

Anti-Inflammatory and Therapeutic Activities of Omega-3-Polyunsaturated Fatty Acid Oil Extract on Acute Inflammation in Animal Models

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Abstract: Background: When prescribing painkillers for acute, chronic, and degenerative pain disorders, one must take into account safety issues for continuous long-term use of anti-inflammatory drugs due to the side effects. As a result, the therapeutic safety of anti-inflammatory supplements utilizing natural forms, such as omega -3 fatty acids (n-3 fatty acids), is more important than its effectiveness. Method: The nutritional intervention of n-3 fish oil extract (owing to its eicosapentaenoic acid, EPA, and docosahexaenoic acid, DHA contents) in experimental animal for acute inflammatory models was investigated in this work using standard methods (Carageenan, Arachidonic acid, and PGE₂). Result: Results obtained demonstrated a notable reduction in inflammation caused by fish oil due to its EPA and DHA content. For groups 1, 2, 3, 4, and 5, the percentage inhibition of the carrageenan-induced paw edema inflammation after 24 hours was 78.57%, 57.14%, 50.00%, 100.00% and 92.85%, respectively. Arachidonic acid-induced ear edema was inhibited by 30, 24, 27, 37, and 40%, whilst the PGE-2 test was inhibited by 44.16, 52.48, 61.34, 74, and 80.05%. Conclusion: The findings demonstrated that eicosapentanoic acid (EPA) and docosahexanoic acid (DHA), the active components in omega-3 polyunsaturated fatty acids, have the ability to disrupt the prostaglandin metabolic pathway by competing with arachidonic acids for the COX active site and thereby inhibiting their synthesis. The novelty of the work is demonstrated in the use of diet instead SAIDs to resolve inflammation.

Keywords: Key words: Docosahexanoic acid, Eicosapentanoic acid, Inflammation, Inhibition, prostaglandin

1. Introduction

Fatty acids play vital biochemical, structural, and functional roles in the human body. They serve as the primary components of cellular membranes in addition to serving as an energy source¹¹. As a component of membrane phospholipids, they guarantee the fluidity, flexibility, and permeability of the membrane. They also ensure passive transport through the membrane, and they are related to other membrane proteins. Omega-3 and omega-6 polyunsaturated fatty acids (PUFAs) appear to be the most significant fatty acids because of their numerous biological functions, which include influencing the inflammatory cascade, lowering oxidative stress and providing protection for the nervous system and cardiovascular system¹¹. Additionally, they serve as the building blocks for steroid hormones like eicosanoids (prostaglandins, leukotrienes, and thromboxanes)¹⁹ and bioactive

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mediators like cholesterol. The immune system's reaction to adverse stimuli like pathogens, damaged cells, poisonous substances, or radiation is inflammation, which operates by eradicating harmful stimuli and starting the healing process. As a result, it serves as a defense mechanism that is essential to health⁴.

A class of naturally occurring lipids known as omega-3 fatty acids, often referred to as n-3 fatty acids, are found in high concentrations in a number of fish and plants, including flax seed oil, perilla oil, and others⁵. The designation omega-3 ("n-3", "ω-3") denotes that the first double bond is present at the third carbon-carbon bond from the carbon chain's terminal methyl end (ω). Alpha linolenic acid (ALA), eicosapentaenoic acid (EPA), and docosahexaenoic acid are the three most prevalent omega 3 fatty acids (DHA). Flaxseed and canola vegetable oils include ALA, whereas fish and fish oil contain higher concentrations of EPA and DHA¹³. They are present in fish in different amounts, especially in pelagic and cold-water species like menhaden, mackerel, oil sardine and salmon².

Recent research has shown that omega-3 polyunsaturated fatty acids (PUFAs) are important mediators of inflammation, able to enhance anti-inflammatory responses, block hyper-inflammatory reactions, and decrease the incidence of systemic inflammatory response syndrome (SIRS) and infection-related complications^{22, 23}. Reduced NF-κB pro-inflammatory transcription factor activity in response to inflammatory stimuli is one of EPA and DHA's anti-inflammatory actions. This result has been connected to the membrane-mediated activities of EPA and DHA, which suppress inflammatory signaling in its early stages.

However, it appears that EPA and DHA work by directly interacting with membrane receptors on inflammatory cells to suppress inflammatory responses. In macrophages, for instance, binding long-chain fatty acids, particularly DHA, to the GPR120 receptor reduces NF-κB activation and lowers the generation of inflammatory cytokines. This mode of action shows that EPA and DHA can have anti-inflammatory benefits without altering the synthesis of lipid mediators or integrating into cell membranes. Omega-3 fatty acids are also believed to produce mediators known as specific pro-resolving mediators (SPMs). In several disorders, SPMs such as resolvins, protectins, and maresins stimulate the resolution of inflammation^{24, 25, 26,27}.

Omega-3 fatty acids also play a role in controlling how immune cells including macrophages, neutrophils, basophils, eosinophils, T cells, and B cells are activated. According to studies, omega-3 fatty acids are found in the phospholipids that make up neutrophil cell membranes, and by secreting cytokines and chemokines, they enhance immune function by improving macrophage activity and phagocytic capabilities. These results suggest that omega-3 fatty acids may be useful as a pharmaco-nutrient for reducing COVID-19-induced inflammation²⁸. Investigating modifiable risk factors for severe consequences of an inflammatory storm is crucial given the public health concerns surrounding the present COVID-19 outbreak and accompanying mortality. For high-risk patients, one preventive strategy that is also comparatively inexpensive is the use of diets and supplements rich in unsaturated fatty acids, especially omega-3 PUFA.

In a certain study, the outcomes suggested that omega-3 leads to a reduction in inflammation, improved body composition, enhanced exercise capacity, higher quality of life, and lower exacerbation occurrences. The study concluded that omega-3 supplementation in COPD management showed promising results, considering its efficacy in slowing down COPD progression with minimal side effects, cost-effectiveness, and feasibility²⁹. Furthermore, cardiovascular disease (CVD) is the world's most recognized and notorious cause of death. It is known that increased triglyceride-rich lipoproteins (TRLs) and remnants of triglyceride-rich lipoproteins (RLP) are the

major risk factor for CVD. Furthermore, hypertriglyceridemia commonly leads to a reduction in HDL and an increase in atherogenic small dense low-density lipoprotein (sdLDL or LDL-III) levels. Thus, the evidence shows that Ω -3 fatty acids (eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) have a beneficial effect on CVD through reprogramming of TRL metabolism, reducing inflammatory mediators (cytokines and leukotrienes), and modulation of cell adhesion molecules³⁰.

Omega-3 PUFAs such as EPA, DPA and DHA are also available at sites of inflammation for enzymatic conversion to bioactive mediators. The anti-inflammatory effect of omega-3 PUFAs is thought to occur not only by competing with the formation of eicosanoids from AA, but also by providing alternative metabolites with less potent activity than that of AA-derived mediators³¹⁻³³. Recent advances in liquid chromatography–tandem mass spectrometry (LC–MS/MS)-based lipidomics uncovered a novel series of lipid mediators derived from omega-3 PUFAs³⁴⁻³⁵. Emerging evidence suggests that uncontrolled inflammation may contribute to a progression to chronic inflammatory states, including cardiovascular diseases, autoimmune diseases, fibrosis and cancer³⁶⁻³⁷. Understanding the molecular mechanisms of how elevated omega-3 PUFA levels control inflammation and tissue homeostasis will lead to a new class of therapeutic applications.

The precise determination of the molecular mechanisms that control inflammation and the resolution process will aid in the development of a new class of anti-inflammatory therapies. Omega-3 PUFA-derived specialized pro-resolving mediators such as resolvins, protectins and maresins are potential candidates to be developed as new therapeutics³⁸⁻³⁹. Also oxygenation at the site of the omega-3 double bond that distinguishes omega-3 PUFAs from other fatty acids may play an important role for the beneficial effects of dietary omega-3 PUFAs in keeping human health and tissue homeostasis⁴⁰. Therefore, enhancement of this metabolic pathway may have therapeutic implications in controlling inflammation and related diseases.

The therapeutic effects of omega-3 FAs on inflammation have been widely studied for many years, with an overall consensus that increasing consumption results in the reduction of pro-inflammatory agents. There are several mechanisms underlying the anti-inflammatory actions of omega-3 FAs. The regulation of inflammatory gene expression, namely the suppression of inflammatory factor nuclear factor kappa B (NFkB) transcription and expression of peroxisome proliferator activated receptor g (PPARg), lead to a reduction in the production of inflammatory cytokines, such as TNF- α . Further, omega-3 FAs play an important role in reducing the generation of pro-inflammatory eicosanoids, such as prostaglandin E2 (PGE2) and leukotriene B4 (LTB4), through altering the cell membrane fatty acid composition.

Although the mechanism of action of omega-3 fatty acids' anti-inflammatory activity in both human and animal models is still being extensively researched, a number of research and in-depth studies have shown that this fatty acid is one of the natural supplements with excellent therapeutic safety and potent anti-inflammatory activity. Therefore, the purpose of this study is to explore the use of diet and omega-3 PUFA supplements as a therapeutic alternative to SAID medications.

2. Materials and Methods:

Chemicals and drugs

The chemicals and reagents used for this study were of analytical grade and were obtained from Merck (Germany), (Sigma, St. Louis, Missouri, USA), BDH Chemicals Limited (Poole, England), May and Baker Ltd, (England), Riedel-de-Haen, (Hannow, Germany), Hopkins and Williams (Essex, England) except otherwise stated. Omega -3 fatty acid capsules were gotten from Ibadan, Nigeria.

Animals

Healthy male and female albino rats (120 – 200 g) of Wistar strain and mice (40 – 65 g) were obtained and used. Animals were maintained at standard lighting and temperature conditions in the Animal House for one week to acclimatize in solid-bottom cages before commencement of the experiment. Food pellets and water were provided ad libitum to eliminate the effect of stress. Animals were handled according to prescribed ethical guidelines for Care and Use of Laboratory Animals by the committee for Ethical Care and Use of Laboratory Animals of the University.

Methods

Drug treatment

Content of one 200 mg capsule was dissolved in 10 ml phosphate buffer saline (PBS) solution to obtain a 20 mg/ml concentration⁸. The required amount of drug (1 ml) was then given to the rats orally.

Experimental design

Animals were distributed in six groups of five each (n = 5) as follows;

Group 1 received a dose of 180 mg/ml EPA + 120 mg/ml DHA, Group 2 received 360 mg/ml EPA + 240 mg/ml DHA, Group 3, 540 mg/ml EPA + 360 mg/ml DHA, Group 4 was administered a combined dose of 180 mg/ml EPA + 120 mg/ml DHA + 20 mg/ml Celecoxib, Group 5 was given 20 mg/ml celecoxib as the standard group while Group 6 took 2 ml PBS as the vehicle control group. All doses were administered orally and per kilogram body weights of the rats and mice.

Acute Inflammation studies

Carrageenan-induced paw edema

This method was carried out as described by Winter²⁰ with slight modifications from more recent sources³. The animals were divided into six groups (n = 5), deprived of food overnight but given water ad libitum prior to the experiment. One hour after drug dosing and 6 weeks of omega-3 fatty acids supplementation, test rats were then challenged by a subcutaneous injection of 0.1ml of 1% solution of carrageenan in sodium chloride into the sub-plantar side of the left hind paw following inhalation of 5% isoflurane (v/v) for 15 seconds while the vehicle control group was injected with the carrageenan solution. The basal paw thickness was then measured using a vernier caliper⁹. The paw thickness was measured again at 1, 2, 3, 4, and 5 hours after the challenge. The increase in paw thickness was then calculated as a percentage compared with the basal thickness (the zero hour). The difference of average values among treated and untreated groups was calculated for each time interval and evaluated statistically. The percent inhibition was calculated using the formula as follows: % edema inhibition = $[1 - (Tt/Tc)] \times 100$

(Tt and Tc are edema thickness in the test and control groups, respectively).

Arachidonic acid – induced ear edema

This model is based on the principle of metabolism of arachidonic acid by cyclooxygenase (COX), as described by Romay¹⁷, leading to the generation of prostaglandins and thromboxanes that mediate pain and edema associated with inflammation. Inhibition of these mediators by the extract and test drug is then evaluated. In this method, Inflammation was induced by topical application of arachidonic acid (2 mg in 20 µml of acetone) to both surfaces of the right ear of each mouse. The left ear was considered as control. One hour later, the thickness of the ear was determined using a

Digital Caliper. The percentage of the ear edema was calculated based on the left ear which is the control¹⁸.

PGE₂- Induced Hind Paw Edema

This model was used for the determination of anti-inflammatory activity following the modified method of Kasahara¹⁰. Animals were grouped in six groups of five each and were given either oil extract, standard drug or just physiological salt solution in the case of the control group. One hour after oral administration of the extract, drug or vehicle, each rat received 5 μ l of freshly prepared suspension of 1mg/ml PGE-2 in Tyrode's solution by injection into the sub-plantar tissue of the right hind paw. For the vehicle control group, 5 μ l of Tyrode's solution was injected into the left hind paw. Thereafter, edema was measured at 15minutes interval for 75minutes. Difference in the thickness of the footpad was measured relative to time zero¹.

Statistical analysis

All analyses were performed using Graph prism version 5 software and subjected to Bonferroni test for the separation of means. Results were expressed as Mean \pm SEM. Differences between means were considered statistically significant at $p < 0.05$.

Results

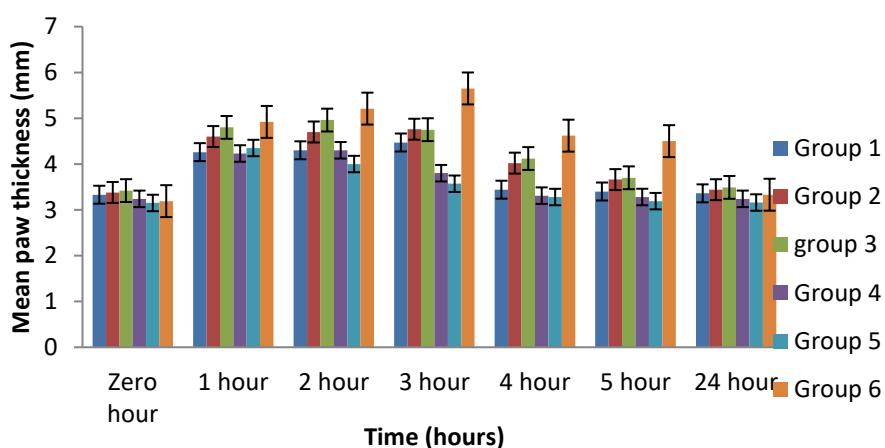


Figure 1. Mean paw thickness (mm \pm SEM) of rats induced with carrageenan

Mean paw thickness (mm \pm SEM) of rats induced with carrageenan solution at 0,1,2,3,4,5 and 24 hours. Edema was induced by a subcutaneous injection of 0.1ml of 1% carrageenan solution into the sub plantar side of the left hind paw. Group 1 received a dose of 180 mg/ml EPA + 120 mg/ml DHA, Group2 received 360 mg/ml EPA + 240 mg/ml DHA Group 3, 540 mg/ml EPA + 360 mg/ml DHA, Group 4 was administered a combined dose of 180 mg/ml EPA+ 120 mg/ml DHA + 20 mg/ml Celecoxib, Group 5 was given 20 mg/ml celecoxib as the standard group while Group 6 took 2 ml PBS as the vehicle control group.

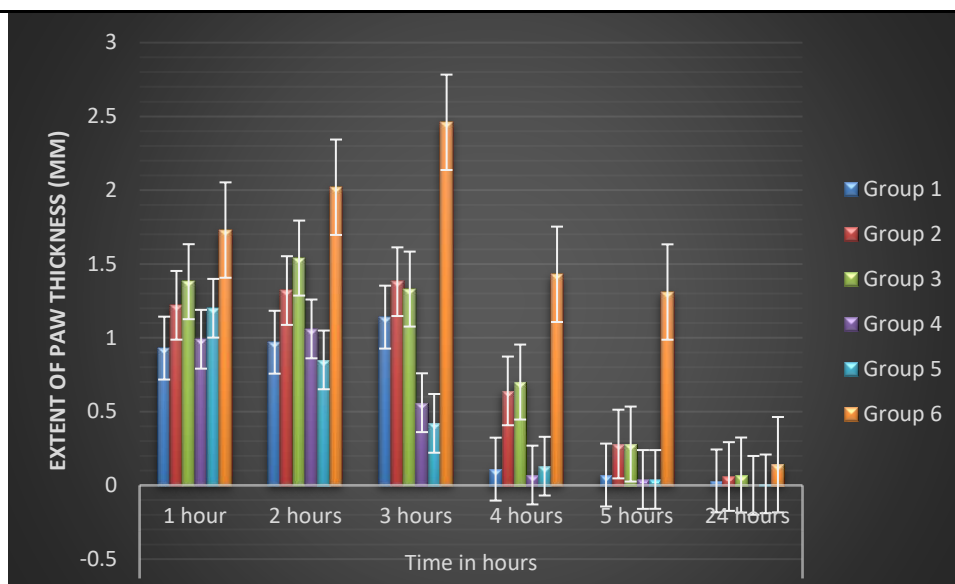


Figure 2. Progressive decrease in paw thickness in albino rats from 1 – 24 hours after induction

Progressive decrease in paw thickness in albino rats from 1 – 24 hours after induction with carrageenan solution and treatment with extract. This increase was calculated relative to t= 0. Group 1 received a dose of 180 mg/ml EPA + 120 mg/ml DHA, Group2 received 360 mg/ml EPA + 240 mg/ml DHA Group 3, 540 mg/ml EPA + 360 mg/ml DHA, Group 4 was administered a combined dose of 180 mg/ml EPA+ 120 mg/ml DHA + 20 mg/ml Celecoxib, Group 5 was given 20 mg/ml celecoxib as the standard group while Group 6 took 2 ml PBS as the vehicle control group.

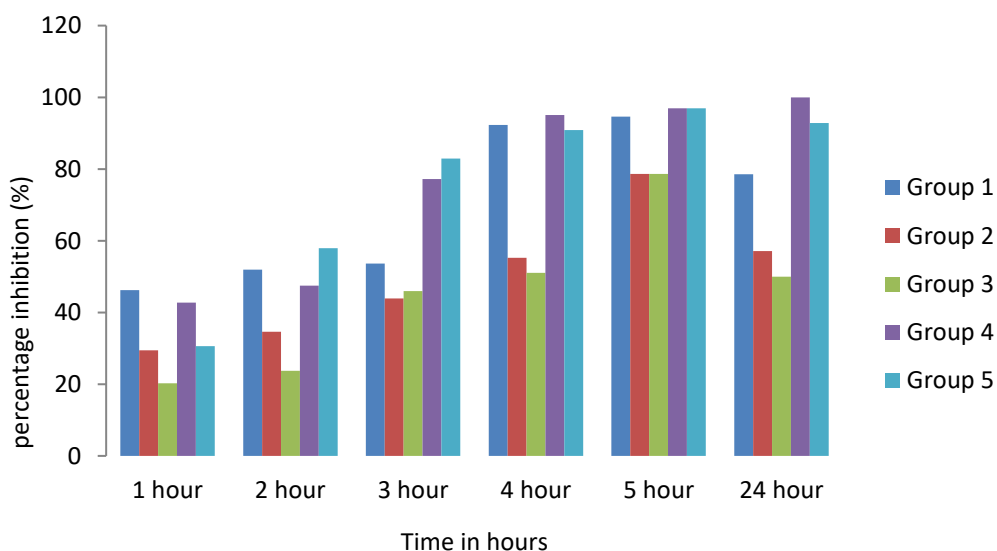


Figure 3. Percentage inhibition of paw edema in carrageenan - induced rats

Percentage inhibition of all extracts and standard used from 1, 2, 3, 4, 5 to 24 hours after induction of inflammation and commencement of treatment with extracts. Percentage inhibition of edema was

gotten with respect to the carrageenan control. Inhibition was observed to be indirectly proportional to the increase in paw thickness from 1 hour to the 24th hour. Animals in group 4 had 100% recovery rate recorded after 24 hours, other groups had their highest inhibition rates after the fifth hour

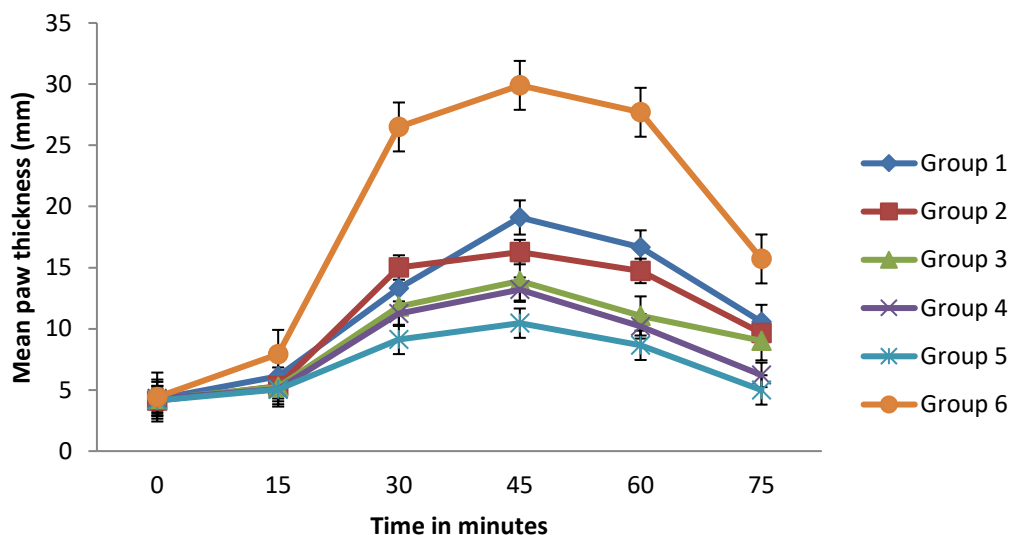


Figure 4. Paw thickness of albino rats after a one –time induction with Prostaglandin E2. Paw thickness was measured over a course of 75 minutes after the challenge. All groups reacted in a dose – dependent manner with the vehicle control having the highest effect of edema. Group 1 received a dose of 180 mg/ml EPA + 120 mg/ml DHA, Group2 received 360 mg/ml EPA + 240 mg/ml DHA Group 3, 540 mg/ml EPA + 360 mg/ml DHA, Group 4 was administered a combined dose of 180 mg/ml EPA+ 120 mg/ml DHA + 20 mg/ml Celecoxib, Group 5 was given 20 mg/ml celecoxib as the standard group while Group 6 took 2 ml PBS as the vehicle control group.

3. Discussion

A vast array of inflammatory mediators are activated as a result of inflammation, which is a healthy host reaction to environmental alterations or cellular injury and completes the repair of tissue structure and function¹⁵. Animal models were used to supplement omega-3 fatty acids to test and assess their anti-inflammatory capabilities. The anti-inflammatory effect of various doses of omega-3 fatty acids was examined in order to obtain different quantities of EPA and DHA from fish oils containing 1000 mg/ml, and these doses were provided in accordance with the kg body weights of the rats. Carrageenan-induced paw edema, arachidonic acid-induced ear edema, and prostaglandin E2-induced paw edema are anti-inflammatory models used to assess acute inflammation.

Rats' carrageenan-induced paw edema is an efficient way to monitor the occurrence of change and assess acute inflammation (Fig 1). Histamine and bradykinin are released by mast cells during the first phase of the reaction, which typically lasts between 0 and 2 hours and alerts the immune system to an injury or challenge. This causes a rise in vascular permeability, which causes fluids from the circulation to exude into the interstitial space²². The second phase then follows, beginning around the third hour and frequently characterized by the formation of prostaglandins¹³, an inflammatory mediator. The oil extract's and the standard employed in this study's mechanism of action is inhibition of prostaglandin production.

The results of the mean paw thickness (MPT) of the carrageenan-induced paw edema in the rat model (Fig. 1) are 3.33 ± 0.14 , 3.38 ± 0.06 , 3.42 ± 0.21 , 3.24 ± 0.07 , 3.15 ± 0.03 , and 3.19 ± 0.27 mm,

respectively, for groups 1, 2, 3, 4, 5 and 6. Inflammation began to develop an hour after induction, with MPT readings of 4.26 ± 0.28 , 4.60 ± 0.07 , 4.80 ± 0.31 , 4.23 ± 0.09 , 4.35 ± 0.04 , and 4.92 ± 0.35 mm. After 24 hours, the inflammation was almost totally reduced by MPT, with 3.36 ± 0.21 , 3.44 ± 0.21 , 3.49 ± 0.13 , 3.24 ± 0.27 , 3.16 ± 0.23 , and 3.33 ± 0.28 mm indicating, respectively, 78.57%, 57.14%, 50.00%, 100.00%, and 92.85% percentage inhibition. This outcome (Fig. 3) demonstrated the superiority of supplementation, as demonstrated in group 4 by 100% complete resolution of the inflammation. The oil extract's and the study's standard's mechanism of action is inhibition of prostaglandin production. At the conclusion of the observation period, the oil extract employed was discovered to effectively prevent and resolve edema. At the conclusion of the observation period, it was discovered that the oil extract utilized efficiently inhibited and resolved edema, and the pattern of resolution is consistent with that reported by Girish⁷.

From Fig. 2, it was clear that the paw thickness had not changed significantly even two hours after the extract had been given. After 3 hours, though, resolution was apparent. Following administration of the extract for 4 and 5 hours, the decrease in paw thickness was remarkably noticeable. Edema was fully resolved after 24 hours, and the simultaneous resolution of the rate of paw thickness reduction in groups 4 and 5 demonstrated the significance of supplementation in comparison to the norm. The metabolites of arachidonic acid (AA), among a broad list of mediators that also includes histamine, serotonin, the kinins, etc., have recently drawn attention. The cyclooxygenase and lipoxygenase pathways' of AA products are capable of generating vasodilatation, hyperemia, pain, edema, and cellular infiltration alone or in the right mix to produce these indications of inflammation²¹. The impact of fish oil extract on the metabolism of AA—which produces prostaglandins and thromboxanes that can trigger an inflammatory cascade that results in discomfort and fluid exudation that causes edema—was particularly striking, as shown in Table 1. Due to the release of inflammatory mediators that are drawn to the inflamed site, which results in the characteristic swelling within the dermal region and increased vascular permeability frequently associated with inflammation⁶, topical application of AA to mice's ears increased ear thickness, redness, and edema⁶. According to reports^{6, 16} direct application of AA to a mouse's ear results in an instantaneous vasodilatation, erythema, and development of severe edema.

The synthesis of leukotriene and prostaglandins, serotonin and histamine are responsible for the formation of the ear edema observed¹⁶. Arachidonic acid-induced ear edema in the rat models is given by ear weight and percentage inhibition. These were gotten to be, 0.118 ± 1.00 , 0.129 ± 2.03 , 0.123 ± 1.50 , 0.107 ± 2.10 , 0.101 ± 1.40 and 0.169 ± 3.60 mg corresponding to 30.18, 23.67, 27.22, 36.69 and 40.24% percentage inhibitions for groups 1, 2, 3, 4 and 5 respectively (Table 1). The weight of group 4, which is the supplemented group and that of 5, the positive control group as well as percentage inhibitions, were not statistically different ($p < 0.05$). Result obtained shows marked inhibition of edema in the test groups when compared to the control group ($p < 0.05$).

Omega-3 fatty acids (O3FAs) have been shown to reduce inflammation via several mechanisms, including inhibiting leukocyte chemotaxis, producing eicosanoids and inflammatory cytokines, and T-helper 1 lymphocyte reactivity⁴¹. The O3FAs eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) give rise to inflammation-reducing resolvins, protectins and maresins, and attenuate oxidative stress-induced DNA damage. The cardioprotective effects of O3FAs have been studied in both primary and secondary prevention settings, using dietary and fish oil interventions. Higher fish consumption associates with lower mortality rates in both primary and secondary prevention settings. Studies of fish oil, however, have shown greatest benefit specifically with EPA supplementation. The JELIS study, which randomised patients with hypercholesterolemia to either a statin or a statin

plus EPA (1.8 g daily), demonstrated a significant, 19% reduction in major coronary events in the statin plus EPA group⁴¹.

The discovery of novel substances for the prevention of inflammation frequently calls for the evaluation of anti-inflammatory activity. Prostaglandin E-2 (PGE₂) was used in this work to assess the effects of omega-3 fish oil extract on paw edema in rats. When newly generated PGE-2 in tyrode solution is subcutaneously injected into a rat paw, AA is massively mobilized from the membrane and then processed through the COX metabolic pathway to form PGs that regulate pain and edema. After a period of delayed reaction, or the initial phase, the majority of prostaglandin reactions successfully begin. Sensory nerves are notified of any hazard at this stage. This stage is swiftly followed by a period of excruciating pain and swelling (edema) as well as redness brought on by an increase in vascular permeability, fluid exudation, and leukocyte infiltration to the troubled tissue.

From Table 2 and Fig 4, Omega-3 fish oil extract inhibited PGE₂-induced paw edema significantly ($p < 0.05$) and the percentage inhibition were monitored after every 15 minutes for 75 minutes. Groups 4 and 5 showed no difference all through (group 4:5; 77.93:74.50; 68.57: 77.40; 65.07:75.19; 74.65:80.62 and 82.90:92.38 %) when compared but significantly different when compared to the control at $p < 0.05$ although this was not the case for the vehicle control group, which had the worst hit from the induction. The standard group however, showed maximum inhibition of edema although the other test groups also showed remarkable synergy in activity compared to the standard.

The scientific literature demonstrates how EPA plays a beneficial function in the regulation of endothelial tone. The endothelial cells, in fact, release nitric oxide (NO), which has the ability to modulate the vasomotor tone in response to acetylcholine and other vasoactive agonists⁴². Under physiological conditions, there is, in the body, an endothelium-dependent vasodilation due to the release of NO. If one has endothelial dysfunction, then NO release is reduced or absent. In this case, we are witnessing the appearance of toxic effects due to reactive oxygen costs, including peroxynitrite (ONOO₋₁)^{43, 44}. EPA is able to significantly reduce the formation of reactive oxygen species, as well as the expression of adhesion molecules, the release of pro-inflammatory cytokines, and the apoptotic cascade, as demonstrated by the *Din in vitro* studies conducted on HUVEC cells⁴⁵. EPA was also found to be able to reduce or inhibit lipid peroxidation in membrane vesicles with even high cholesterol levels. The highlighted effect is also easy due to the presence of statins⁴⁶. It is known that glucose contributes to the development of lipid peroxidation, the direct consequence of which is the appearance of high cholesterol levels. In this process, EPA also plays a key role, since it inhibits the formation of lipids⁴⁷. The key cells of the inflammatory process are rich in arachidonic acid n-6 fatty acid, but their content can vary through oral administration of EPA and DHA. The increase in the membrane content of EPA and DHA causes a change in the production pattern of eicosanoids and probably also of resolvins. Given the involvement that n-3 marine PUFAs have in modulating inflammatory responses, it is understood how these can be decisive in inflammatory process and resolution. The clinical data obtained from the anti-inflammatory evaluations obtained thanks to the role of EPA have raised the awareness that an increase in the diet could bring a clinical benefit. A univocal and clear dose of EPA to be used has not yet been identified, although it is clear that the therapeutic effects are strictly dose-dependent⁴⁸.

PGE₂ caused edema in the rat models, and the effect was dose-dependent, with the vehicle control showing the greatest edema effect. As the paw thickness returned to the pre-induction level, Groups 4 and 5 (the supplemented group and the standard group) demonstrated superiority, as illustrated in Fig 4. This explains why the oil extract, which is a powerful anti-inflammatory molecule capable of blocking the lipoxygenase and cyclo-oxygenase pathways that result in the formation of

prostaglandins and other inflammatory mediators, confer superior beneficial and therapeutic advantages when used with the usual SAID medication¹².

4. Conclusion

In-vitro and in-vivo prostaglandin production are both inhibited by dietary omega-3 polyunsaturated fatty acid intake, according to this study, particularly when a common medication is added. Therefore, we draw the conclusion that the long-standing use of omega-3 fatty acids as dietary supplements in the treatment of inflammatory disorders is both safe and beneficial.

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Table 1. Table 1 Anti-inflammatory effect of Omega-3-PUFA oil extract on Arachidonic Acid-induced ear edema in albino rats.

Treatment Groups	Ear weight (mg)	Percentage
		Inhibition (%)
1	0.118±1.00 ^b	30.18
2	0.129±2.03 ^c	23.67
3	0.123±1.50 ^b	27.22
4	0.107±2.10 ^a	36.69
5	0.101±1.40 ^a	40.24
6	0.169±3.60 ^d	-

Group 1 received a dose of 180 mg/ml EPA + 120 mg/ml DHA, Group2 received 360 mg/ml EPA + 240 mg/ml DHA Group 3, 540 mg/ml EPA + 360 mg/ml DHA, Group 4 was administered a combined dose of 180 mg/ml EPA+ 120 mg/ml DHA + 20 mg/ml Celecoxib, Group 5 was given 20 mg/ml celecoxib as the standard group while Group 6 took 2 ml PBS as the vehicle control group. Values are reported as Mean ± SEM of five determinations. Columns with the same superscripts are not statistically significant ($p < 0.05$). Percentage inhibition was calculated with respect to the control. For this analysis, the concentration of standard Celecoxib used was 10 mg/kg body weight of the mice.

Table 2. Percentage Inhibition of Inflammation as a result of induction with PGE-2

Treatment groups	Percentage Inhibition (%)				
	15 min	30 min	45 min	60 min	75 min
Group 1	47.00	59.10	41.76	46.78	44.19
Group 2	66.18	50.67	52.31	54.42	51.10
Group 3	71.63	65.67	62.09	70.79	57.75
Group 4	77.93	68.57	65.07	74.65	82.90

Group 5	74.50	77.40	75.19	80.62	92.38
Group 6	-	-	-	-	-

Percentage inhibition of inflammation by samples at 15 minutes interval and relative to the control. Group 1 received a dose of 180 mg/ml EPA + 120 mg/ml DHA, Group 2 received 360 mg/ml EPA + 240 mg/ml DHA Group 3, 540 mg/ml EPA + 360 mg/ml DHA, Group 4 was administered a combined dose of 180 mg/ml EPA+ 120 mg/ml DHA + 20 mg/ml Celecoxib, Group 5 was given 20 mg/ml celecoxib as the standard group while Group 6 took 2 ml PBS as the vehicle control group. After 60 minutes, all groups showed an increase in inhibition, this is the point where significant decrease in paw thickness was observed. This increase continued for rats in groups 4 and 5 until the end of the observation period. Generally, after the investigation period, all groups inhibited the edema in a dose – dependent manner.

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Author's roles

Jamila A. Omale conceived, designed and carried out the study. Lawrence U. S. Ezeanyika and Bennett C. Nwanguma analyzed, interpreted the data and drafted the manuscript. Aminu Omale revised the manuscript, read and made the final corrections. All the authors read and agreed to the publication of the finding as contained in the manuscript.

Conflicts of interest

The authors declare no conflicts of interest.

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Ethical approval

The experimental protocol was approved by the KSU-Ethics Committee and carried out in accordance with the guidelines given by committee for **Ethical Care and Use of Laboratory Animals of Kogi State University, Anyigba- Nigeria.**