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A comparative neurobehavioral study of sesame oil and fish oil on experimental animals

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Abstract: Natural oils are enriched with polyunsaturated fatty acids (PUFAs) which are important for our health. Recent experimental data explained that PUFAs might have a beneficial effect on various brain functions such as anxiety, dementia, epileptic seizures, depression or bipolar and other neurobehavioral diseases. The objective of the current research work was to evaluate the effect of sesame oil, fish oil and mixture of both oils (1:1) on neurobehavioral changes and cognition. For this purpose shark fish oil and sesame oil were extracted out and there poly unsaturated and saturated fatty acids were analyzed by using GCFID that exposed the presence of different PUFs in shark fish oil, sesame oil and mixture of both oils. Neurobehavioral changes were seen after 5ml/kg/day sesame oil, 5ml/kg/day shark fish oil and 1:1 combination of both oil 5ml/kg/day administration on open field, cage crossing, light and dark, stationary rod, forced swimming induced depression test and water maze test. Our GCFID results showed sesame and fish oil enriched with higher amount of PUFs and showed significant anxiolytic and antidepressant like effect after 30 days of treatment ($P<0.05$) however combination of these both oils exhibited greater efficacy ($P<0.01$) in reducing anxiety and depression as imipramine standard drug. Results showed that combination of both oils (sesame oil and fish oil) could be a better option to treat neurobehavioral problems as compared to alone.

Keywords: PUFs, neurobehavioral diseases, sesame oil, fish oil, neuroprotection.

INTRODUCTION

Natural oils are enriched with polyunsaturated fatty acids (PUFAs) like omega-6 (n- 6) fatty acids present in high concentrations in soy, corn, safflower, sesame, and sunflower oils in contrast, omega-3 (n-3) fatty acids present in leafy green vegetables and in flaxseed, canola oil and peanut oil (Johnson *et al*, 2018; James *et al*., 2000). Recent researches highlighted the beneficial effects of PUFAs in the treatment of many metabolic brain and coronary heart disorders (Horikawa *et al*, 2018; Din *et al* 2004), In order to minimize the risk of rapid cardiac loss, memory loss and chronic inflammatory disorders natural oils play significant role for our health (Tsujiguchi *et al*, 2019; Bauman-Fortin *et al*, 2019; Zarate *et al*, 2017). Despite the cardiac vascular system effects there are several other uses of PUFA's have been reported by recent researchers. These studies explained that, n-3 and n-6 fatty acids might have a valuable influence on several brain functions such as epileptic seizures, synaptic plasticity, memory retrieval, motor coordination, depression and anxiety (Lindseth and Petros, 2016; Hussain *et al*, 2013; Gomez-Pinilla, 2008; Hibbeln, 1998 and 2009). It is anticipated that n-3 and n-6 fatty acids exert their neuroprotective effect by limiting neuronal excitability and decreasing the brain inflammation due to inhibiting cytokines and prostaglandins production (Dyall, 2015; Denis *et al*, 2015). Neural inflammation, cognitive decline, Alzheimer's diseases, depressive disorders and Parkinsonism's disease have been associated with an unnecessary stimulation of glutamate receptors, cytokines and prostaglandins production (Naeem *et al*, 2019; Galic *et al*, 2012). Simultaneously excessive stimulation of glutamate receptors is also linked with unnecessary influx of Ca^{2+} ions and leads towards glutamate toxicity. Omega 3 and 6 PUFAs decrease or limit neuronal cells destruction, while decreasing prostaglandins production, oxidative stress and decreasing glutamate poisoning by alteration in Ca^{2+} influx (Frautschy and Cole, 2011; Hein and O'Banion, 2009; Gammone *et al*, 2018). Furthermore previous researches confirmed that the animals consuming PUFAs particularly DHA (Docosahexaenoic acid), EPA (Eicosapentaenoic acid) omega 3 and 6 fatty acids in their regular diet compared with the animals that consumed PUFA's poor in their diet precipitates memory loss, mood disorders and other inflammatory disorders (Frautschy and Cole, 2011; Saini *et al*, 2018).

In current study we have selected two different natural resources including plant and marine derived oils to compare their neuroprotective effect. Traditionally fish oil and sesame oil are being used as pain relieving agents and for treating cardiac related problems as they have many professed health benefits and may be promising

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nutraceuticals (Muller *et al*., 2015). Various biological active compounds are present in sesame seed oil (*sesamum indicums* L.) like sesamolin, sesamin and other omega-3 and omega-6 polyunsaturated fatty acids. Similarly, (EPA), (DHA, n-3 and n-6 fatty acids are also present in fish oil especially in shark fish (*Carcharhinus bleekeri*) (Aslam *et al*., 2017; Saify *et al*., 2003). Previously sesame oil and fish oil have been reported for their different therapeutic actions especially these two oils had been used for the treatment of major depressive disorders and other brain related diseases (Ahmed *et al*., 2006; Hsu *et al*., 2006; Grosso *et al*, 2014; Aslam *et al*., 2007). Lauritzen *et al*, 2000 investigated that fatty acids inactivate neuronal loss via inhibition of glutamatergic transmission. These novel rising function and utilization of fatty acids containing fish oil and sesame oil in the prevention of neurodegenerative diseases now make one of the central beneficial developments of the nutraceuticals.

We designed current experimental study to explore potential neuroprotective role of polyunsaturated fatty acids by mixing sesame and shark fish oil (1:1) for their anxiolytic and antidepressant like effects, as previously combination of these oils were not been studied in determination of neuroprotective effect,

MATERIALS AND METHODS

Sample collection and identification

The seeds of *Sesamum Indicum L.* seeds were procured from local market of Karachi, Pakistan. The identification of plant species was done by Department of Botany University of Karachi, Karachi, Pakistan. The herbarium number is allocated as BDUK-20089. A sample specimen of the *Sesamum indicum* L. seeds was preserved in the Herbarium, Faculty of Pharmacy Hamdard University, Karachi, Pakistan for future reference.

Marine shark fish *(Carcharhinus bleekeri*) were purchased from a local supplier in Karachi and identified by Zoologist of Department of Zoology, University of Karachi, Pakistan. Shark fishes stored at -20C until used for the assay and were dissected out after 5 days, their livers were collected out and placed on filter paper to remove moisture then weighed.

Chemicals and drugs

All standard graded chemicals (sigma Aldrich) such as n-hexane, chloroform, methanol, anhydrous sodium sulfate, ethylenediamine tetraacetic acid and normal saline were used during this experimental work. The standard drug Imipramine (Tofranil 25mg) was used as give sample.

Extraction of sesame oil

The Soxhlet extractor was used to obtain the sesame oil from seeds. The 100 grams of the sesame seeds was initially taken in a Soxhlet apparatus for oil extraction , n-hexane (40 to 60°C B.P) was used as solvent extractor 150 ml volume of n-hexane were taken Soxhlet and extraction were repeated after 6 hours until the required amount were achieved. After oil extraction Rotary evaporator (Eyela, Japan) was used to remove the excess solvent from sesame oil. Thus the sesame oil %age yield was calculated and stored in air tight jar at cold place (refrigerator) (Mohammed and Hamza 2008).

Extraction of shark fish oil

For the extraction of shark fish oil the solvent extraction procedure was used with few modifications (Bligh and Dyer, 1959; Kinsella *et al*., 1977). The 50g chopped shark fish liver in a mixture of methanol 85ml and chloroform 45ml were mixed for 120 second in a blender. After this further 45ml chloroform was added to the mixture and further mixed for 20 to 30 seconds again 45 ml distilled water was added into the mixture and mixed well and then filtered through Whatman. 1 filter paper on a funnel with vacuum suction. After that about 20ml of chloroform was again used to rinse the remainder. At the end, Ethylenediamine tetraacetic acid was added to the extracted shark fish oil as an anti-oxidant to minimize lipid per oxidation (Ghaly *et al*., 2010). After oil extraction Rotary evaporator (Eyela, Japan) was used to remove the excess solvent from filtrate. Then the total extracted oil was weighed and used for percentage yield calculations. The shark fish oil was placed in cold place in air tight jar (refrigerator) wrapped with foils (aluminum) to protect from light exposure.

Gas chromatography flame ionization detection method

Gas chromatography-flame Ionization detection method using the Agilent technologies 7890 B Gas Chromatography systems under the operating condition Agilent HP-88 capillary column was used for analysis of different compounds. GC peak areas according to the concentrations of compound were identified. The FID was recorded at 240^{o}C. The injection temperature was 220^{o}C. The initial temperature maintain during GC-FID was 50^{o}C and hold for 10 minutes. Further 5^{o}C per minute was raised and ended up to 240^{o}C as final temperature. The results for the extraction (sesame oil, fish oil and (SO+FO) oil) was given on the basic of GC elution time.

Animals selection and housing

Long-term administration of sesame oil, fish oil and mixture of both oils (1:1) were carried out on male albino mice for determining the neurobehavioral changes. 25-30 gm of albino mice were obtained from Dow University, Karachi, Pakistan and were kept in the well ventilated cages in animal facility of the pharmacology Department, Hamdard University, Karachi, Pakistan. All mice were kept under a required cycle of 12/12h (light/dark). The standard diet and tap water was provided to all animals and temperature was regulated between 22 and 25°C accordance with the guidelines for the care and use of experimental animals (Helsinki, 2004). The animal study was approved by the Animal Ethics Committee of

Hamdard University, Karachi, Pakistan. All albino mice were divided into 5 groups n=10.

Experimental protocol

All experimental mice were housed before 1 week in a research lab for familiarization. In this study they were categorized in to (05 groups) each contain n=10. For the CNS activities (open field, cage crossing, stationary rod, light and dark, water maze activity and forced swimming induced test) all treated animals were administered their respective doses orally according to the body weight of animal for 30 days. The dosing protocol was as follows:

Group I: Sesame oil (SO) 5ml/kg/day 0.1ml bd (Saleem *et al*., 2013)

Group II: Fish oil (FO) 5ml/kg/day 0.1ml bd (El Daly, 1997)

Group III: Mixture of sesame oil and fish oil (SO+FO) 1:1 combination of SO+FO 0.2ml bd

Group IV: Imipramine (Standard drug) 10mg/kg/day (Upadhyay *et al.,* 2016)

Group V: Normal saline (Control)

Neurobehavioral evaluation

Open field activity

The test imparts a special chance to completely assess novel environment exploration, general locomotor activity and provide a primary screen for anxiety-related behavior in rodents (Prut, and Belzung, 2003; Belzung and Griebel, 2001; Walsh and Cummings, 1976). Apparatus was consisted of twenty five equally designed squares with surrounding walls that helps to avoids escaping of animals. The test is valuable tool to assess anxiety or depression like behavior with locomotive impairment in animal models (Raben *et al*., 2000; Nagaraju *et al*., 2000). All the animals were placed in the central arena of the apparatus then in 10 minutes cut off time, total numbers of peripheral squares crossed were counted along with time spend in central area for each group. This test was repeated after every 10, 20 and 30 days of sesame oil, fish oil, (SO+FO) oils, Imipramine and saline control drug administration.

Cage crossing test

This test was used to evaluate the locomotor behavior in rodents. It is made up of transparent plastic with surrounded walls (26cm length x26cm width x 26cm height). All standard, control, sesame oil, fish oil and mixture oil (treated) animals were kept in the clear cage separately and in ten minutes cut off time, number of total cage crossings were noted down after 10, 20 and 30 days after the administration of sesame oil, fish oil, (SO+FO) oils, Imipramine standard drug and normal saline control drug (Prut and Belzung, 2003).

Stationary rod test

In this test mice were trained for 3 consecutive days prior to the first testing date by repeatedly placing the mice on the rod until they were able to remain on a rod rotating at a constant speed. In this test during the first trial of the day, mice were placed on the stationary rod and allowed to become familiar with the environment for 1 minute. On each testing day, each mouse was tested for three trials with a 10-min inter-trial resting period. For each consecutive trail, mice were allowed to become acquainted with the environment for 30 s. All the mice were then allowed to walk, individually and the time of crossing the rod to another platform was noted before administration of drug and the experiment was repeated after 10days, 20days and 30 days after the administration of sesame oil, fish oil, (SO+FO) oil, Imipramine standard drug and normal saline control drug (Kishioka *et al*., 2009).

Light and dark test

The light and dark tests, is used to examine anxiety-like behavior in rodents. In this test the subject is exposed to a new environment with protected (dark area) and unprotected (light area) (Khan and Haleem, 2008). Mice naturally show a preference for the dark, protected area (Crawley and Goodwin, 1985). The important measure for assessing anxiety-related behavior in this test is a change in motivation to explore the illuminated, unprotected area, reflected in increases or decreases in the number of transitions between the compartments and in time spent in each compartment, during a 10-min test session were recorded for both control and for sesame oil, fish oil & (SO+FO) oils treated groups for 10 minutes (Peng *et al*., 2000).

Morris water maze test

The Morris water maze (MWM) is a special procedure of spatial learning for rodents. There were many water mazes have been developed, but a unique method to assess spatial or place learning is referred as the Morris water maze (MWM) (Nunez, 2008). However, several characteristics have contributed to the prevalent use of the MWM, such as the lack of required pre training, its high reliability across a wide range of tank configurations and testing procedures. Mice were trained in the absence of the platform on the first day for 60 s. The trial sessions were separated by 1 day. Trial sessions were stopped when the mouse can't find the platform within two minutes, and an escape latency of two minutes was recorded. After training session the trail session was started in which the retention latency was recorded (RL; the time taken by each rat to place the unseen platform) and the experiment was repeated the experiment after 10,20 and 30 days after the administration of sesame oil, fish oil, (SO+FO) oils, Imipramine standard drug and normal saline control drug.

Forced swimming induced test

In this test the animals are forced to swim in a cylinder from which they cannot escape (Porsolt *et al*. 1977). FST apparatus was made up of acrylic glass cylinder with twenty centimeter height and six centimeter diameter

(Matsuda *et al*., 1995). For developing swimming induced depression in animals, the FST container filled with water (25±2°C) up to twelve centimeter high. All control and oil treated animal groups were presented in the cylinder separately for induction of depression and struggling time was noted of each animal with cut off 10 minutes time, after 10, 20 and 30 days administration of sesame oil, fish oil, (SO+FO) oils, Imipramine as standard drug and normal saline control drug.

STATISTICAL ANALYSIS

Data are expressed as means ± S.E M and were analyzed by using software SPSS version 21. Descriptive statistics were performed by *t* test. The experimental data on the effect of all treatment oils on, open field activity, cage crossing activity, stationary rod activity, light and dark area activity, water maze activity and forced swimming induced depression was analyzed by one way ANOVA, the significance of the differences between averages of above experimental results were determined by Bonferroni test, Whereas P-value less than 0.05 was considered significant, P-value < 0.01 was considered as highly significant.

RESULTS

% age yield of Lipid/Oil Content

The overall percentage yield of the oil (lipid content) of shark fish liver samples in this study was 19.97g/50g of wet liver tissue. However total yield of sesame oil by soxhlet method was 58.93g/100g.

Gas chromatography analysis of sesame oil, fish oil and (SO+FO) oil

Sesame oil, fish oil and mixture oil were examined and analyzed by gas chromatography using Agilent technologies 7890 B Gas Chromatography system under the operating condition Agilent HP-88 capillary column. The % age composition of fatty acids were obtained from electronic integration measurement by using flame Ionization detection (FID) method. All the compounds and their relative FID area % of the characterized components of sesame oil, fish oil and mixture oil as shown in fig. (1 a), (1b) and (1 c) respectively. Results indicated a large variation between sesame seed oil, shark liver oil and oil mixture of sesame and fish oil. Twenty-three individual fatty acids from sesame seed oil, twenty-six fatty acids from shark fish oil and thirty-one fatty acids from oil mixture (SO+FO) were analyzed.

Neurobehavioral studies

Effect of sesame oil, fish oil and mixture oil on open field activity

In the open field test the squares crossing activity of sesame oil, fish oil and (SO+FO) oil treated animals significantly (P<0.05) increased after 10th day (159.50±19.18, 271.40±22.57 and 247.10±13.63) respectively and at 20th day of dosing (221.60±21.19, 235.80±19.23 and 287.70±8.79) as compared to control group while this activity was significantly reduced (P<0.000) after 30 days of treatment (157.70±16.01, 215.70±19.04 and 231.70±13.25) respectively as compared to control group. Data illustrated in table 1 analyzed by post hoc Bonferroni test explained that mixture oil treated group exhibited highly significant anxiolytic effect on day 10 and day 20 as compared to sesame and fish oil treatment group (F(4, 45)=8.12, P<0.01) however after 30 days of mixture oil treatment moderate reduction was seen in number of square crossing activity (P<0.05). In contrast sesame oil treated animals crossed significantly reduced number of square after 30 days of treatment as compared to control, fish oil and (SO+FO) oil treated group (F (4, 45)=9.16,P<0.05) table 1.

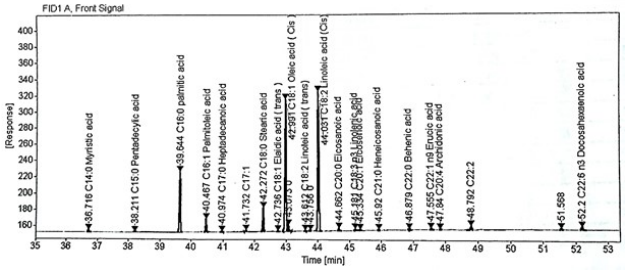

Fig. 1(a): Gas chromatography analysis of sesame oil

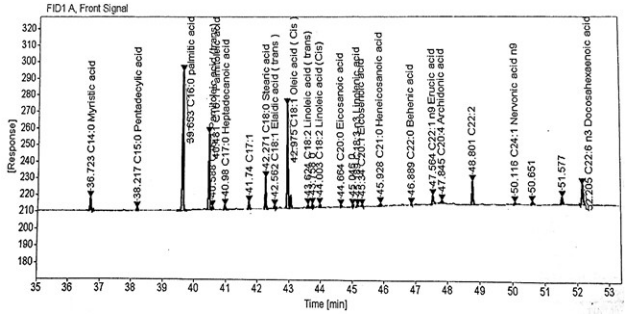

Fig. 1(b): Gas chromatography analysis of shark fish oil

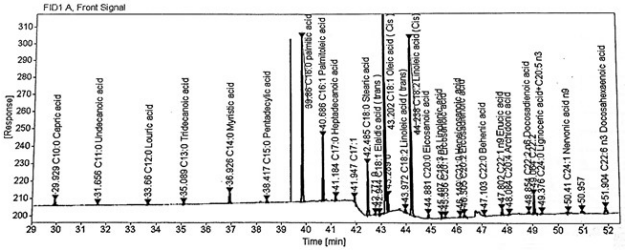

Fig. 1(c): Gas chromatography analysis of mixture oil (SO+FO)

Effect of sesame oil, fish oil and mixture oil on cage crossing test

The result of cage crossing test showed significant differences among the sesame oil, fish oil and (SO+FO) oil treatment groups as sesame and fish oil treated animals showed highly significantly (F (4, 45)=166.2,P<0.01)

improved cage crossing activity after 10th(60.50±0.87 and 61.10±0.92) and 20thday (44.20±1.83 and 48.90±1.94) as compared to control while (SO+FO) oil treated group exhibited moderate significance (F(4, 45)= 24.5, P<0.05) after 10 (41.30±1.19) and 20th(40.80±1.67) days of treatment as compared to control. After 30th days of sesame oil and fish oil treatment significantly (F (4, 45)= 132.8, P<0.01) reduced cage crossing activity (41.90±0.72, 40.50±1.73) as compared to control. table 2 analyzed by post hoc Bonferroni test represented that (SO+FO) oil treated group after 30th days of dosing significantly improved cage crossing activity (50.30±1.19) as compared to sesame oil treated, fish oil treated and control group (P<0.05). The Imipramine animals showed similar effects just like to mixture oil.

Effect of sesame oil, fish oil and mixture oil on stationary rod test

The results of the stationary rod activity showed that the time to reach opposite plat form of sesame oil and fish oil treated group were significantly reduced after 10th (9.00±0.63 and 10.90±0.52) and 20th (6.70±0.59 and

Table 1: Effect of sesame oil, fish oil and mixture oil on open field activity

Days	No. of squares crossed in 10 minutes					P Value
	No. of Groups					
	Control	Sesame oil	Fish oil	Mixture oil	Standard	
10	175.50±3.60	159.50±19.18*	271.40±22.57***	247.10±13.63**	233.20±14.01**	P<0.000
20	184.40±9.29	221.60±21.19*	235.80±19.23**	287.70±8.79***	280.10±11.94***	P<0.000
30	180.00±8.99	157.70±16.01*	215.70±19.04*	231.70±13.25**	264.20±9.47***	P<0.000

Table 2: Effect of sesame oil, fish oil and mixture oil on cage crossing test

Days	No of cage crossed in 05 minutes					P Value
	No. of Groups					
	Control	Sesame oil	Fish oil	Mixture oil	Standard	
10	32.80±1.38	60.50±0.87**	61.10±0.92**#	41.30±1.19*	54.20±1.32**	P<0.000
20	29.80±1.87	44.20±1.83*	48.90±1.94**	40.80±1.67 *	50.30±0.70**	P<0.000
30	29.70±1.07	41.90±0.72*	40.50±1.73*	50.30±1.19 **	54.10±1.26**	P<0.000

Table 3: Effect of Sesame oil, fish oil and mixture oil on stationary rod test

Days	Time to reach Opposite Plat form (sec)					P Value
	No. of Groups					
	Control	Sesame oil	Fish oil	Mixture oil	Standard	
10	11.50±0.71	9.00±0.63	10.90±0.52	12.40±1.04	10.90±0.62	P<0.032
20	13.80±1.03	6.70±0.59**#	7.30±0.51**#	10.60±0.52*	11.10±0.54	P<0.000
30	13.70±0.87	8.20±0.71**	8.70±0.76**	9.50±0.58*	10.10±0.52*	P<0.000

Table 4(a): Effect of sesame oil, fish oil and mixture oil on light area test

Days	Time spent in light area (minutes)					P Value
	No. of Groups					
	Control	Sesame oil	Fish oil	Mixture oil	Standard	
10	2.51±0.01	3.39±0.10**	3.46±0.14**	4.59±0.14**	4.32±0.03**	P<0.000
20	2.56±0.05	1.92±0.11** #	3.00±0.13*	4.17±0.06** #	3.38±0.02*	P<0.000
30	3.24±0.14	2.77±0.09*	2.87±0.34	3.05±0.02	3.10±0.05	P<0.334

Values are expressed in mean ± S.E.M data analyzed by one way ANOVA, POST HOC analysis by Bonferroni test which represent *P < 0.05, **P<0.01 and ***P<0.000 as compared to control whereas #P < 0.05, ##P<0.01 and ###P<0.000 as compared to standard.

Table 4(b): Effect of Sesame oil, fish oil and mixture oil on dark area test

Days	Time spent in dark area					P Value
	No. of Groups					
	Control	Sesame oil	Fish oil	Mixture oil	Standard	
10	7.48±0.01	6.59±0.09*	6.53±0.14 *	5.40±0.14**	5.67±0.03**	P<0.000
20	7.43±0.05	7.07±0.11 #	6.99±0.13*	5.82±0.06** #	6.61±0.02*	P<0.000
30	6.75±0.14	7.22±0.09	7.12±0.34	6.94±0.02	6.89±0.05	P<0.334

Table 5: Effect of sesame oil, fish oil and mixture oil on water maze (Trial Session)

Trial Session	Time to reach Platform (sec.)					P Value
	No. of Groups					
	Control	Sesame oil	Fish oil	Mixture oil	Standard	
	30.50±1.82	29.90±2.06	31.20±1.96	30.90±1.95	30.80±1.90	P<1.000

Table 6: Effect of sesame oil, fish oil and mixture oil on water maze (Test Session)

Days	Time to reach Platform					P Value
	No. of Groups					
	Control	Sesame oil	Fish oil	Mixture oil	Standard	
10	6.80±0.41	9.90±0.56 $^{**\#\#}$	7.40±0.73	7.80±0.51*	5.90±0.64	P<0.000
20	6.50±0.40	5.70±0.42	6.10±0.48	5.70±0.42	7.10±0.64*	P<0.460
30	6.80±0.41	4.30±0.91 $^{**\#\#}$	4.90±0.52$^{*\#\#}$	4.50±0.40 $^{**\#\#}$	9.70±0.68**	P<0.000

Table 7: Effect of sesame oil, fish oil and mixture oil on forced swimming induced test

Days	Struggling Time					P Value
	No. of Groups					
	Control	Sesame oil	Fish oil	Mixture oil	Standard	
10	1.89±0.09	2.60±0.36	3.25±0.33*	4.11±0.59**	5.02±0.64***	P<.000
20	2.09±0.14	3.10±0.54*	4.02±0.63**	4.35±0.60**	4.97±0.44***	P<.002
30	1.93±0.27	3.60±0.55*	3.88±0.29**	3.60±0.35*	4.55±0.34 ***	P<.000

Values are expressed in mean ± S.E.M data analyzed by one way ANOVA, POST HOC analysis by Bonferroni test which represent *P<0.05, **P<0.01 and ***P<0.000 as compared to control whereas $^{\#}$P < 0.05, $^{\#\#}$P<0.01 and $^{\#\#\#}$P<0.000 as compared to standard.

7.30±0.51) days of dosing (F (4, 45)= 18.91, P<0.001) as compared to control group while (SO+FO) oil treated animals showed insignificant result as compared to control animals after10th (12.40±1.04) and 20th (10.60±0.52) days of dosing (P<1.000). However after 30th day of sesame oil, fish oil and (SO+FO) oil treatment the stationary rod activity was significantly reduced (F (4, 45)= 2.90, P<0.05) as compared to control however as illustrated in table 3 analyzed by post hoc Bonferroni test explained that the sesame oil, fish oil and (SO+FO) oil treated group significantly reduced stationary rod activity (8.20±0.71, 8.70±0.76 and 9.50±0.58) as compared to control group (P<0.000).

Effect of sesame oil, fish oil and mixture oil on light and dark area test

a) Effect of Sesame oil, Fish oil and Mixture oil on Light Area Test

The light and dark area box activity showed that the time spent in light area of sesame oil, fish oil and (SO+FO) oil treated groups significantly increased after 10th (3.39±0.10, 3.46±0.14 and 4.59±0.14) and 20th (1.92±0.11, 3.00±0.13 and 4.17±0.06) days (F (4, 45)=63.72, P<0.000) as compared to control animals. After 30thdays of sesame oil, fish oil and (SO+FO) oil treatments (2.77±0.09, 2.87±0.34 and 3.05±0.02) all treated groups significantly spent less time in light area (F (4, 45)= 1.17, P<0.01) as compared to control and showed similar effect as standard Imipramine group. table 4 (a) analyzed by post hoc Bonferroni test represented that the sesame oil, fish oil and (SO+FO) oil treated group significantly improved time spent in light area activity as compared to control group (P<0.01).

b) Effect of sesame oil, fish oil and mixture oil on dark area test

The result of the light and dark area box activity showed the time to spent in dark area of sesame oil, fish oil and (SO+FO) oil treated groups significantly decreased after 10th (6.59±0.09, 6.53±0.14 and 5.40±0.14) and 20th (7.07±0.11, 6.99±0.13 and 5.82±0.06) days (F (4, 45)= 64.67, P<0.000) as compared to control group. However after 30th day of treatment sesame oil, fish oil and (SO+FO) oil (7.22±0.09, 7.12±0.34 and 6.94±0.02) the time spent in dark area was insignificantly increased (F (4, 45)= 1.18, P<0.334) as compared to control and just like standard Imipramine group. table 4 (b) analyzed by post hoc Bonferroni test explained that the sesame oil, fish oil and (SO+FO) oil treated group significantly decreased time spent in dark area activity as compared to control group (P<0.000).

Effect of sesame oil, fish oil and mixture oil on water maze test

a) Effect of Sesame oil, Fish oil and Mixture oil on Water Maze (Trial Session)

The result of the water maze activity in the trial session showed that the time to reach plat form of sesame oil, fish

oil and (SO+FO) oil treated group showed insignificant differences (29.90±2.06, 31.20±1.96 and 30.90±1.95) as compared to control and standard Imipramine treated group (F (4, 45)= 0.064, P>0.05) as illustrated in table 5 analyzed by post hoc Bonferroni test explained that the sesame oil, fish oil and (SO+FO) oil treated group showed insignificant differences compared to control and standard Imipramine treated group.

b) Effect of sesame oil, fish oil and mixture oil on water maze (Test Session)

The results of the water maze activity showed that the time to reach plat form (escape latency) of sesame oil, fish oil and (SO+FO) oil treated groups were at 10^{th} day (9.90±0.56, 7.40±0.73 and 7.80±0.51), at 20^{th} day (5.70±0.42, 6.10±0.48 and 5.70±0.42) and after 30^{th}day (4.30±0.91, 4.90±0.52 and 4.50±0.40) respectively. The analysis by post hoc Bonferroni test explained significant differences between all treatment groups as sesame oil, fish oil and (SO+FO) oil treated animals showed significantly decreased escape latency after 10^{th} days (F (4, 45)=0.92, P<0.05), 20^{th} days (F (4, 45)=4.97, P<0.05) as compared to control however after 30^{th} days (F (4, 45)=35.17, P<0.01) all these treatment groups showed significant decreased escape latency as compared to control and even Imipramine group as shown in table 6 suggesting that all the treatments improved memory after chronic use.

Effect of sesame oil, fish oil and mixture oil on forced swimming induced test

The result of the forced swimming activity showed that the Struggling time of sesame oil, fish oil and (SO+FO) oil treated groups significantly increased (P<0.01) after 10^{th} days (2.60±0.36, 3.25±0.33 and 4.11±0.59) 20^{th} days (3.10±0.54, 4.02±0.63 and 4.35±0.60) and after 30^{th}days (3.60±0.55, 3.88±0.29 and 3.60±0.35) when compared to control group. As illustrated in table 7 analyzed by post hoc Bonferroni test explained that the sesame oil, fish oil and (SO+FO) oil treated group significantly shortened the immobility time interval after 20^{th} day as compared to control group (F (4, 45) =7.40, P<0.001) similarly this effect was significantly sustained (F(4, 45)= 6.51, P<0.05) after 30^{th} days of treatment with all treatment groups in comparison to control group. The standard Imipramine animals showed similar effects just like to sesame, fish and (SO+FO) oil.

DISCUSSION

Researches have established that the consumption of PUFs might beneficially affect neuronal composition including regulation of mood, neurotransmission and cognitive functioning. Epidemiological data emphasized that people who usually consume a diet with rich content of n-3 and n-6 fatty acids are at lower risk of developing major depression, anxiety and bipolar disorder (Tsujiguchi *et al*, 2019; Hibbeln 2009; Golding *et al*., 2009; Noaghiul and Hibbeln 2003). Pharmacological knowledge about the dietary sources enriched with PUF i,e plants or aquatic animals like fish would allow us to evaluate central nervous system activity, which could be used to treat anxiety type of disorders.

In the present study we compared the anxiolytic and antidepressant like effect of sesame oil, Fish oil and mixture of SO+FO and found that All these two oils significantly contain linoleic acid, oleic acid, palmitic acid, stearic acid, palmitoleic acid, Myristic acid and other PUFAs shows in (Fig 1(a, b, c)). However we found higher concentrations of different PUF in SO+FO as compared to single SO and FO. PUFAs exert several biological role in human body regulation especially in brain it regulates memory and neuronal plasticity (Müller *et al*., 2015) This is also in correlation to our study in which we found significant antideprassant effect in open field test after 20 days administration of sesame oil, shark fish oil and mixture of these two oils reflected by an increase in central area crossings with increase in the number of rearing. Whereas at day 30 we observed decreased number of squares crossing in open field (table 1) which reflects anxiolytic effect of S0, FO and SO+FO. Our results are in agreement with previous studies which explained that PUFAs improves dopamine levels in brain especially in mid brain and improves locomotion and motor coordination mediated by nigral dopaminergic system (Sublette *et al*., 2014). PUFAs are essential part of neuron and play crucial role in brain development. As reported PUF's perform vital role in neuronal cell regeneration and neuronal growth, as well as by inhibitng oxidative stress may develop synaptic processing of neural cell that intensify memory and mood (Kavraal *et al*., 2012). Colucia *et al*., (2009) reported that prolong administration of PUFAs in adult rats improve motor performance and coordination.

In the cage crossing activity SO, FO and SO+FO treated mice showed significant increase in the cage crossing activity after 10 and 20 days of treatment. As shown (table 2) that represents all of these oils gradually increased number of cage crossing after 10 and 20 days of treatment which indicates their anxiolytic effect. As sesame oil contains different PUFAs like omega n-3 and n-6 fatty acid (Asghar and Majeed, 2013). Previous researchers explained that fatty acid exert their physiological effects due the presence of ketone bodies. These ketone bodies upon consumption are converted in to Gamma-Aminobutyric acid (GABA) responsible for reducing action potential and give calming effect just like to other anxiolytics (McNally and Hartman, 2012). Another in vitro study suggest that rats brain cerebrum after PUFAs treatment showed advanced N- methyl D-aspartic acid (NMDA) response in proximal neurons after the activation of glycine (Kato, 2015). This activated NMDA receptor may increase the inhibitory Gabaergic transmission onto hippocampus and result in decrease fear

and anxiety (Lu *et al*., 2012). Similarly, after 30 days of chronic dosing the cage crossing activity were found highly significantly decreased. These results taking together indicate that, in contrast to imipramine the SO, FO and SO+FO showed antidepressant like effects after 30days without affecting locomotor activity. These results are in accordance with research by Su *et al*., (2013) who reported that PUFAs are involved in reducing anxiety by involving in brain biogenic amine regulation.

In stationary rod experiment SO, FO and SO+FO treated mice showed improved motor coordination as mice took less time to reach to opposite platform as compared to control group which indicates all these oils can improve motor control and attentional processes which are represent in (table 3). Sesame oil and fish oil contains (linoleic acid, oleic acid, palmitic acid, stearic acid, palmitoleic acid, myristic aid) and other PUFAs, which improves dopamine levels in brain especially in mid brain and improves locomotion and motor coordination mediated by nigral dopaminergic system (Sublette *et al*., 2014). Furthermore Colucia *et al*., (2009) reported that prolong administration of fish derived PUFAs in adult rats improve motor performance and coordination.

One of the most widely used animal models for screening putative anxiolytic effect is the light and dark test and the number of transitions between these two compartments reflects locomotor activity. The locomotors activity is considered to be a sign of alertness that represent normal behavior (Thakur and Mengi, 2005). Our result represents that sesame oil, fish oil and SO+FO treated animals spent more time in light area than normal animals after 20th days however gradually reduced after 30th day of treatment than control group. This behavior showed their anxiolytic activity after 20 day of dosing and antidepressant like effect after 30 days of dosing. Experimental researchers suggest that PUFAs deficiency responsible for decreasing production of dopamine in the brain that ultimately exert depression and stress (Mocking *et al*, 2016; Sarris *et al*., 2015; Rucklidge *et al*., 2013; Grosso *et al.*, 2014). PUFAs decreases the degradation of dopaminergic receptors and up regulation the dopamine levels in mid brain and cortex thus prevent anxiety and depression (Liu, 2018). Sesame oil, fish oil and SO + FO treated mice initially had decreased stay time in dark area after 10 days dosing and gradually increased after 20 and 30 days of dosing which represents its anxiolytic effect first than after chronic dosing antidepressant like effect. This effect can be attributed to its sesamin and sesamolin content, as Guo *et al*., (2016) reported that in chronic pain experimental animal model sesame oil diminishes anxiety and alter mood by modifying excitatory and inhibitory transgenic in the mice brain and exert benzodiazepine like effect. Similarly fish oil long-term dietary supplementation positively impacts on anxiety and cognitive performances (Vinot *et al*., 2011).

In Morris water maze test sesame oil, fish oil and oil mixture treated mice showed insignificant change in latency time to reach platform after 10 and 20 days treatment however after 30 days treatment sesame oil, fish oil and oil mixture treated mice have significantly taken lesser time to reach the platform in water maze that showed reduced latency and improved memory. As discussed earlier the sesame oil and fish oil are capable of producing neuroprotection due to their EPA and DHA type of PUF's which exert strong inflammation resolving effects in brain and inhibits IL-1 β-stimulated expression of cyclooxygenase-2 (COX-2) in glial cells intern limits neuroinflammation and improve memory (Mayurasakorn *et al*., 2011). Downregulation of COX2 derived prostaglandins ultimately decreases the release of IL1 and TNF resulting in improved memory and neuronal plasticity (Bensten, 2018; Yirmiya and Goshen, 2011). Further it is described that PUF's may responsible of stabilising dopamine and acetyl choline levels in brain (prefrontal cortex) and accelerate memory consolidation (Chang *et al*., 2018). As Bauer *et al*., (2014) explained dietary rich n-3 and n-6 fatty acids increases memory consolidation and cognition (Rodríguez-Landa *et al*., 2013; Nielsen *et al*., 1988). In another study reported by Muldoon *et al*., (2014) the lower utilization of n-3 fatty acid like EPA and DHA is linked to late neurodevelopment and memory impairment which ultimately lead towards Alzheimer's disease (AD) in late stage of life.

In the forced swimming test the struggling time activity of sesame oil, shark fish oil and SO+FO treated groups showed gradually increased after 10^{th}, 20^{th} and 30^{th} days of treatment (table 7). These results suggest that both oils and its combination have dose dependent depressant effects. The daily consumption of n-3 and n-6 PUFAs profoundly increases dopamine development in the cerebral cortex inhibit the monoamine oxidase (MAO-B) activity thereby increasing the availability of neurotransmitters at synapse thus improve stress by reducing depression (Arachchige *et al*., 2006; Liu *et al*, 2015). We reported that mixture oil exert better effect than single oils because of (S.O and F.O) simultaneously containing sesamin and highly purified EPA or DHA in the form of ethyl esters that exert neuroprotective effect. Our present study suggest a linked between PUFAs and neurobehavioral changes and confirm that daily intake of in rich PUFAs containing diet may prevent neurobehavioral problems and give neuroprotective effect (Grosso *et al*., 2014; Ide *et al*., 2004; Pusceddu *et al*, 2016).

CONCLUSION

We concluded that supplementation with sesame oil and shark fish oil could protect the anxiety and depression due to their enriched PUFs contents, and the combination of

SO+FO might produce more effective anxiolytic effects than the separate nutrient did. Further we speculate that neuroprotective effects are largely attributable to the reduction of oxidative stress, Although further preclinical and clinical studies are required to explain the antioxidant or other underlying molecular mechanism.

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