doi.org/10.36721/PJPS.2021.34.2.REG.499-506.1

Comparative antioxidant and analgesic effect of sesame oil, fish oil and their combination in experimental animal model

Sadaf Naeem[1,2]*, Liaquat Ali[2], Rahila Ikram[3], Saira Saeed Khan[3], Huma Shareef[1], Yousra Shafiq [1], Nausheen Alam[4] and Anum Adil[3]

[1]Institute of Pharmaceutical Sciences, Jinnah Sindh Medical University, Karachi, Pakistan
[2]Department of Pharmacology, Faculty of Pharmacy, Hamdard University, Karachi, Pakistan
[3]Department of Pharmacology, Faculty of Pharmacy & Pharmaceutical Sciences, University of Karachi, Karachi, Pakistan
[4]Department of Pharmacology, Faculty of Pharmacy, Federal Urdu University Science and Technology, Karachi, Pakistan

Abstract: Natural oils are rich in polyunsaturated fatty acids (PUFs) like omega 3, omega 6 and other nutrients that boost physical and mental health. Traditionally these oils have been used to treat joint pain associated with several inflammatory conditions. In this study, we investigated the antioxidant and analgesic properties of the sesame oil (SO), fish oil (FO) and combination of these two oils (SO+FO). Different concentrations of the SO, FO and SO+FO combination 0.02-4mg/ml were used for assessing the free radical scavenging activity by DPPH method and the IC50 value was calculated. Acetic acid-induced abdominal writhing test, tail immersion and hot plate models were used to determined analgesic effect. Results showed that both oils were well tolerated as no signs of toxicity or death were noticed during the observational study period. SO+FO combination showed the best antioxidant properties as shown by DPPH assay. Similarly in analgesic models, SO and FO significantly reduced the number of abdominal contractions ($p<0.05$) however, SO+FO (1:1) exhibited highly significant results ($p<0.001$) in writhing reflex test. Furthermore, SO and FO both increased the reaction time on a hot plate as well as in tail flick test ($p<0.05$) whereas, SO+FO significantly increased reaction time ($p<0.001$) in hot plate and in tail flick test as compared to SO and FO single treatments. Conclusively, our results suggest that the combination of both oils (SO+FO) exhibited significant antioxidant and analgesic potential that it could be considered as one of the active combinations for relieving pain in adjunctive treatment for joint pain associated with rheumatoid arthritis.

Keywords: Polyunsaturated fatty acids, sesame oil, fish oil, analgesic activity, antioxidant activity, rheumatoid arthritis.

INTRODUCTION

Pain is a common nonspecific manifestation of many disease conditions (Shojaii *et al*., 2015). It is a physiological and pathological condition initiated by any physical and chemical agents and can be treated with medication, as well as by nutritional strategies or using dietary supplements (Ballou *et al*., 2000; Fujino *et al*., 2003). Prostaglandins (PGs) are released after tissue damage and act as important mediators of inflammation, fever and pain, Inhibition of PGs reduces the pain perception and inflammation (Abdulkhaleq *et al*., 2018; Kupeli *et al*., 2003; Singh and Majumdar, 1995). Non-steroidal anti-inflammatory drugs are used to relieve pain, fever and inflammation associated with these harmful stimuli by inhibiting PGs however; these drugs have some side effects including gastric ulcer, bronchoconstriction, kidney failure and cardiac abnormalities (Park *et al*., 2013). Therefore, scientists are now focusing on plants and aquatic sources derived oils as their oils are enriched with PUFs especially n-3 and 6 fatty acids.

Natural fixed 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 physiological functions by exhibiting analgesic, anti-inflammatory, antioxidant and immunomodulating properties (Kristensen *et al*., 2018; Calder, 2017). Various biologically active compounds are present in natural oils like sesame oil contain sesamolin, sesamin & other omega 3 & 6 polyunsaturated fatty acids (PUFAs) as well as fish oil contain eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA) & omega 3 & 6 fatty acids (Goldberg and Katz, 2007). These EPA (eicosapentaenoic acid) and DPA (docosapentaenoic acid) exert a vital health-enhancing role in reducing inflammation & its related disorders. Fish derived omega 3 and 6 fatty acids has molecular mechanisms of inhibiting prostaglandins & cytokines that are involved in anti-inflammatory actions, which makes it a potential health aid in dealing with chronic pain conditions (Lee *et al*., 2012; Abou-Gharbia *et al*., 2000).

Sesame oil (*Sesamum indicum* L. seeds; *Pedaliaceae*) has been employed in the food and pharmaceutical industries due to its high lipids, protein content and distinctive flavor (Brar and Ahuja, 1980). It contains polyunsaturated fatty acids such as oleic acid, palmitic acid, stearic acid, and linoleic acids (Cheng *et al*., 2006). Various studies confirmed that sesame oil showed antioxidant and anticancer properties due to the presence of sesamin,

**Corresponding author:* e-mail: sadaf.naeem@jsmu.edu.pk

sesamolin, omega 3 and 6 poly unsaturated fatty acids (Mohammed and Hamza, 2008).

Shark fish oil *(Carcharhinus bleekeri,* Liver*)* is rich in eicosapentaenoic acid (EPA, C20: 5n-3) and docosahexaenoic acid (DHA, C22: 6n-3). According to Saify *et al*. (2003) 16 shark fish oil contain several saturated fatty acid and unsaturated fatty acids. Among unsaturated fatty acids monoenoic e.g. oleic, linoleic acid and palmitoleic acids were the major constituents and traces of dienoic and trienoic fatty acids were also found (Maroon & Bost, 2006; Kinsella, 1977).

Considering the applications of these oils as medicinal food in pharmaceutical industries due to the presence of PUFs, we designed the present study for the first time to evaluate the antioxidant and analgesic activities of sesame oil, shark fish oil and also the combination of these two oils in animal model. This study will provide scientific evidence to justify the combined analgesic effect of sesame oil and fish oil (1:1).

MATERIALS AND METHODS

Study area

The study was carried out from the month of January to July 2019 in the Department of Pharmacology, Institute of Pharmaceutical Sciences, Jinnah Sindh Medical University, Karachi, Pakistan.

Sample collection and identification

The seeds *of Sesamum Indicum* L. were procured from local market of Karachi, Pakistan. The identification of plant species was done by Botany Department of Karachi University, 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.

Marine shark fish *(Carcharhinus bleekeri)* were purchased from a local supplier in Karachi and identified by Zoologist of Zoology Department, Karachi University, Pakistan. Shark fishes were stored at -20ºC 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 to extract the oil.

Chemicals and drugs

All standard graded chemicals (purchased from 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 Acetyl Salicylic Acid (Aspirin) was procured from local Pharmacy, Karachi, Pakistan.

Extraction of sesame oil

The Soxhlet's extractor was used to obtain the sesame oil from seeds. For extraction purpose, 100 grams of sesame seeds were initially taken in an extraction chamber for oil extraction; n-hexane (40 to 60°C B.P.) was used as solvent. 150ml volume of n-hexane was taken in Soxhlet and extraction was continued for 6 hours to acquire the required amount. After oil extraction, rotary evaporator (Eyela, Japan) was used to remove the excess solvent from sesame oil. At the end, the total extracted oil was weighed to calculate percentage yield and stored in air tight jar at cold place (refrigerator) (Bligh and Dyer, 1959).

Extraction of shark fish oil

For the extraction of shark fish oil, the reported solvent extraction procedure was used with few modifications. Fifty gram of chopped shark fish liver in a mixture of methanol 85 ml and chloroform 45ml was mixed for 120 second in a blender. After this, again 45ml chloroform was added to the mixture and further mixed for 20 to 30 seconds. Again 45ml distilled water was added into the mixture and mixed well and then filtered through filtration assembly through Whattman No. 1 filter paper on a funnel with vacuum suction. After that, about 20 ml of chloroform was again used to rinse the remainder. At the end, ethylenediamine tetra acetic acid (EDTA) was added to the extracted shark fish oil as an anti-oxidant to minimize lipid peroxidation (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 to calculate percentage yield. The shark fish oil was placed in a cold place (refrigerator) in an air tight jar wrapped with aluminum foils to protect from light exposure.

Animals selection and housing

Acute analgesic effect of sesame oil, fish oil and mixture of both oils (1:1) was carried out on adult male mice. 25-30 gm of mice were purchased from Dow University of Health Sciences, Karachi, Pakistan & were kept in well ventilated cages in the animal facility of Pharmacology Department, Jinnah Sindh Medical 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 22-25°C in 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 mice were divided into 8 groups n=6.

Acute toxicity

The determination of the median (LD50) of sesame and fish oil were performed according to the methods described by Lorke (1983) with some modifications. Sixty mice were fasted overnight and were then divided into two sets. Sesame oil was administrated to set I (n=30) orally at the doses of 5, 10 and 15ml/kg while set II (n=30) were treated with fish oil at the same doses orally.

The mice were allowed to access food and water ad libitum and observed for 24h after treatment for signs of acute toxicity or death.

Dosing protocol

A total of 48 male Albino mice were divided into 8 groups (n=6 per group) for each experiment separately, Group A and B: orally treated with 5ml and 10ml/kg/day of SO respectively; Group C and D: orally treated with 5ml and 10ml/kg/day of FO respectively; Group E and F orally treated with 5ml and 10ml/kg/day of SO+FO (1:1) respectively; Group G: orally treated with aspirin 150mg/kg (as positive control) and Group H: on distilled water (control group).

DPPH antioxidant assay

The DPPH assay was performed as described by Fernandes *et al*. (2016). Each concentration (0.02 – 4mg/ml) of sesame oil, fish oil and combination (SO+FO) sample (10 µL) in methanol: Water was added to 190µL of 150µM DPPH in methanol. After vortex mixing, the mixture was incubated for 10 minutes at room temperature and the absorbance values were measured at 517 nm. The sample mixture was kept in dark for 20 minutes, and the absorbance was measured until the reading reached plateau. Ascorbic acid at concentration of 5 mg/ml was used as standard. The differences in absorbance between test sample and Control (DPPH alone) was taken and the IC50 values were determined as the concentration of the sample that gave a 50% decrease in the absorbance from the blank test.

The antioxidant activity was calculated using the following formula:

$$\% \text{ Inhibition} = \frac{\text{Absorbance of control - Absorbance of sample}}{\text{Absorbance of control}} \times 100$$

Acetic-acid induced abdominal writhing test

The writhing test was based on the method described by Koster *et al*. (1959) with modifications in timing of observations. Animals were injected with acetic acid (6%, 10mL/kg) intraperitonially to induce abdominal writhing. A total of 48 mice were divided into 8 groups (n=6 per group) and were pretreated 30 min prior to acetic acid injection to their respective doses. The number of writhing were counted twice (at 15 min intervals) over a period of 5 min after a 5-min lag period after the injection of acetic acid and expressed as % of constriction inhibition. The percentage of reduction of writhes in 15 minutes was calculated as follows:

$$\% \text{ age reduction} = \frac{\text{Mean control group Mean treated group}}{\text{Mean of control group}} \times 100$$

Hot-plate test in mice

This test was performed according to Turner method (Turner, 1965). Al animals were placed on hotplate (Eddy's hot plate, Germany) kept at a temperature of 53 ± 0.5^0C. The reaction time was then observed over a period of 120mins (at 30, 60, 90 and 120 mins) after oils and standard drug administration. The reaction time was considered as the time elapsed between placing of the mouse on the hotplate and licking of the hind paw or jumping. The increase in reaction time was calculated for each oil and drug-treated groups and is expressed as percentage. %age protection against thermal stimulus was calculated as follows:

% protection against thermal stimuli = Treatment – Control / Control x 100

Tail flick activity

Antinociceptive (analgesic) activity of the sesame oil and fish oil was evaluated by the tail-flick method (Alam *et al*., 2013). After dosing, about 5 cm from the distal end of the tail of each mouse was immersed in water bath maintained at 50°C. The reaction time (in seconds) was the time taken by the mouse to flick its tail due to pain. The first reading was omitted and reaction time was taken as the average of the next two readings. The reaction time was recorded before (0 min) and at 30, 60, 90, 120, 150 and 180 mins after the administration of the treatments.

MPA= Post drug latency (T) – Pre drug latency (T^o)

STATISTICAL ANALYSIS

The data obtained is presented as Mean ± Standard Error Mean (SEM) and analyzed using one-way ANOVA followed by Tukey's post hoc test. SPSS version 21 software was used for data analysis with $p<0.05$ considered as statistically significant.

RESULTS

Oil extraction

The overall percentage yield of the oil of shark fish liver samples in this study was 19.97 g/50g of wet liver tissue. However, the total yield of sesame oil by Soxhlet's method was 58.93g/100g.

Acute toxicity

We found that both oils were nontoxic after twenty four hours of treatment at the administered doses. No death was seen at any dose and overall signs of toxicity, like cyanosis, piloerection, tremors, convulsions, ataxia, hypnosis, writhing, ptosis, red urine and diarrhea were not noticed. Likewise, the motor performances such as respiration, corneal reflex, righting and withdrawal, body tone and amount of pats were also found unaffected. Based on these results, the effective dose was fixed at 5ml/kg/day and 10ml/kg/day for performing the analgesic study.

DPPH Antioxidant Assay

The antioxidant reactivity of the shark fish oil, sesame oil and the combination SO+FO were analyzed with DPPH, a

Table 1: Analgesic effect of sesame oil, fish oil and SO+FO by writhing reflex method

Groups	No. of writhes	% protection
Control	33.5±2.41	0
Sesame oil 5ml/kg/day	18.50±1.72**	44.71
Sesame oil 10ml/kg/day	14.50±1.13***	56.7
Fish oil 5ml/kg/day	19.89±2.01**	40.7
Fish oil 10ml/kg/day	13.35±1.11***	60.14
SO+FO (1:1) 5ml/kg/day	16.92±1.54**	49.49
SO+FO (1:1) 10ml/kg/day	11.48±0.92***	65.7
Aspirin 150mg/kg/day	11.21±0.87***	66.53

Effects of the sesame oil (SO), fish oil (FO) and SO+FO on Acetic acid induced writhing reflex in mice. Data are mean ± S.E.M. Control: p<0.001***, p<0.01**, p<0.05*

Table 2: Analgesic effect of sesame oil, fish oil and SO+FO by Tail-flick method in mice

Reaction time in seconds (mean ± SD)							
Groups	0min	30min	60min	90min	120min	150min	180min
Control	0.78±0.02	0.79±0.02	0.79±0.00	0.79±0.01	0.80±0.00	0.78±0.00	0.76± 0.00
SO 5mg/kg	0.82±0.78	1.29±0.11	**2.09±0.28	**2.09±0.28	**2.36±0.38	**2.23±0.39	*1.67±0.15
SO 10mg/kg	1.05±0.06	*1.5±0.10	**2.3±0.22	**2.81±0.96	**2.97±0.51	**2.80±0.47	*1.93± 0.20
FO 5mg/kg	0.93±0.07	1.09±0.06	*1.38±0.13	*1.77±0.18	**2.86±0.53	*1.64±0.20	*1.33± 0.22
FO 10mg/kg	0.84±0.04	**2.04±0.80	**2.84±0.47	***3.16± 0.34	***3.84±0.83	**2.16±0.34	*1.75± 0.07
SO+FO 5mg/kg	**2.16±0.75	**3.25±0.38	***#5.50±1.04	***#6.24±0.75	***6.83±0.82	***#5.76±0.46	***4.73±0.98
SO+FO 5mg/kg	**2.00±0.63	***5.33±1.03	***##6.65±1.21	***##7.16±0.98	***##7.83±1.42	***#6.57±1.23	***#5.33±0.51
Aspirin	1.83±0.03	**2.37±0.11	**3.27±0.31	***5.01±0.06	***5.93±0.25	***4.22±0.05	**2.01±0.06

Effects of Sesame oil (SO), Fish oil (FO) and SO+FO on tail flick activity in mice. Data are mean ± S.E.M. Control: p<0.00***, p<0.01**, p<0.05* Standard: p<0.00###, p<0.01##, p<0.05#

stable free radical. As DPPH picks up one electron in the presence of a free radical scavenger, the absorption decreases and the resulting discoloration is stoichiometrically related to the number of electrons gained. All three oils showed a dose dependent inhibitory effect on DPPH radical scavenging activity as shown in the fig. 1. The maximum inhibition shown by SO+FO combination was 98.22±0.76% and that of ascorbic acid was 97.18±0.40% at 4mg/ml. Sesame oil and fish oil had the lowest IC50 value (12.96±0.40lg/mL, 10.31±74lg/mL respectively) however, combination of SO+FO exhibited more significant lowering in IC50 values (7.29±0.54lg/mL) just like the standard antioxidant ascorbic acid (6.11±0.44lg/mL).

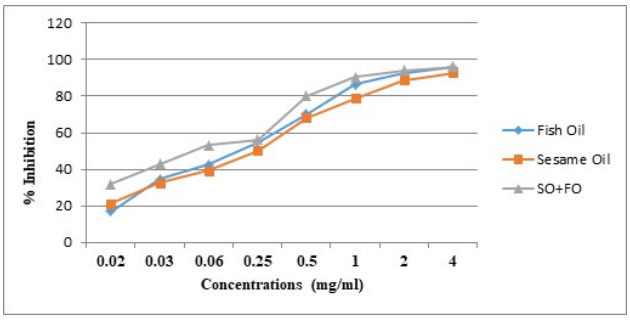
Effect of sesame oil and fish oil on Acetic-Acid Induced Abdominal Writhing Test

Treatment with SO and FO showed significant reduction in the writhes count as compared to control group (44.71%, p<0.05 and 56.7%, p<0.001, respectively) (table 1). Treatment with SO+FO at both doses (5ml and 10ml/kg/day) significantly inhibited acetic acid-induced writhing response and increased pain threshold of mice as evidenced by significant decrease in number of writhes and increased inhibition ratio (16.92±1.54, 49.49%; 11.48±0.92, 65.7% respectively) as compared with control. Similarly, Aspirin showed significant (66.53%, p < 0.001) reduction in the writhes count. Post hoc analysis by Tukey's test revealed that, treatment of animals with sesame oil and fish oil (5ml and 10ml/kg/day) produced a significant (p<0.05) dose-dependent inhibition of the abdominal writhes as compared to control group. However, combination of SO+FO produced highly significant (p<0.01) amelioration of abdominal writhes persuaded by acetic acid as compared to control.

Effect of sesame oil and fish oil on Hot Plate latency

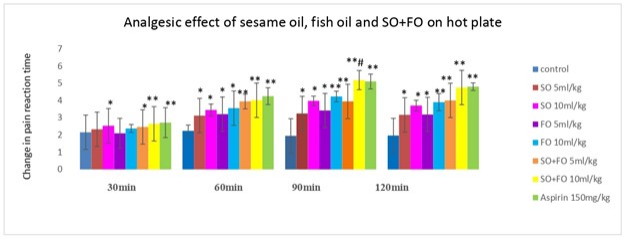
In hot plate test, SO and FO 5ml and 10ml/kg/day showed significant analgesic effect as both oils showed increased latency time at different doses and at different time intervals (fig. 2 and fig. 3). Initially, we observed insignificant antinociceptive effect after 30 minutes of dosing while, after 60 minutes, all doses of SO and FO except 5ml/kg of sesame oil showed significant increase in the latency time when compared to control group. As can be seen in fig. 2 and 3, the maximum activities were obtained after 90 minutes. In contrast, combination of SO+FO 5ml/kg/day and 10ml/kg/day exhibited highly significant increase in pain reaction time in comparison to control (p<0.01). Post hoc analysis by Tukey's test revealed that after 90 min of treatment, the doses 10ml/kg/day of sesame oil as well as fish oil (78.12% and 102.56 %; p<0.01 and p<0.001, respectively) and 5ml/kg/day and 10ml/kg/day SO+FO (147.91%; 152.71%; p< 0.001) significantly improved pain reaction time. The most significant result was shown by SO+FO at 10ml/kg/day which was started from 60min and remains significant till 120 min (90.17%, 152.71%, 145.16% respectively) just like aspirin (94.21%, 153.81%, 146.22%). Whereas, sesame and fish oils alone showed

significant analgesic effect at 60min and this effect was started to decrease at 120min.



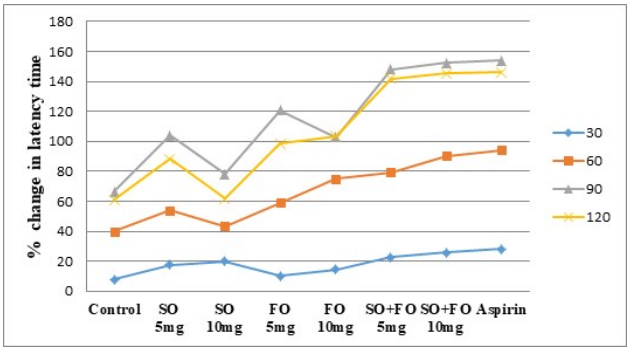
% inhibition of Fish oil, Sesame oil and SO+FO in DPPH Assay, Data expressed as mean ± S.E.M.

Fig. 1: DPPH Free Radical Inhibitory Activity of Fish oil, Sesame oil and SO+FO



Effects of Sesame oil, Fish oil and SO+FO on hot plate activity in mice. Data are mean ± S.E.M. Control: P<0.00***, p<0.01**, p<0.05*; Standard: p<0.00$^{###}$, p<0.01$^{##}$, p<0.05$^{#}$

Fig. 2: Analgesic effect of sesame oil (SO), fish oil (FO) and SO+FO (Hot Plate Method)

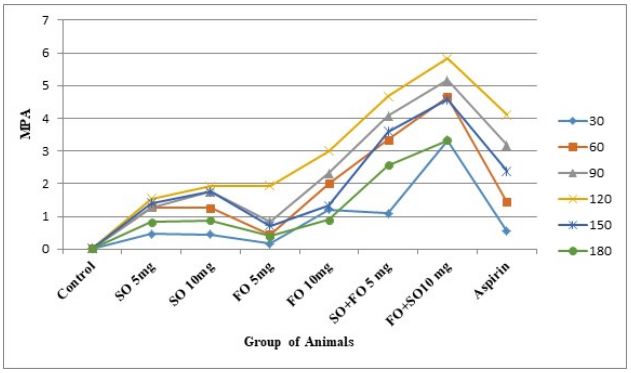


Change in pain latency time after sesame oil, fish oil and SO+FO administration on hot plate activity in mice. Data are mean ± S.E.M.

Fig. 3: Change in reaction time after different treatments of sesame oil (SO), fish oil (FO) and SO+FO on Hot plate method

Effect of sesame oil and fish oil on Tail Flick Latency

Table 2 demonstrates the tail flick latencies of SO, FO and SO+FO at 5ml and 10ml/kg/day doses. Both oils (SO and FO) at different doses showed significant difference in the reaction time on tail flick throughout the whole observation time (p<0.05) and 10ml/kg/day (p<0.01, p< 0.01, p<0.001) significantly increased the latency time on the tail-immersion in hot water after 60min of treatment. At 90min, maximum tail flick latency was seen with sesame oil and fish oil (p<0.01). However, SO+FO combination showed highly significant results (p<0.001) at 120mins, sesame oil (10ml/kg/day, p<0.001), fish oil (5ml/kg/day, p<0.05; 10ml/kg/day, p<0.01) and SO+FO (5ml/kg/day, p<0.01; 10ml/kg/day, p<0.001) respectively improved the pain reaction time. The maximum effect was observed after 120 min of treatment with sesame oil, fish oil and SO+FO combination (p<0.05; p<0.01; p<0.001) respectively. The maximum possible analgesic effect of SO, FO and SO+FO at different doses were calculated and expressed in fig. 4.



Maximum possible analgesic effect of Sesame oil, Fish oil and SO+FO on tail flick activity in mice. Data expressed as mean ± S.E.M.

Fig. 4: Maximum possible Analgesic effect of sesame oil (SO), fish oil (FO) and SO+FO by tail-flick method in mice

DISCUSSION

Oxidative stress play a focal part in analgesia associated with inflammation by releasing free radicals (Jiang *et al*., 2004). Cyclooxygenase is a free radical forming enzyme like peroxidase, xanthine oxidase and other enzymes (Braca *et al*., 2001; Koleva *et al*., 2002). These free radicals accumulate in the body counter with biological molecules and disturb the normal structure of cells, leading to free radical-induced diseases such as inflammation, metastasis and cardiovascular disorders (Arshiya, 2013; Ainooson *et al*., 2009). Therefore, the identification of natural antioxidant may reduce the risk of various chronic diseases involved in free radicals especially inflammation (Qnais *et al*., 2017). In current study SO, FO and SO+FO showed significant %age inhibition and lowest IC50 values in DPPH assay that represents these oils have excellent antioxidant property. Our results are in accordance with Farvin *et al*., 2014 and Suja *et al*., 2004 who reported that sesame oil and fish oil possess free radical scavenging property in DPPH assay.

Three analgesic models: Acetic acid-induced writhing reflex, tail flick and hot plate models were used to evaluate the analgesic activity of sesame oil, shark fish oil

and combination of SO+FO. Since, tests of analgesic drugs commonly measure nociception and involve the reaction of animals to painful stimuli (Ezeja *et al*., 2011; George *et al*., 2009). The stimulus may be thermal (tail immersion or hot plate tests), chemical (acetic acid-induced writhing or formalin tests) or mechanical (tail or paw pressure tests) (Ribeiro *et al*., 2000). The results of the present study showed that the oil mixture SO+FO elicited potent antinociceptive activities that were assessed using different pain models (Toma *et al*., 2003; Zhang *et al*., 2018). The present results also demonstrate that the sesame oil and fish oil act both centrally and peripherally (Calder, 2013; Tapiero *et al*., 2002). The pain models used in this study were selected such that both centrally and peripherally mediated effects were measureable. In writhing reflex test, sesame oil, fish oil and SO+FO significantly decreased the mean number of abdominal constrictions or writhes which was dose dependent. Also these oils increased the percent inhibition of abdominal constriction from 0% in the control group to 65% at the dose of 10ml/kg/day and that was similar to aspirin standard drug. Acetic acid induced writhes in mice by producing pain sensation and initiation of inflammatory response which resulted in arachidonic acid release from tissue (Le Bars, 2001). Arachidonic acid further converted into prostaglandins particularly PGE2 and PGF2α, as well as lipoxygenase products in the peritoneal fluid of mice by sensitization of pain receptors (nociceptor) (Abbott and Melzack, 1982). The analgesic effect of SO, FO and SO+FO seen in this experiment may be mediated through peripheral pain mechanism and/or via inhibition of prostaglandin pathway. EPA and DHA are omega 3 fatty acids enriched in shark fish and sesame oil (Nemirovsky *et al*., 2011; Saleem *et al*., 2013). Wagner *et al*., 2014 has reported that DPA exert a vital health-enhancing role in reducing pain, inflammation & its related disorders by inhibiting prostaglandins & cytokines that are involved in anti-inflammatory actions.

Nociceptive reaction towards thermal stimuli in tail flick and hot plate test is a well-validated animal model for the detection of central analgesic effect of different plant extracts (Bukhari *et al*., 2010). The hot plate is a specific central antinociceptive test in which opioid agents exert their analgesic effects via supra spinal and spinal receptors (Tarawneh *et al*., 2013). The SO and FO showed dose dependent anti-nociceptive activity by increasing the latency to discomfort in hot plate test. This action could be due to activating the periaqueductal gray matter to release endogenous peptides (endorphins or enkephalins) (Monteiro *et al*., 2014). These peptides inhibit process of nociception and transmission of pain impulses at the synapse in the dorsal horn (Nakamoto *et al*., 2011). The possible analgesic mechanism of SO and FO could be due to their action on the central opioid receptors or by releasing endogenous opioid peptides.

Similarly in hot plat test, our results demonstrated that the oral administration of the SO and FO exerted significant prolongation in the latency time to the heat stimulus however, effect of SO+FO was superior than single oils even in comparison with aspirin (fig. 2 and table 1). In the tail flick test, the fish oil and sesame oil showed dose dependent maximum possible analgesic (MPA) effect however increased MPA was seen with SO+FO 10ml/kg/day when compared to the control and positive control group. This dose dependent analgesic effect clearly indicates the centrally mediating analgesic effect of the SO+FO oil that is in line with the previous research which demonstrated that sesame oil produced a similar effect to morphine that significantly increased the latency time to the nociceptive response when compared with the control group. Furthermore, fish oil contain eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), these acids facilitate the release of β-endorphin and finally the stimulation of μ- and δ-opioid receptors thus induced antinociception (Nakamoto *et al*., 2011). These reported pharmacological actions make these oils a potential health aid in dealing with chronic pain conditions. Thus the two common food ingredients, oils from sesame and fish and its combination, have desirable biochemical properties to develop potent analgesic effect which make their combination beneficial as a therapeutic agent. These results provide the rationale that the combined effect of polyunsaturated fatty acids with two different natural sources may be used as adjuncts to NSAIDs or morphine in pain treatment, which might help to increase analgesia and decrease adverse effects.

CONCLUSION

In conclusion, sesame oil, fish oil and the combination (SO+FO) treatment exhibited analgesic effect in both chemical and thermal pain models. However, maximum analgesic effects were reported with combination of sesame and fish oil at 10ml/kg/day. Thus, the combination of these two oils in treating arthritis and other joint pains could be beneficial in future.

ACKNOWLEDGEMENT

The authors would like to thanks the Department of Pharmacology, Faculty of Pharmacy & Pharmaceutical Sciences, University of Karachi, Karachi, Pakistan for providing the facilities to conduct the study.

REFERENCES

Abbott FV and Melzack R (1982). Brainstem lesions dissociate neural mechanisms of morphine analgesia in different kinds of pain. *Brain. Res*., **251**(1): 149-155.

Abdulkhaleq LA, Assi MA, Abdullah R, Zamri-Saad M, Taufiq-Yap YH and Hezmee M (2018). The crucial roles of inflammatory mediators in inflammation: A review. *Veterinary World*, **11**(5): 627-635.

Abou-Gharbia HA, Shehata AAY and Shahidi F (2000). Effect of processing on oxidative stability and lipid classes of sesame oil. *Food Res. Int.*, **33**(5): 331-340.

Ainooson GK, Woode E, Obiri DD and Koffour GA (2009). Antinociceptive effects of Newbouldia laevis (P. Beauv.) Stem bark extract in a Rat model. *Pharmacogn. Mag.*, **5**(17): 49.

Alam B, Akter F, Parvin N, Pia RS, Akter S, Chowdhury J, Sifath-E-Jahan K and Haque E (2013). Antioxidant, analgesic and anti-inflammatory activities of the methanolic extract of *Piper betle* leaves. *Avicenna J Phytomed.*, **3**(2): 112.

Arshiya S (2013). The antioxidant effect of certain fruits:- A review. *J. Pharm. Sci. Res.*, **5**(12): 265.

Ballou LR, Botting RM, Goorha S, Zhang J and Vane JR (2000). Nociception in cyclooxygenase isozyme-deficient mice. *Proc. Natl. Acad. Sci.*, **97**(18): 10272-10276.

Bligh EG and Dyer WJ (1959). A rapid method of total lipid extraction and purification. *Can. J. Biochem. Physiol.*, **37**(8): 911-917.

Braca A, De Tommasi N, Di Bari L, Pizza C, Politi M and Morelli I (2001). Antioxidant principles from *Bauhinia tarapotensis*. *J. Nat. Prod.*, **64**(7): 892-895.

Brar GS and Ahuja KL (1980). Sesame: Its culture, genetics, breeding and biochemistry. *Annu. Rev. Plant Sci*., **1**: 245-313.

Calder PC (2013). Omega-3 polyunsaturated fatty acids and inflammatory processes: nutrition or pharmacology?. *Br. J. Clin. Pharmacol.*, **75**(3): 645-662.

Calder PC (2017). Omega-3 fatty acids and inflammatory processes: From molecules to man. *Biochem. Soc. Trans.*, **45**(5): 1105-1115.

Cheng FC, Jinn TR, Hou RC and Tzen JT (2006). Neuroprotective effects of sesamin and sesamolin on gerbil brain in cerebral ischemia. *Int. J. Biomed. Sci*., **2**(3): 284.

Ezeja MI, Omeh YS, Ezeigbo II and Ekechukwu A (2011). Evaluation of the analgesic activity of the methanolic stem bark extract of *Dialium guineense* (Wild). *Ann. Med. Health Sci. Res*., **1**(1): 55-62.

Farvin KS, Andersen LL, Nielsen HH, Jacobsen C, Jakobsen G, Johansson I and Jessen F (2014). Antioxidant activity of Cod (*Gadus morhua*) protein hydrolysates: *In vitro* assays and evaluation in 5% fish oil-in-water emulsion. *Food Chem.*, **149**: 326-334.

Fernandes RDP, Trindade MA, Tonin FG, Lima CG, Pugine SMP, Munekata PES, Lorenzo JM and De Melo MP (2016). Evaluation of antioxidant capacity of 13 plant extracts by three different methods: Cluster analyses applied for selection of the natural extracts with higher antioxidant capacity to replace synthetic antioxidant in lamb burgers. *J. Food Sci. Technol.*, **53**(1): 451-460.

Fujino S, Andoh A, Bamba S, Ogawa A, Hata K, Araki Y, Bamba T and Fujiyama Y (2003). Increased expression of interleukin 17 in inflammatory bowel disease. *Gut*, **52**(1): 65-70.

Ghaly AE, Dave D, Budge S and Brooks MS (2010). Fish spoilage mechanisms and preservation techniques. *Am. J. Appl. Sci.*, **7**(7): 859.

Goldberg RJ and Katz J (2007). A meta-analysis of the analgesic effects of omega-3 polyunsaturated fatty acid supplementation for inflammatory joint pain. *Pain*, **129**(1-2): 210-223.

Jiang J, Borisenko GG, Osipov A, Martin I, Chen R, Shvedova AA, Sorokin A, Tyurina YY, Potapovich A, Tyurin VA and Graham SH (2004). Arachidonic acid-induced carbon-centered radicals and phospholipid peroxidation in cyclo-oxygenase-2-transfected PC12 cells. *J. Neurochem.*, **90**(5): 1036-1049.

Kinsella JE, Shimp JL, Mai J and Weihrauch J (1977). Fatty acid content and composition of freshwater finfish. *J. Am. Oil Chem. Soc.*, **54**(10): 424-429.

Koleva II, Van Beek TA, Linssen JP, Groot AD and Evstatieva LN (2002). Screening of plant extracts for antioxidant activity: A comparative study on three testing methods. *Phytochem Anals.,* **13**(1): 8-17.

Kong W, Yen JH, Vassiliou E, Adhikary S, Toscano MG and Ganea D (2010). Docosahexaenoic acid prevents dendritic cell maturation and *in vitro* and *in vivo* expression of the IL-12 cytokine family. *Lipids Health Dis*., **9**(1): 1-10.

Koster R (1959). Acetic acid for analgesic screening. *In*: *Fed Proc* **18**: 412.

Kristensen S, Schmidt EB, Schlemmer A, Rasmussen C, Johansen MB and Christensen JH (2018). Beneficial effect of n-3 polyunsaturated fatty acids on inflammation and analgesic use in psoriatic arthritis: A randomized, double blind, placebo-controlled trial. *Scand J. Rheumatol.*, **47**(1): 27-36.

Küpeli E, Erdemoglu N, Yeşilada E and Şener B (2003). Anti-inflammatory and antinociceptive activity of taxoids and lignans from the heartwood of *Taxus baccata* L. *J. Ethnopharmacol.,* **89**(2-3): 265-270.

Le Bars D, Gozariu M and Cadden SW (2001). Animal models of nociception. *Pharmacol. Rev.*, **53**(4): 597-652.

Lee YH, Bae SC and Song GG (2012). Omega-3 polyunsaturated fatty acids and the treatment of rheumatoid arthritis: A meta-analysis. *Arch. Med. Res.*, **43**(5): 356-362.

Maroon JC and Bost JW (2006). Omega-3 fatty acids (fish oil) as an anti-inflammatory: An alternative to nonsteroidal anti-inflammatory drugs for discogenic pain. *Surgical neurology,* **65**(4): 326-331.

Mohammed MI and Hamza ZU (2008). Physicochemical properties of oil extracts from *Sesamum Indicum* L. seeds grown in Jigawa State, Nigeria. *J. Appl. Sci. Environ. Manage.*, **12**(2): 58-62.

Monteiro EMH, Chibli LA, Yamamoto CH, Pereira MCS, Vilela FMP, Rodarte MP, de Oliveira Pinto MA, Da

Penha Henriques do Amaral M, Silverio MS, de Matos Araújo ALS and Da Luz Andre de Araujo A (2014). Antinociceptive and anti-inflammatory activities of the sesame oil and sesamin. *Nutrients*, **6**(5): 1931-1944.

Nakamoto K, Nishinaka T, Ambo A, Mankura M, Kasuya F and Tokuyama S (2011). Possible involvement of β-endorphin in docosahexaenoic acid-induced antinociception. *Eur. J. Pharmacol*., **666**(1-3): 100-104.

Nemirovsky A, Chen L, Zelman V and Jurna I (2001). The antinociceptive effect of the combination of spinal morphine with systemic morphine or buprenorphine. *Anesth. Analg*., **93**(1): 197-203.

Park Y, Lee A, Shim SC, Lee JH, Choe JY, Ahn H, Choi CB, Sung YK and Bae SC (2013). Effect of n-3 polyunsaturated fatty acid supplementation in patients with rheumatoid arthritis: A 16-week randomized, double-blind, placebo-controlled, parallel-design multicenter study in Korea. *J. Nutr. Biochem*., **24**(7): 1367-1372.

Qnais E, Bseiso Y, Wedyan M and Alkhateeb H (2017). Evaluation of analgesic activity of the methanol extract from the leaves of *Arum palaestinum* in mice and rats. *Biomed. Pharmacol. J.*, **10**(3): 1159.

Ribeiro RA, Vale ML, Thomazzi SM, Paschoalato AB, Poole S, Ferreira SH and Cunha FQ (2000). Involvement of resident macrophages and mast cells in the writhing nociceptive response induced by zymosan and acetic acid in mice. *Eur. J. Pharmacol.*, **387**(1): 111-118.

Saify ZS, Akhtar S, Khan KM, Perveen S, Ayattollahi SAM, Hassan S, Arif M, Haider SM, Ahmad F, Siddiqui S and Khan MZ (2003). A study on the fatty acid composition of fish liver oil from two marine fish, *Eusphyra blochii* and *Carcharhinus bleekeri*. *Turk. J. Chem.*, **27**(2): 251-258.

Saleem MT, Chetty MC and Kavimani S (2013). Putative antioxidant property of sesame oil in an oxidative stress model of myocardial injury. *J. Cardiovasc. Dis. Res*., **4**(3): 177-181.

Shojaii A, Motaghinejad M, Norouzi S and Motevalian M (2015). Evaluation of anti-inflammatory and analgesic activity of the extract and fractions of *Astragalus hamosus* in animal models. *Iran J. Pharm. Res*., **14**(1): 263.

Singh S and Majumdar DK (1995). Anti-inflammatory and antipyretic activities of *Ocimum sanctum* fixed oil. *Int. J. Pharmacogn*., **33**(4): 288-292.

Suja KP, Jayalekshmy A and Arumughan C (2004). Free radical scavenging behavior of antioxidant compounds of sesame (*Sesamum indicum* L.) in DPPH• system. *J. Agric. Food Chem*., **52**(4): 912-915.

Tapiero H, Ba GN, Couvreur P and Tew KD (2002). Polyunsaturated fatty acids (PUFA) and eicosanoids in human health and pathologies. *Biomed. Pharmacother.*, **56**(5): 215-222.

Tarawneh AH, Leoon F, Radwan MM, Wang X, Dale OR, Husni AS, Rosa LH and Cutler SJ (2013). Fatty acids with in vitro binding affinity for human opioid receptors from the fungus *Emericella nidulans*. *J. Agric. Food Chem.*, **61**(44): 10476-10480.

Toma W, Gracioso JDS, Hiruma-Lima CA, Andrade FD, Vilegas W and Brito AS (2003). Evaluation of the analgesic and antiedematogenic activities of *Quassia amara* bark extract. *J. Ethnopharmacol*., **85**(1): 19-23.

Turner R (1965). Depressants of the central nervous system in screening methods. *Pharmacol.*, pp.69-86.

Wagner K, Vito S, Inceoglu B and Hammock BD (2014). The role of long chain fatty acids and their epoxide metabolites in nociceptive signaling. *Prostag. Oth. Lipid. M.,* **113**: 2-12.

Zhang L, Terrando N, Xu ZZ, Bang S, Jordt SE, Maixner W, Serhan CN and Ji RR (2018). Distinct analgesic actions of DHA and DHA-derived specialized pro-resolving mediators on post-operative pain after bone fracture in mice. *Front. Pharmacol.*, **9**: 412.