0.01$), repeated measure (days) x stress interaction ($F = 149.121$ df 1, 30 $p < 0.01$) and interaction of repeated measure (days) x omg-3 x stress ($F = 179.499$ df2, 30 $p < 0.01$). Effects of omg-3 ($F = 3.076$ df 2, 30 $p > 0.05$) and stress ($F = 0.350$ df 1,30 $p > 0.05$) were not significant.

Post-hoc analysis showed that time spent and number of entries in open arms in water treated stressed animals were decreased after single and repeated immobilization stress compared to the respective unstressed group. Both the parameters were increased at low-dose stressed animals compared to the unstressed group. High doses decreased the time spent and the number of entries after single immobilization stress but increased after repeated immobilization stress compared to the respective unstressed group. Low-dose and high-dose unstressed groups decreased both the time spent and the number of entries after single and repeated immobilization stress. These results suggest that omg-3 PUFAs had anxiogenic effects in an unstressed condition however, it reduced stress-induced anxiety when given to stress animals.

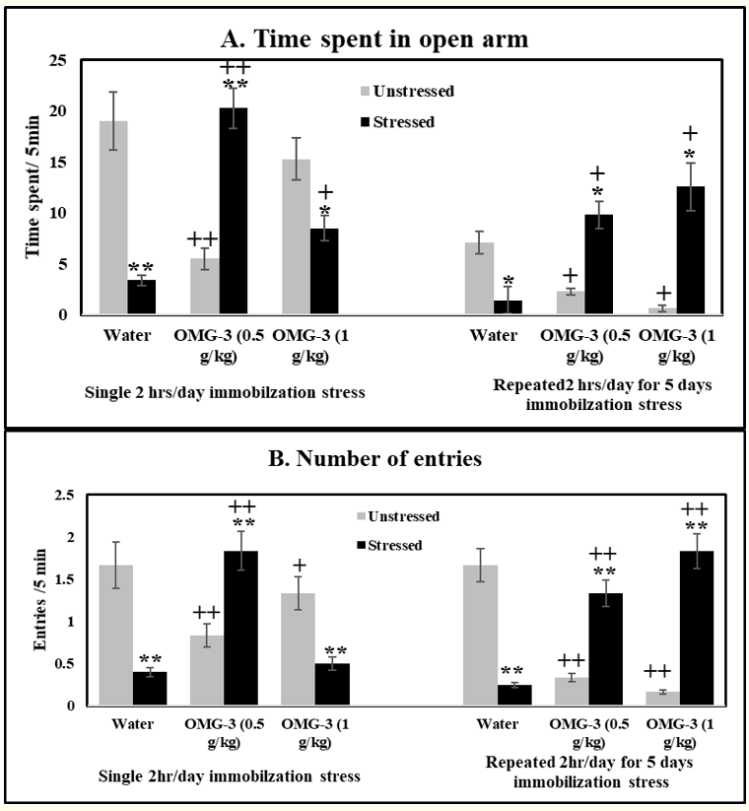


Figure 3: *Effects of single and repeated immobilization stress with and without omg-3 (0.5 g/kg and 1 g/kg) administration on behavior in EPM test with prior 15 days of treatment of omg-3. Data represents as mean ± SD (n = 6). Significant difference by Tukey's test *p < 0.05 and **p < 0.01) from respective unstressed animals, +p < 0.05 and ++p < 0.01) from respective water-treated animals following three-way ANOVA repeated measure design.*

Figure 4: Effects of single and repeated immobilization stress on activity in an open field on rats pre-treated with omg-3 for 15 days

Data on several squares crossed in open field test after single and repeated (2hrs for 5 days) immobilization stress was analyzed by three-way ANOVA (repeated measure design) and showed significant effects of omg-3 ($F = 19.274$ df2, 30 $p < 0.01$), stress ($F = 322.803$ df1, 30 $p < 0.01$) and interaction between omg-3 x stress ($F = 43.678$ df2, 30 $p < 0.01$), repeated measure (days) ($F = 27.094$ df 1,30 $p < 0.01$), the interaction of repeated measure(days) x omg-3 ($F = 6.310$ df 2,30 $p < 0.05$), repeated measure(days) x stress interaction ($F = 29.932$ df1, 30 $p < 0.01$). The effect on the interaction of repeated measure x omg-3 x stress ($F = 2.740$ df2, 30 $p > 0.05$) was not significant. Post-hoc analysis showed that omg-3 (0.5 g/kg and 1 g/kg) increased activity in an open field in stressed animals as compared to the water treated stressed animals after single stress but decreased when compared to the unstressed group. also, after repeated immobilization stress omg-3 (0.5 g/kg and 1 g/kg) increased activity in an open field in stressed animals as compared to the water treated stressed animals after single stress but decreased when compared to the unstressed group, suggesting that omg-3 stress-induced increased in motor activity. These results suggested that omg-3 attenuated stress-induced decreased motor activity in the open-field test.

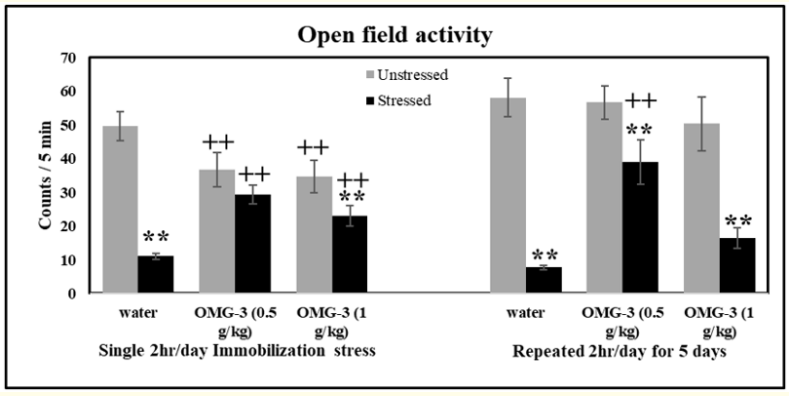


Figure 4: *Effects of single and repeated immobilization stress with and without omg-3 (0.5 g/kg and 1 g/kg) administration on activity in an open field test with prior 15 days treatment of omg-3. Data represents as mean ± SD (n = 6). Significant difference by Tukey's test *p < 0.05 and **p < 0.01) from respective unstressed animals, +p < 0.05 and ++p < 0.01) from respective water-treated animals following three-way ANOVA repeated measure design.*

Discussion

The present study showed that the administration of omg-3 at a low dose (0.5 g/kg) attenuated repeated immobilization stress-induced decrease in body weight. However, stress-induced decrease in food intake was attenuated at the high dose of omg-3 (1 g/kg). Omg-3 produced anxiogenic effects in stress-induced anxiety in stressed animals. Stress-induced decrease in motor activity was also inhibited by omg-3 treatment.

Previous studies suggested that omg-3 fatty acids were known to reduce visceral fats in obese rodents but that is less certain in humans [10]. However, clinical studies of Munro IA., *et al.* [11] showed that omg-3 cannot reduce weight in humans but another study [12] showed the reduction of body fat only in women and reported against any weight gain effects of omg-3 in men. While the pre-clinical study of [13] showed that omg-3 PUFA has a very important role in body restoration in obese diabetic mice following adiponectin regulation and also it can enhance insulin sensitivity over prolonged administration. Conversely, the studies of [14-16] observed no statistical difference in body weight after the supplementation of omg-3 LC-PUFA compared with control in rodents. Rats fed with the omg-3 enriched diet had a lower stress-induced weight loss but no changes were observed in total food intake [17]. Further, the study of [18] showed the dissociation between restraint-induced inhibition of food intake and body weight loss. The present study showed that omg-3 attenuated stress-induced decreases in body weight and food intake.

Previous studies showed both anxiogenic and anxiolytic effects of omg-3 in OF test and EPM activity after restraint and immobilization stress. The omg-3 diet did not affect general activity in the open field, but rats consuming omg-3 PUFA showed less reactivity in OF [19]. Further, the study of [20] showed that in restraint stress the control stress lowered the entries to the open arm of the EPM while the supplementation of omg-3 increased the number of entries to the open arm of the EPM suggesting that it reduced stress-induced anxiety, however, locomotor effects in OF were not affected. Parallel to that another study of [1] also found the same results with the supplementation at chronic restraint stress. The present study showed that omg-3 produced an anxiogenic effect in unstressed animals at both doses (0.5 g/kg and 1 g/kg). However, in line with previous research, the current study showed that the administration of omg-3 at a dose of

0.5 g/kg and 1g/kg enhanced the number of entries and time spent in the open arm of the EPM, suggesting that omg-3 inhibited stressed induced anxiety and produced anxiolytic effects only in stress condition. Stress-induced decrease in locomotor activity in the arena of OF was also reduced by the administration of omg-3.

Conclusion

In conclusion, the present study shows the beneficial effects of omg-3 in stress conditions, which suggests that it will improve stress-induced behavioral deficit.

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Volume 18 Issue 1 January 2023
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