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Anti-pain and anti-inflammation like effects of Neptune krill oil and fish oil against carrageenan induced inflammation in mice models: Current statues and pilot study

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ABSTRACT

Although inflammation is a reactive to injurious stimuli and considered as beneficial process in body, but it causes some discomforts, such as pain. Murine dietary contains appreciable amounts of fatty acids and antioxidants which encourages researchers to focus on their potential therapeutic effects. This study is aimed to examine the analgesic and anti-inflammatory activity of Neptune krill oil (NKO) and fish oil (FO) in rodent model which are two well-known sources of rich content of n-3 polyunsaturated fatty acids (n-3 PUFAs), mostly eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). NKO and FO were used at the same dose of 500 mg and also balanced at similar doses of EPA: 12 in NKO vs. 12 in FO wt%, DHA: 7 NKO vs. 8 FO wt%. Application of NKO and FO in acetic acid-induced writhing effect, hot plate, and formalin induced test, indicated the nociceptive activity of the two tested drugs in comparison with normal saline. Also, the anti-inflammatory effect of these supplements was confirmed by carrageenan test. Analysis of cytokines levels in the blood samples of the mice after induction inflammation by carrageenan indicated decreased levels of those proteins compared to that in the normal groups. Both tested drugs, effectively could reduce severe inflammation and pain in rodents in comparison with the references drugs (depends on the tests); however, NKO was found to be more effective.

1. Introduction

Inflammation and pain which could affect body health seem to be one of the hottest health topics [1]. This process could be defined as the immediate defensive response by vertebrate tissue against harmful stimulus, such as injury and infection which are caused by chemical and physical agents. It is shown that a series of biochemical mechanisms which are managed by pro-inflammatory factors could initiate and mature the inflammatory response [1,2]. Some chemical responses of inflammation, such as interleukin 6 (IL-6) and IL12, interferons (INFsγ), tumour necrosis factor (TNF) develop the persistence of a build-up of fluid into the tissues, causing edema and all sorts of pain, such as chronic or acute, peripheral or central type [3]. For instance, the production of cytokines by cells in either immune or central nervous system (CNS) activate the peripheral nociceptors [4], also PGEs are hormone-like molecules typically originate in the body, and induce many adverse effects, some of which are, fever, inflammation, and peripheral pain [5,6].

To relieve pain and reduce inflammation, the growing researches are introducing a variety of effective natural and chemistry therapeutics.

A range of existing diverse chemical classification drugs which are widely used to alleviate pain from inflammation, are as non-steroidal anti-inflammatory drugs (NSAIDs). NSAIDs contain the analgesic and antipyretic effects. Any unwanted effects are considered as a crucial subject for researcher to focus natural compounds, which possess short onset time activity coupled with minimum adverse effects.

Impact of essential fatty acids on inflammation may be the key answer to how dietary fats could affect health promoting. Saturated fatty acids and linoleic acid promote anti-inflammatory process in body and are potential potent anti-inflammatory natural agents. The n-3 PUFAs decrease the production of some inflammatory agents such as n-6 PUFAs, cytokines, reactive oxygen species (ROS) [7]. Evidence reveals that oral supplementation of both eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) diminish the production of some pro-inflammatory mediators,

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such PGs, leukotrienes (LTs), and also interleukins which lead to alleviate inflammation [8].

Unlike omega-3, omega-6 involve in initiating inflammatory process because it can induce the production of PGs and LTs [9]. Therefore, a diet of a lot of omega-3 and not much omega-6 are strongly suggested for improving body health.

It is definite that fish oil (FO) and krill oil (KO) are very well-known sources of PUFAs and antioxidants which are widely extended consuming supplements [10,11]. Neptune Krill Oil (NKO) which is extracted from Antarctic krill contain higher levels of omega3 fatty acids than other types of KO [11]. Albite, both FO and KO contain a high proportion of EPA and DHA, but in contrast together, fatty acid are mainly stored as triglycerides (TAGs), KO possesses a major portion (30%–65%) of the named fatty acids in form of phospholipids (PLs), mainly PC [12].

Animal studies have demonstrated that KO can improve both absorption and delivery of DHA inside body better than FO [13]. Interestingly, some rodent and human studies illuminate that oral consumption of phosphatidylcholine show beneficial effects of inflammation [10,14]. Existing of antioxidant astaxanthin, mainly in esters form only exists in KO and not in FO is another possible reason which makes KOs more potent in bioactivities than FO [15].

In some studies which compare the bio-effects of n-3 PUFA extracted from KO and FO, the standard features of FO products are fully verified in order to use and repeat the same protocols and experiments [16]. Because of some limiting factors, such as the differences levels of EPA and DHA in KO and FO and also other factors, namely the methods which are used to extract fish and krill, therefore there are no define explanation to compare the bioactivity of KO and FO [10,17].

The aim of this pilot comparative study was to investigate and compare the anti-pain and anti-inflammation impacts of NKO and FO in rodent model. Also the expression level changes of IL6 and TNF-α before and after treatments was measured.

2. Material and methods

2.1. Animals

One hundred sixty eight adult male Swiss mice (25–35 g) were kept in standard animal housing conditions at the temperature of $23 \pm 1\,^{\circ}C$ and suitable humidity ($55 \pm 5\%$) with dark-light cycles (12/12 h). The animals were kept in home cages prepared with Plexiglas (65 cm × 25 cm × 15 cm) with free access to water, libitum and standard laboratory chow. The mice were acclimatized to laboratory condition for 1 day before the experiments were carried out without having any access to food. The experimental processes including the protocols in this study were approved by the Animal Care Committee of the Faculty of Medicine's Animal House, Institutional Animal Care and Use Committee; University Malaya (UM), according to the guideline and policy of University Malaya ; regarding the care and use of animals for scientific purposes with the reference number. For running the assays, mice were divided into 4 groups (n = 6).

2.2. Testing dietary drugs for the animals

The administered testing drugs were: 500 mg Neptune brand krill oil (NKO®, Neptune Technology & Bioressources Inc (NEPT), Darmstadt, Germany), containing substantially high level of phospholipids bound form of DHA and EPA and also astaxanthin (a powerful free radical scavenger); also, 500 mg fish oil (Nature Made® Fish oil, California, USA). The two administered drugs were balanced for their DHA and EPA contents. The diets were based on the AIN-93 G formulation for oily consumption in rodents [18]. The diets were stored in vacuum bags to prevent (n-3) PHFA oxidation.

Table 1
NKO and FO nutraceutical products compared in this study. Subsamples from two separate patches of each product were pooled for phospholipids, triglycerides, DHA and EPA analysis.

Supplement Fact	Neptune Krill oil (NKO™),500 mg Patch NO: 060822	Fish oil (Nature's Made ®), 500 mg Patch NO: 031604026622
Omega-3 fatty acids, mg	115 (23 g/100 g)	–
Eicosapentaenoic Acid (EPA), mg	60 (12 g/100 g)	90 (12 g/100 g)
Docosahexaenoic Acid (DHA), mg	35(7 g/100 g)	60 (8 g/100 g)
Phospholipids, mg	195(39 g/100 g)	–
Triglycerides, mg	–	150 (18.75 g/100 g)
Esterified Astaxanthin, mcg	375(0.075 g/100 g)	–

Those products representative of the NKO and FO manufacturers were selected by omega-3 oils category. Full details of selected products and batch numbers are listed in Table 1. For making a balance of EPA and DHA (EPA + DHA), we used 0.5 mg/100 *g* of the NKO and 0.32 mg/100 *g* of the FO were also used for the mice in the all of the assays. Treated animals with FO and NKO were labelled as the group I and group II (treated groups) and those treated with 5% sterile normal saline solution (negative control, Merck, Darmstadt, Germany) as the NS group. Indomethacin (Merck, Darmstadt, Germany), Pentazocine (Jinan Limin Pharmaceutical Co., Ltd.), Aspirin (Merck, Darmstadt, Germany), Carageenan (Aladdin Chemistry Co. Ltd.), also was used exclusively in each animal experiments for induction iflammation and, in normal saline, 0.1 ml/mice was used as control in all the groups. At the end of feeding period, the animals were killed under general anesthesia using 2% isoflurane (Merck, Darmstadt, Germany). Blood was collected by cardiac puncture in BD Vacutainer tubes which contained 7.5% EDTA. The blood samples were chilled on ice for at least one quarter of hr and then were centrifuged for preparing plasma and were frozen at -80 C for measuring cytokines.

3. Assessment of anti-inflammatory activity

3.1. Carrageenan-induced mice paw edema test

The treatments were orally given to the four groups of animals 60 min before carrageenan injection. Acute inflammation (edema) was produced via injection of 0.1 ml of 1% carrageenan (in 1% carboxyl-methylcellulose, w/v) into the subplantar region of right hind paw of the mice based on the method described by Levy [19]. The measurement of volume was immediately done before sub plantar injection, and 1, 2, 3, hr thereafter, using a hydro-pletismometer which was particularly modified for the measurement of small volumes [20]. The increase in paw volume was calculated by subtracting the initial paw volume (basal) from the paw volume measured at the three different hr. The inhibitory effects were calculated according to the following formula [15];

Percentage of inhibition = $[(V_t–V_0)_{control} – (V_t–V_0)_{treated}]/(V_t–V_0)_{control} \times 100]$,

Where $(V_t–V_0)_{control}$ is the difference in the size of paw at 3 h in control mice, and $(V_t–V_0)_{treated}]$ is the difference in the size of paw at 3 h in mice treated with the treatments

3.2. Measurement of cytokines

For harvesting serum for measuring the cytokines, the blood samples were collected from mice. Levels of serum cytokines (TNF-α and IL-6) were measured by TNF alpha Mouse ELISA Kit, High

Sensitivity (Catalog # BMS607HS) and IL-6 Mouse ELISA Kit (KMC0061) in ELISA, Thermos Fisher according to manufactures instruction.

4. Assessment of analgesic activity

4.1. Formalin-induced nociception

Formalin test used in this test was performed according the protocol prescribed by Hunskaar and Hole [21]. After the acclimatization time, the formalin (5% in 0.9% saline; 20 μl/paw) was injected intra-plantar into the right hind paws of the mice. Right after the formalin injection, the animals were put back into the glass cone chamber and monitored for 30 min A mirror was sited in front of the glass cone to permit a passable view of the formalin injected paw. The time (sec) that animals spent in licking, shaking and retracting the injected paw was recorded, using a chronometer and expressed as the total licking time in the early phase (0–5 min) and late phase (10–15 min). Those recorded times were named as a reflection for the current nociception in the animals.

4.2. Hot plate assay

The hot-plate assay was carried out according to the protocol which was prescribed by Eddy and Leimback [22] based on the response latencies. The reaction in the animals including the paw licking and jumping were evaluated based on their response times, hence the temperature were maintained constant ($55 \pm 0.5\,^{\circ}C$) during the experiment. Both animal reactions were considered to be supra spinally integrated responses. The mice were put for a 10 s (maximum time) to avoid any thermal injury/damage in their paws. As soon as the mice started licking their fore- and hind-paws, and then jumping from the hot plate, reaction times were recorded before (basal/0 min) and after 1, 2, and 3 hs, following administration of the all treatments. The percentage of reaction time increasing was calculated by the following formula:

$$\text{Percent increase} = (T_T/T_c - 1) \times 100,$$

Where, TC and TT are defined as the mean analgesic time at basal and after treatment (1, 2, and 3 hs), respectively.

4.3. Acetic acid-induced abdominal constriction test

Acetic-acid-induced abdominal constriction test was performed based on the method described by Collier et al. [23]. After sixty min of administration of the particular test solution, the animals were injected by a phlogistic solution (0.6% acetic acid in 10 ml/kg NaCl) intra-peritoneally (i.p.). The animals were immediately turned back to the separate glass cages for 5 min, and then they were permitted to be free. The abdominal constriction was because of the injection of acetic acid which was coupled with one or both hind limb severe stretch. The nociceptive behaviour intensity was quantified by counting the total number of writhes occurring in 0–25 min. Anti-nociceptive activity was considered as the drastic reduction in the mean number of abdominal constrictions (writhes) in the treated groups in comparison with the control group which was expressed as writhing score over a period of 25 min. The percentage protection of analgesic activity was calculated by using the following formula:

$$\text{Percentage inhibition} = (1 - W_T/W_c) \times 100,$$

Where, W_T and W_C mean as the number of writhing in the treated group the NS group, respectively.

4.4. Statistical analysis

The data are presented as the mean ± standard error, and all of the statistical analyses were performed using SPSS 16.0 statistical software. For the numbers of writhing episodes and the licking/biting times, repeated measures ANOVAs and one-way ANOVA followed by the post hoc Bonferroni test were applied. The data for the onset time of writhing, the locomotor activity, the paw edema and the western blots were analysed using one-way ANOVA followed by the post hoc Bonferroni test. Tukey's post hoc analysis was used to compare any differences among the groups. P values <0.01, 0.05, and 0.001 were considered as statistically significant.

5. Results

5.1. Anti-inflammatory activity

5.1.1. Dietary supplementation of NKO and FO reduces hind paw thickness in mice

The subcutaneous injection of carrageenan cause paws volumes in mice due to edema. The volume of paw and the percentages of inhibition by the treatments are listed in Table 2 and shown in Fig. 1, respectively. As shown at the third hr, indomethacin, NKO, and FO produce 57.4%, 43.6%, 35.1% inhibition of carrageenan-induced hind paw edema, respectively, compared to the normal saline. According to the data shown in Table 2, the inhibition achieved after the 2nd and 3rd hours were significantly different between FO and NKO.

5.1.2. Effect of NKO and FO on expression of IL-6 and TNF-α

The levels of IL-6 and TNF-α were significantly increased in the serum of animals with induced acute inflammation in their paw by the injection of carrageenan. In comparison with the cytokines levels in the control mice and the treated with NKO, FO and indomethacin, the results indicated that the three treatments

Table 2
Effects of NKO and FO on carrageenan (0.1%)-induced paw edema.

Treatment	Dose	Paw volume (ml)			
		Time			
		0hr	1 hr	2hr	3hr
Control (Na.CMC)	0.1 ml/mice,ip	0.6 ± 0.02	0.85 ± 0.04	0.9 ± 0.02	0.94 ± 0.01
Reference (Indomethacin)	10 mg/kg,ip	0.62 ± 0.04	0.47 ± 0.01**(44.7)	0.41 ± 0.01**(54.4)	0.4 ± 0.0**(57.4)
FO	500(mg) Orally	0.64 ± 0.02	0.75 ± 0.01*(11.8)	0.58 ± 0.04**(35.5)	0.61 ± 0.01** (35.1)
NKO	500(mg) (orally)	0.61 ± 0.02	0.68 ± 0.03*(20)	0.49 ± 0.04**(45.5)	0.53 ± 0.02**(43.6)

The values in the parentheses indicate the percentage of inhibition. Values are mean ± SD of six samples in each group. * Significant ns – Not significant Inhibition of carrageenan-induced mouse paw edema by indomethacin, NKO and FO measured at 1, 2, 3 h, after carrageenan injection.

* =$p < 0.05$.

** =$p < 0.01$.

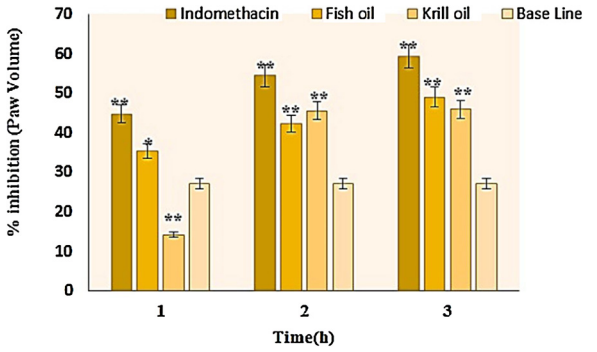


Fig. 1. Inhibition percentage of paw volume in carrageenan induced-inflammation.

significantly decreased the expression of these pro-inflammatory cytokines. But, NKO could significantly reduce cytokines levels more than FO (Figs. 2 and 3).

5.1.3. Effect of NKO and FO on lick duration in formalin test

Formalin-induced paw licking response is one of the most valuable test of clinical pain including the first phase or early phase, consists of direct chemical which stimulate nociceptors. While the second phase (late phase) is reliant on peripheral inflammation and alterations in central mechanism [24,25]. Both NKO and FO did not have significant anti-noceptive activity in comparison with the control in reducing lick duration formalin-induced nociceptive responses during the first and second phases. While, indomethacin during the second phase could reduce the lick duration compared to the control group, significantly ($p < 0.01$) (Fig. 4).

5.1.4. Effect of NKO and FO on latency time in hot plate test

The results of the analgesic activity of NKO and FO, using hot plate method are presented in Table 3. NKO ($p < 0.01$) and also FO ($p < 0.05$), significantly increased the post drug pain reaction time. However, after the administration of NKO and FO, the most activity was observed at the 2nd hour. There was no significant different in increasing the pain reaction time between the both treatments.

5.1.5. Effect of NKO and FO on acetic acid induced writhing number

Regarding the results found for NKO and FO in the acetic acid induced writhing test, a significant reduction in the writhes counts was recorded by NKO ($p < 0.01$), FO ($p < 0.05$), and aspirin ($p < 0.001$) compared to the normal saline (Table 3). Aspirin

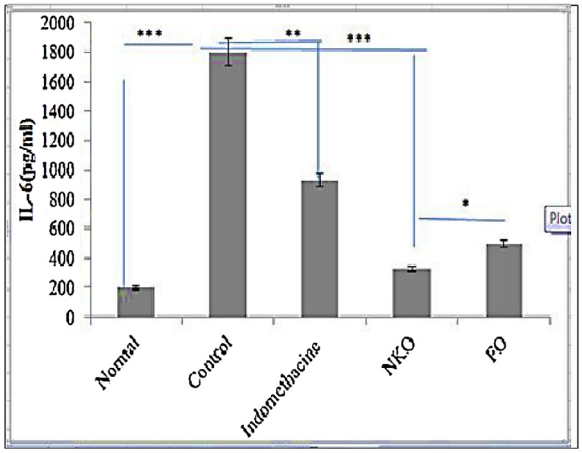


Fig. 2. Levels of IL-6 in the serum of animals with carrageenan induced-inflammation before and after treatments.

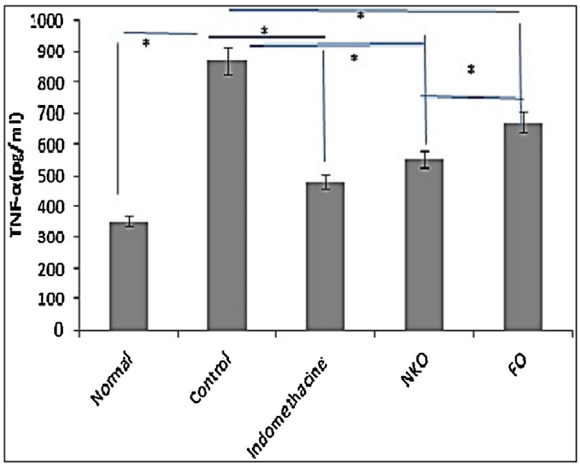
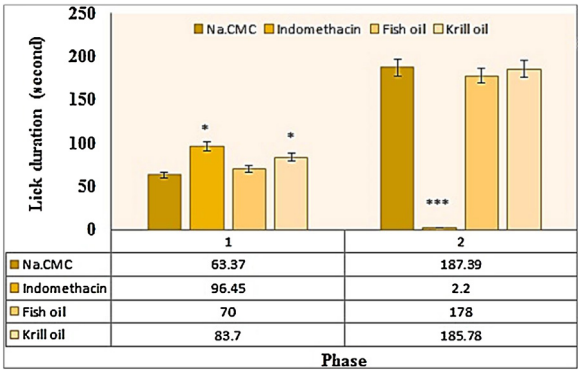


Fig. 3. Levels of TNF-α in the serum of animals with carrageenan induced-inflammation before and after treatments.



	1	2
Na.CMC	63.37	187.39
Indomethacin	96.45	2.2
Fish oil	70	178
Krill oil	83.7	185.78

Fig. 4. FO and NKO did not have any analgesic effect using formalin test in mice.

(100 mg/kg) showed the highest protection against the acetic acid induced writhing (70%), while FO and NKO showed 22.5% and 50%, respectively reducing of the writhes counts. It was found that NKO was significantly more potent compared to FO in the percentage of inhibition ($p < 0.01$)

6. Discussion

In our study, the oral supplementation of NKO and FO at the same dose and level of EPA and DHA (EPA + DHA) were chosen to study of their anti-inflammation and anti-nociceptive activities and also the results were analysed to find out which treatment was more effective. We used 500 mg/ml of the both supplements which are closely equivalent to the dose recommended for normal human (300–500 mg/kg/day) were used [26]. Meanwhile, the EPA:DHA ratio was 1.6 which may be equally beneficial, since both fatty acids are in the most appropriate matching in the range of 2:1 to 1:2 as recommended in the literature [27]. Although there are several studies focused on the inflammatory and nociceptive effects of KO and FO on human [28,29], there are almost no reports for rodents and evaluations of the changes of inflammatory factors in response to the treatments after inducing inflammation. Both supplements showed significant anti-inflammatory and anti-nociceptive activities compared to that of the normal saline. However, NKO showed significant higher level of activities than FO.

Rat and mice are the most commonly used models for studies of nociception and inflammation in animals. It was noted that in rodents neurogenic inflammation and pain could be evaluated by

Table 3
Effects of NKO and FO on writhing induced by acetic acid and change in latency time in hot-plate in mice[#], *=p < 0.05,[##] and **=p < 0.01 and ***=p < 0.001. #=different value effect between FO and NKO.

		Hot plate test mean reaction time (h ± SD)					
Groups	Dose	0	1 hr	2hr	3hr	Number of writhing	% inhibition
Control (Na.CMC)	0.2 ml/mice, ip	2.58 ± 0.02	2.28 ± 0.02	2.62 ± 0.03	2.66 ± 0.04	40	0
Reference	100	2.58 ±	6.98 ± 0.0	8.11 ± 0.0	7.51 ± 0.0	12***	70
Aspirin		0.02	1**	0**	1**		
	10		(44.1)	(51.3)	(47.5)		
Pentazocine (for hot plate)	(mg/kg),ip						
FO	500(mg)	2.41 ± 0.01	3.4 ± 0.01*(24.1)	5.49 ± 0.01**(39)	3.01 ± 0.01**(21.2)	31*	22.5
NKO	500	2.48 ± 0.01	3.82 ± 0.02*(23.4)	6.53 ± 0.02**(44.1)	3.52 ± 0.02	20**#	50##
	(mg)				(20)		

The values in the parentheses indicate the percentage of inhibition. Values are mean ± SD of six samples in each group. * Significant ns – Not significant Inhibition of hot plate test by Pentazocine and writhing number in acetic acid induced writhing by Aspirin, NKO and FO measured at 1, 2 and 3 h in hot plate assay.

several chemical stimulants, including carrageenan, formalin, acid acetic, and physical stimulants, such as heat and cold [30,31]. Although behavioural symptoms of those phenomena, such as edema of the injected paw and pain which lead to lick or bite responses are similar regardless the nature of the chemicals and physicals stimuli used, it has been assumed that these stimulus have definite and different mechanisms of actions which might obtain different results for the same treatments [32].

Carrageenan-injection developed edema and inflammation which is biphasic and involves several mediators released in sequential order [33], such as histamine, bradykinin, and serotonin releasing during the first phase, i.e., 30–60 min. Those mediators are released by the cells neighbouring the carrageenan injection zone provoking edema, hyperalgesia and erythema which are considered as signs of inflammation. Second phase, i.e., 2–3 hr is mediated by the release of prostaglandins (PGE1, PGE2), the main culprit responsible for acute inflammation, which are formed from ARA through the action of COX isoenzymes [33,34]. The rapid up-regulation of expression of COX-2 mRNA in the CNS are detectable and more prominent in the spinal cord compared to the all tissue of brain [32]. In the current study, NKO and FO, significantly decreased the mice paw edema at the first, second and third hr, with a peak at the second hr compared to the control, suggesting the possible mechanism of action may inhibit COX synthesis, thus decreasing PG (in mice $PGF_2\alpha$-EA) concentrations contribute to provoke of both pain perception and nociceptive neuron hyperactivity of dorsal horn [35]. Such inhibitory could act in the same pathway which non-steroidal anti-inflammatory drugs, such as indomethacin involve. Although the paw volume at the third hr treated by NKO increased compared to the second hr, but it was still smaller than those measured in the first hr and base line. It was found that the anti-inflammatory potentials were significantly different between NKO and FO, due to different level of omega-3 PUFAs [36]. No similar work to the current study was found by our finding were somehow similar to the study performed by Miles et al. [10] who worked on inflammation of rheumatoid arthritis in a mice model. The study found that KO could decrease paw thickness and arthritic score more effectively than FO for the same oral EPA + DHA dose.

Several studies suggested that microglial cells reveal to play a vital role in the initiation of processes involving persistent pain states. C-fibre nociceptive input from the sciatic nerve induces glial activation, and thus, such activation indicates to be vital to provoke acute inflammatory and inflammatory pain in rodent models. Activated glial cells are characterized by the proliferation, hypertrophy, and increased production of inflammatory cytokines, such as IL-1β, IL-6, IL-2, PGE2 and TNF-α [37]. Out of these cytokines, TNF-α is a more crucial factor in inflammatory reactions, producing native protective responses by stimulating T cells and also macrophages, which then could activate the production of others inflammatory cytokines [38]. IL-6 is also one of the most vital cytokines which is released by several cells at the injured sites [39]. The results of this study indicated that pre-treatment of carrageenan mice with NKO, FO and also indomethacin decreased the TNF-α and IL-6 levels in plasma of those mice blood when compared to normal saline in the control animals. This fact indicated the anti-inflammatory effect of both NKO and FO and also showed that there was significant different between NKO and FO in decreasing both TNF-α and IL-6 levels, however, NKO was found more effective than FO. The present findings are in accordance with earlier results where NKO and also PUFAs (omega-3) declined some pro-inflammatory factors, including IL-6 and TNF-α in inflammatory joint pain and chronic inflammation in arthritic symptoms [28,40].

The formalin-induced paw edema consists of migration of neutrophils, macrophages and proliferation of fibroblasts which are considered as distinctive biphasic nociceptive phases. The early phase pain is because of a direct impact of formalin on nociceptors and PGE1, PGE2 do not have any important roles within this phase. The late phase pain is believed as a result of acute inflammation response termed neurogenic and inflammatory factors [21]. The present study indicated that NKO and FO were failed to indicate nociceptive effect via this test. The disability of the two treatments to have any effects on the both phases showed that they may not contain active anti-nociceptive and anti-inflammatory principle acting both centrally and peripherally. The indomethacin (10 mg/kg) exhibited the reduction of paw licking time of 30 min. only in the second phase. Based on a study performed by Lotz et, al. [41], The present study could assume that both treatments may not possess inhibitory activity on the proliferation of fibroblasts and also probably connective tissue modulation effect. However, further studies may be needed to explore the cause of such effects.

Hot plate animal test elucidates that the central mediated analgesic activity or supra-spinal analgesia and spinal reflex after induced pain, may be resulted from any treatments depleting LC-noradrenergic neurons [42]. This sort of stimuli is found to be centrally selective and sensitive analgesic to COX inhibitors, but not peripherally. In the present work, NKO and FO significantly increased the hot-plate latency compared to normal saline, which was related to their analgesic impact probably through inhibiting the synthesis of some related components, such as prostaglandins which highlight the role of omega-3 PUFAs in such activities. Nobre et al. [43] indicated that omega-3 PUFAs are known to have ability to increase significantly the withdrawal threshold by both peripheral and central mechanisms in the hot plate test suggesting anti-nociceptive properties, probably by inhibiting microglial release of matrix metalloproteinases.

In the acetic acid test which is considered as chemical stimuli intraperitoneal administration of acetic acid excites the pain nerve endings which could be either central and or peripheral analgesia.

The abdominal constriction is associated to sensitization of nociceptive receptors to prostaglandins [44]. Various nociceptive mechanisms are suggested to be effective because of releasing the biogenic amines such as histamine and serotonin and also by inhibiting the COXs and their metabolites, such as PGE2 and PGF2α as well as the sympathetic nervous system (opioid system). In the present study, NKO and FO, efficiently inhibited the writhing in the mice, compared to the control. However, NKO was found to be more potent than FO.

The present study for the first time examined the anti-nociceptive and anti-inflammatory effects of NKO and FO, in rodents via induction acute inflammation in rodent model. So, at the present time, the comparison of our results with other published data seems unreachable. Nevertheless, the anti-edema effects exhibited by omega-3 pre-treated animals, suggesting that they are COX enzymes and microglial inhibitors, leading to reduction of the release of pro-inflammatory cytokines, such as TNF-α, IL-6 among other factors [28].

It was also found in our study that both treatments probably contained anti-nociceptive activity in peripheral pain perception type. However, because of the complexity of peripheral pain perception and related responses found in animal models, it seems that more detailed studies will be needed.

Laidlaw et al [45], reported that there is no difference in bioavailability of DHA from FO and KO. De Boer et al. [46] also, showed that PUFAs could alleviate inflammation, mainly by enhancing adiponectin and decreasing the intensity of adipocyte-macrophage cross-linking. For example, EPA could increase the EPA mediators that are less inflammatory compared to those produced from ARA. It is found that such PUFAs can regulate dendritic and T cells function and production which contribute to supress reactive oxygen species activity.

Batetta et al. [47] indicated that in Zucker rats, EPA and DHA from KO acted more efficiently compared to those from FO in arthritis. Moreover, a higher incorporation of DHA into brain was also reported after KO administration to Zucker rats [48]. Although the type of inflammation and the rats used in the mentioned study were different from those used in the present study, therefore it could be hypothesized that the cause of higher bioactivity of NKO compared to FO, could be possibly due to the follows reasons: 1) different structure of PLs in NKO and TAG in FO. This is because of differences in the pharmacokinetic properties between those fatty acids originating from PLs versus TAG [49]. In fact, PLs are more capable to accelerate the absorption, intestinal metabolism, passing through blood brain barrier (BBB), cell membrane, mitochondria and distribution of omega-3 fatty acids than TAG. 2) Lower amount of ARA, polyunsaturated omega-6 fatty acid (20:4). When omega-6 fatty acids predominate in the PLs of cell membranes, the production of pro-inflammatory type-2 PGs and type-4 LTs are encouraged; whereas, the presence of omega-3 fatty acids promotes the production of anti-inflammatory PGs and LTs. 3) The key role of antioxidants, such as anthocyanins, edaravone, and especially astaxanthin in KO [50].

In summary, it is concluded that FO and NKO can exert analgesic and anti-inflammatory activity by down regulation of the expression of pro-inflammatory cytokines such as TNF-α, and IL-6.

Declaration

The author(s) declare no competing financial interests.

Author contributions statement

Parstoo Mojtahedzadeh-Ardabili: Running the experiments, Data Analysis, Writing the main manuscript.

Sima Kianpour Rad: Data Analysis, Writing the manuscript.

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References

[1] B.-M. Larsson, Induction of a Non-Allergic Inflammation in the Human Respiratory Tract by Organic Dust, (2001) .
[2] A.K. Nussler, T.R. Billiar, Inflammation, immunoregulation, and inducible nitric oxide synthase, J. Leukoc. Biol. 54 (1993) 171–178.
[3] P.A. Ward, Acute and Chronic Inflammation, Fundam. Inflamm. (2010) 1–16.
[4] M.I. Johnson, The physiology of the sensory dimensions of clinical pain, Physiotherapy 83 (1997) 526–536.
[5] C.D. Funk, Prostaglandins and leukotrienes: advances in eicosanoid biology, Science 294 (2001) 1871–1875.
[6] D.L. Simmons, R.M. Botting, T. Hla, Cyclooxygenase isozymes: the biology of prostaglandin synthesis and inhibition, Pharmacol. Rev. 56 (2004) 387–437.
[7] H. Tapiero, G.N. Ba, P. Couvreur, K. Tew, Polyunsaturated fatty acids (Pufa) and eicosanoids in human health and pathologies, Biomed. Pharmacother. 56 (2002) 215–222.
[8] U.N. Das, Nutritional factors in the pathobiology of human essential hypertension, Nutrition 17 (2001) 337–346.
[9] J.G. Gormaz, R. Rodrigo, L.A. Videla, M. Beems, Biosynthesis and bioavailability of long-chain polyunsaturated fatty acids in non-alcoholic fatty liver disease, Prog. Lipid Res. 49 (2010) 407–419.
[10] E.A. Miles, P.C. Calder, Influence of marine N-3 polyunsaturated fatty acids on immune function and a systematic review of their effects on clinical outcomes in rheumatoid arthritis, Br. J. Nutr. 107 (2012) S171–S184.
[11] M.P. Barros, S.C. Poppe, E.F. Bondan, Neuroprotective properties of the marine carotenoid astaxanthin and Omega-3 fatty acids, and perspectives for the natural combination of both in krill oil, Nutrients 6 (2014) 1293–1317.
[12] S.M. Ulven, K.B. Holven, Comparison of bioavailability of krill oil versus fish oil and health effect, Vasc. Health Risk Manag. 11 (2015) 511.
[13] L. Burri, N. Hoem, S. Banni, K. Berge, Marine Omega-3 phospholipids: metabolism and biological activities, Int. J. Mol. Sci. 13 (2012) 15401–15419.
[14] C. Capini, et al., Antigen-specific suppression of inflammatory arthritis using liposomes, J. Immunol. 182 (2009) 3556–3565.
[15] A.R. Rao, R. Sarada, G.A. Ravishankar, Stabilization of astaxanthin in edible oils and its use as an antioxidant, J. Sci. Food Agric. 87 (2007) 957–965.
[16] N. Salem, C.N. Kuratko, A reexamination of krill oil bioavailability studies, Lipids Health Dis. 13 (2014) 137.
[17] P.D. Nichols, S.M. Kitessa, M. Abeywardena, Commentary on a trial comparing krill oil versus fish oil, Lipids Health Dis. 13 (2014) 2.
[18] P.G. Reeves, F.H. Nielsen, G.C. Fahey Jr, Ain-93 purified diets for laboratory rodents: final report of the American Institute of Nutrition Ad Hoc Writing Committee on the reformulation of the Ain-76a rodent diet, J. Nutr. 123 (1993) 1939–1951.
[19] L. Levy, Carrageenan paw edema in the mouse, Life Sci. 8 (1969) 601–606.
[20] M. Bucci, et al., In vivo delivery of the Caveolin-1 scaffolding domain inhibits nitric oxide synthesis and reduces inflammation, Nat. Med. 6 (2000) 1362–1367.
[21] S. Hunskaar, K. Hole, The Formalin test in mice: dissociation between inflammatory and non-inflammatory pain, Pain 30 (1987) 103–114.
[22] N.B. Eddy, D. Leimbach, Synthetic analgesics. Ii. Dithienylbutenyl-and dithienylbutylamines, J. Pharmacol. Exp. Ther. 107 (1953) 385–393.
[23] H. Collier, L. Dinneen, C.A. Johnson, C. Schneider, The abdominal constriction response and its suppression by analgesic drugs in the mouse, Br. J. Pharmacol. 32 (1968) 295–310.
[24] P. Antonisamy, S. Ignacimuthu, Immunomodulatory, analgesic and antipyretic effects of violacein isolated from Chromobacterium violaceum, Phytomedicine 17 (2010) 300–304.
[25] D.M. Sayah, E. Sokolskaja, L. Berthoux, J. Luban, Cyclophilin a retrotransposition into Trim5 explains owl monkey resistance to Hiv-1, Nature 430 (2004) 569–573.
[26] I. Jensen, H. Mæhre, S. Tømmerås, K. Eilertsen, R. Olsen, E. Elvevoll, Farmed atlantic salmon (Salmo salar L.) is a good source of long chain Omega-3 fatty acids, Nutr. Bull. 37 (2012) 25–29.
[27] W.S. Harris, Fish oil supplementation: evidence for health benefits, Cleve. Clin. J. Med. 71 (2004) 208–221.
[28] R.J. Goldberg, J. Katz, A meta-analysis of the analgesic effects of Omega-3 polyunsaturated fatty acid supplementation for inflammatory joint pain, Pain 129 (2007) 210–223.
[29] J. Tur, M. Bibiloni, A. Sureda, A. Pons, Dietary sources of omega 3 fatty acids: public health risks and benefits, Br. J. Nutr. 107 (2012) S23–S52.
[30] E.J. Cobos, E. Portillo-Salido, "Bedside-to-Bench" behavioral outcomes in animal models of pain: beyond the evaluation of reflexes, Curr. Neuropharmacol. 11 (2013) 560–591.
[31] N.S. Gregory, A.L. Harris, C.R. Robinson, P.M. Dougherty, P.N. Fuchs, K.A. Sluka, An overview of animal models of pain: disease models and outcome measures, J. Pain 14 (2013) 1255–1269.
[32] R. Bunea, K. El Farrah, L. Deutsch, Evaluation of the effects of Neptune krill oil on the clinical course of hyperlipidemia, Altern. Med. Rev. 9 (2004) 420–428.

[33] S. Sireeratawong, et al., Anti-inflammatory, analgesic, and antipyretic activities of the ethanol extract of Piper interruptum opiz and Piper Chaba Linn, ISRN Pharmacol. 2012 (2012).
[34] D. Guo, et al., Anti-inflammatory activities and mechanisms of action of the petroleum ether fraction of Rosa multiflora Thunb. Hips, J. Ethnopharmacol. 138 (2011) 717–722.
[35] T.V. Brito, et al., Anti-inflammatory effect of a sulphated polysaccharide fraction extracted from the red algae Hypnea musciformis via the suppression of neutrophil migration by the nitric oxide signalling pathway, J. Pharm. Pharmacol. 65 (2013) 724–733.
[36] P. Reddy, B.R. Lokesh, Studies on anti-inflammatory activity of spice principles and dietary N-3 polyunsaturated fatty acids on carrageenan-induced inflammation in rats, Ann. Nutr. Metab. 38 (1994) 349–358.
[37] K. Ren, R. Torres, Role of Interleukin-1β during pain and inflammation, Brain Res. Rev. 60 (2009) 57–64.
[38] P. Ben-Av, L.J. Crofford, R.L. Wilder, T. Hla, Induction of vascular endothelial growth factor expression in synovial fibroblasts by prostaglandin e and Interleukin-1: a potential mechanism for inflammatory angiogenesis, FEBS Lett. 372 (1995) 83–87.
[39] H. Tilg, C.A. Dinarello, J.W. Mier, Il-6 and Apps: Anti-Inflammatory and Immunosuppressive Mediators, Immunol. Today 18 (1997) 428–432.
[40] L. Deutsch, Evaluation of the effect of Neptune krill oil on chronic inflammation and arthritic symptoms, J. Am. Coll. Nutr. 26 (2007) 39–48.
[41] M. Lotz, J.H. Vaughan, D.A. Carson, Effect of neuropeptides on production of inflammatory cytokines by human monocytes, Science 241 (1988) 1218–1222.
[42] T. Kudo, T. Kushikata, M. Kudo, T. Kudo, K. Hirota, A central neuropathic pain model by Dsp-4 induced lesion of noradrenergic neurons: preliminary report, Neurosci. Lett. 481 (2010) 102–104.
[43] M.E.P. Nobre, et al., Eicosapentaenoic acid and docosahexaenoic acid exert anti-inflammatory and antinociceptive effects in rodents at low doses, Nutr. Res. 33 (2013) 422–433.
[44] S. Ito, E. Okuda-Ashitaka, T. Minami, Central and peripheral roles of prostaglandins in pain and their interactions with novel neuropeptides nociceptin and nocistatin, Neurosci. Res. 41 (2001) 299–332.
[45] M. Laidlaw, C.A. Cockerline, W.J. Rowe, A randomized clinical trial to determine the efficacy of manufacturers' recommended doses of Omega-3 fatty acids from different sources in facilitating cardiovascular disease risk reduction, Lipids Health Dis. 13 (2014) 99.
[46] H.-J. de Geus, I. Aidos, J. de Boer, J.B. Luten, A.T. Udo, Characterisation of fatty acids in biological oil samples using comprehensive multidimensional gas chromatography, J. Chromatogr. A 910 (2001) 95–103.
[47] B. Batetta, et al., Endocannabinoids may mediate the ability of (N-3) fatty acids to reduce ectopic fat and inflammatory mediators in obese Zucker rats, J. Nutr. 139 (2009) 1495–1501.
[48] V. Di Marzo, et al., Dietary krill oil increases docosahexaenoic acid and reduces 2-arachidonoylglycerol but not *N*-Acylethanolamine levels in the brain of obese Zucker rats, Int. Dairy J. 20 (2010) 231–235.
[49] D. Lemaitre-Delaunay, C. Pachiaudi, M. Laville, J. Pousin, M. Armstrong, M. Lagarde, Blood compartmental metabolism of docosahexaenoic acid (Dha) in humans after ingestion of a single dose of [13c] dha in Phosphatidylcholine, J. Lipid Res. 40 (1999) 1867–1874.
[50] F.J. Pashkow, D.G. Watumull, C.L. Campbell, Astaxanthin: a novel potential treatment for oxidative stress and inflammation in cardiovascular disease, Am. J. Cardiol. 101 (2008) S58–S68.