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(RESEARCH ARTICLE)

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Effect of dietary omega-3-polyunsaturated fatty acid in animal models of experimentally-induced granulomatous inflammation

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Abstract

Recent advances have shown how polyunsaturated fatty acids (PUFAs) regulate molecular signaling, especially in the context of inflammation. Nutritional intervention/ supplementation with PUFAs in experimental animal models have exhibited anti-inflammatory properties. In this study, chronic inflammation was assayed by cotton pellet-induced granuloma method to evaluate the anti-inflammatory and anti-granuloma effects of omega-3-polyunsaturated fatty acid using experimentally induced inflammatory models in rats. Obtained results indicate that omega-3 oil extract significantly ($p<0.05$) reduced the granuloma tissue formation (58.36 ± 3.52, 65.40 ± 1.30, 57.78 ± 3.89, 50.93 ± 0.60 and 42.17 ± 0.70 mg) representing 33.78, 25.79, 34.44, 42.21 and 52.15 % inhibition respectively for the proliferative phase when compared to the control. These results showed that omega-3 oil extract studied, possess both anti-inflammatory and anti-granuloma properties.

Keywords: Omega-3 polyunsaturated fatty acids; Chronic inflammation; Cotton- pellet assay; Granuloma; supplementation; Proliferation

1. Introduction

Inflammation is part of the complex physiological response to harmful stimuli such as pathogens, damaged cells, toxins, and irritants [1]. The primary functions of inflammation are to eliminate the initial cause of injury, launch defense mechanisms, remove necrotic cells and tissues, and initiate tissue repair [1]. Properly regulated inflammation represents an efficient physiological mechanism that protects the host from infection and other insults and is thus essential to health. Inflammation can be classified as either acute or chronic according to the duration of the response. It can also be categorized according to the intensity of the process [2].

Inflammation is usually accompanied by redness, heat, pain and loss of function, and there are several stages to the process of inflammation that explain these. Firstly, the body tries to get as much blood as is possible to the damaged area. This is because of different components in the blood that can help to stop any further damage and also try to destroy or eliminate the cause [3].

The body then dilates the blood vessels of the area, allowing more blood to enter. This increased blood flow accounts for the hotness and redness of the inflamed area compared to the surrounding areas. After increasing the blood flow to the area, the body needs to get the chemicals and the immune cells out of the blood vessels and into the tissues. To do this, the cells that make up the blood vessels start to pull apart and create tiny holes in their walls. This allows very

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small molecules like water and some chemicals to escape into the tissues, while keeping larger molecules and cells on the inside [4]. There are different chemical compounds (mediators) that are involved in inflammation. Some are listed below:

- Histamine
- Serotonin
- Prostaglandins
- Leukotrienes
- Tumor Necrosis Factor-alpha (TNF-a) and Interleukin-1 (IL-1)

The mechanism of action of most anti-inflammatory medications is the inhibition of the enzyme (cyclooxygenase) responsible for catalyzing the biosynthesis of prostaglandins involved in inflammation. They are basically of two types:-

- Steroidal anti-inflammatory drugs (SAIDs)
- Non-steroidal anti-inflammatory drugs (NSAIDs)

Persistent long-term use safety concerns must be considered when prescribing pain relief medications for chronic and degenerative pain conditions. Due to serious side effects often experienced with non-steroidal anti-inflammatory drugs, natural alternative anti-inflammatory supplements have proven to be a better option. Although nonsteroidal medications can be effective, herbs and dietary supplements may offer a safer and effective alternative treatment for pain relief, especially for long-term use.

Nutritional intervention with n-3 PUFA (mostly due to its eicosapentaenoic acid, EPA and docosahexaenoic acid, DHA contents), has been observed to possess anti- inflammatory properties in experimental models. Some important mechanisms involved are: alteration of eicosanoids synthesis, cell membrane fluidity, and modulation of gene expression. Omega-3 PUFAs, primarily found in dietary fish oil are derived also from plants and are substrates, able to reduce or limit inflammation in critical illness. The biological effects of omega-3 PUFAs are also mediated by the production of pro-resolving mediators, which have been proposed to modulate and likely resolve inflammatory responses [5]. This study was conducted to evaluate the anti-granuloma activity of omega -3 polyunsaturated fatty acids (fish oil supplements), using chronic inflammation model. The chronic phase is usually characterized by proliferation, fibrin formation and eventual tissue repair.

2. Material and methods

2.1. Materials

2.1.1. Animal handling

Healthy male and female albino rats (120-200g) of Wistar strain and mice (40-65g) were obtained from the Animal Unit of the Faculty of Veterinary Medicine, University of Nigeria, Nsukka, and Enugu State of Nigeria. The 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.

2.1.2. Chemicals and Reagents

The chemicals and reagents used for this study are of analytical grade 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.

2.2. Methods

2.2.1. Drug treatment

Celecoxib, is a non-steroidal anti-inflammatory and analgesic drug known to potentially inhibit COX-2. Content of one 200mg capsule was dissolved in 10ml phosphate buffer saline (PBS) solution to obtain a 20mg/ml concentration. The required amount of drug (1ml) was then given to the rats orally. Doses were calculated based on body weights of the animals according to the OECD's guidelines.

2.2.2. Chronic Inflammation

Cotton-Pellet Induced Granuloma

This method was adopted from [6]. The principle of this method is based on the presence of granuloma, which are masses formed in response to inflammatory conditions, mostly, chronic. This inflammatory condition is induced by subcutaneous implantation of compressed cotton pellets in rats. Days after implantation, granuloma masses can be observed along with exudates. The amount of newly formed connective tissue can be measured by weighing the pellets after removal. The rats were divided into five groups (n = 5), fasted overnight, but allowed free access to water. The animals were administered the vehicle, standard drug and omega-3 oil extract. One hour after the first dosing, the animals were anesthetized with anesthetic ether then one each of 20 mg of sterile cotton pellet was inserted in each axilla of the rats by making small subcutaneous incision. The incisions were then sutured appropriately [6], the animals were sacrificed by excess anesthesia on 8th day and cotton pellets were removed surgically. Pellets were then separated from extraneous tissue and dried at 60℗C until weight became constant. The net dry weight, which is after subtracting the initial weight of the cotton pellet, was determined. The average weight of the pellets for both control and test groups were calculated. The percent inhibition of the granuloma weight relative to vehicle control (which is the anti-inflammatory activity) was determined and statistically evaluated [7], using the formula:

$$\% \text{ inhibition} = Wc - Wd/Wc \times 100.$$

Where;
Wd = difference in pellet weight of the treated group
Wc = difference in pellet weight of the control group.

2.3. Statistical analysis

Analysis was performed using Graph Pad Instat software and subjected to Bonferroni test for the separation of means. Results were expressed as Mean ± SEM. Differences between means were considered statistically significant at $p < 0.05$.

3. Results

Table 1 Anti-Granuloma Activity of Omega-3-Fatty Acid oil extract using Cotton Pellet Model

Treatment Groups	Change in weight of cotton pellet (mg)		
	Wet weight of cotton pellet (mg)	Dry weight of cotton pellet (mg)	Net difference (mg)
1	79.75 ± 4.50^{b}	58.36 ± 3.52^{c}	21.39 ± 2.30^{c}
2	88.26 ± 3.30^{c}	65.40 ± 1.30^{d}	22.86 ± 2.00^{c}
3	75.99 ± 1.40^{b}	57.78 ± 3.89^{c}	18.21 ± 1.32^{b}
4	70.43 ± 2.30^{a}	50.93 ± 0.60^{b}	19.50 ± 1.70^{b}
5	65.68 ± 1.21^{a}	42.17 ± 0.70^{a}	23.51 ± 0.92^{d}
6	100.33 ± 1.10^{d}	88.14 ± 0.70^{e}	12.19 ± 0.50^{a}

Values are reported as Mean of cotton pellets ± SEM. Columns with the same superscripts are not statistically significant ($p<0.05$). Groups 1, 2, 3- 300 mg, 600 mg, and 900 mg omega 3 fatty acid oil extract respectively. Group 4- 300 mg+ 20 mg standard Celecoxib. Group 5- 20 mg standard Celecoxib. Group 6 -2 ml PBS solution. Net difference, also referred to as the transudative phase is the net difference between the wet and dry (exudative and proliferative respectively) weights of cotton pellets as a means to measure the weight of the granuloma formed due to the inflammation. This method is often regarded as a chronic model of inducing inflammation.

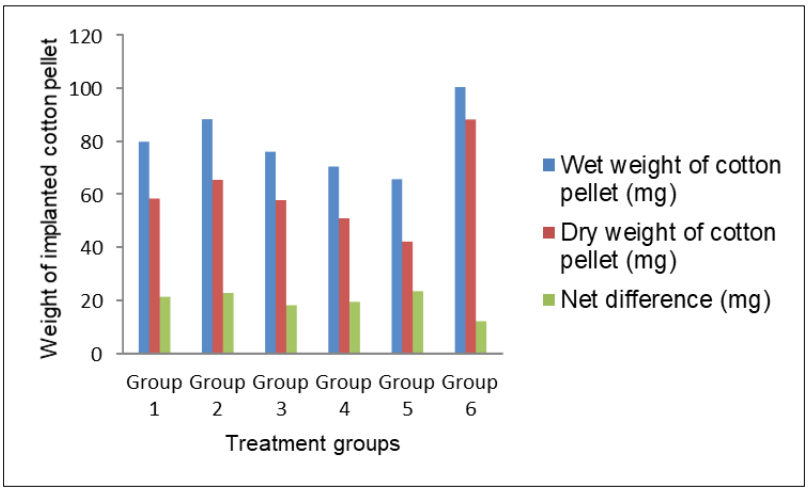

Figure 1 Anti-granuloma effect of the extracts and standard in the reduction of granuloma formation. Here, cotton implantation was carried out for anti-inflammatory activity of both extracts and standard used. This graph shows a significant reduction in the weight of the pellets in the experimental groups when compared to the control

Table 2 Percentage inhibition (%) of the extracts on granuloma formation

Treatment Groups	Inhibition of Granuloma formation		
	Granuloma wet weight (%)	**Granuloma dry weight (%)**	**Increase in Percentage inhibition (%)**
1	20.51	33.78	13.27
2	12.03	25.79	13.76
3	24.25	34.44	10.19
4	29.80	42.21	12.41
5	34.53	52.15	17.62

Percentage inhibition of granuloma formation using the cotton pellet implantation method. Percentage inhibition was calculated relative to the control group and for all stages: dry, wet and the net difference.

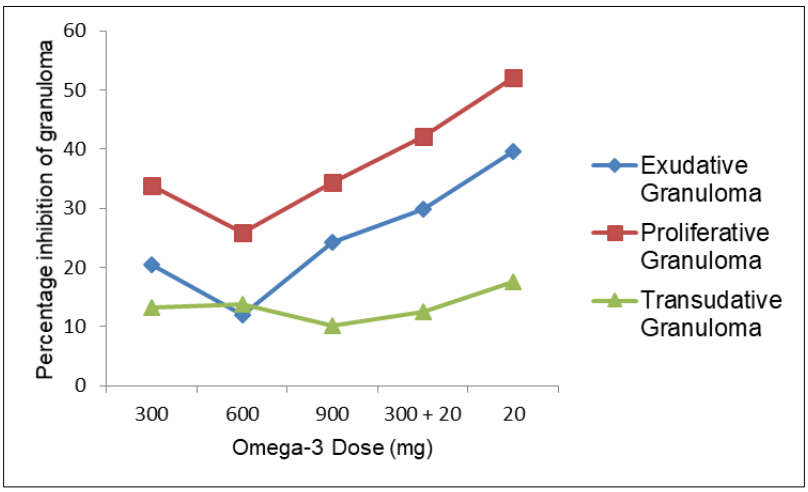

Figure 2 Dose-response relationship of the effect of Omega-3 on the weight of inflammatory exudates as percentage inhibition in cotton pellets-induced granulomas in rat models

4. Discussion

The effect of omega-3 fish oil extract on cotton-pellet induced granuloma in rats is shown in Table 1 and figure 1. The exudative weight of the granuloma for the control group was found to be 100.33 ± 1.10 mg and proliferative weight was 88.14 ± 0.70 mg with a net difference of 12.10 ± 0.50 mg. Treatment with fish oil effectively decreased the exudative weight of granuloma with the standard group (group 5), exhibiting maximum reduction at 65.68 ± 1.12 mg which is not significantly different ($p < 0.05$) from group 4 at 70.43 ± 2.30mg. Both groups have proliferative weight of 42.17 ± 0.70 and 50.93 ± 0.60mg with net difference of 23.51 ± 0.92 and 19.50 ± 1.70 mg respectively. Table 2 and figure 2 showed that all groups administered fish oil inhibited granuloma tissue formation at exudative and proliferative stages with group 5 exhibiting maximum inhibition in all the phases, closely followed by group 4.

Cotton-pellets implantation model often involves the exudation of fluid, containing mostly proteins to the implantation site and macrophage proliferation, results obtained in this study show that the fish oil extract used is effective in suppressing proliferation of the granuloma formed as a result of the implantation thereby making it a natural supplement capable of inhibiting inflammation. The net difference in the granuloma formed for the groups were 21.39 ± 2.30, 22.86 ± 2.00, 18.21 ± 1.32, 19.50 ± 1.70, 23.51 ± 0.92 and 12.19 ± 0.50mg. Group 4, which was administered the combined dose of fish oil and standard celecoxib, has the same rate of granuloma formation that is not statistically significant ($p < 0.05$) from the positive control. The results of this finding showed the effectiveness of omega-3 PUFAs in suppressing fibroblasts and preventing angiogenesis thereby making it an effective and potent supplement capable of inhibiting granuloma formation and suppressing the proliferative phase of inflammation process. This is consistent with literature since it has been reported that in chronic inflammation, the efficacy of anti-inflammatory agents can be indicated by its inhibition of fibroblasts, infiltration of neutrofils and exudation [8; 9].

Studies have shown that in animal models of acute and chronic inflammation, the effects of n-3 long chain polyunsaturated fatty acids (LC-PUFAs) and their bioactive mediators have been demonstrated [10; 11]. Also, supplementation of patients diagnosed with Alzheimer's disease (AD) with a DHA-rich diet led to a reduced release of pro-inflammatory cytokines from blood mononuclear leukocytes [12]. Furthermore, a diet rich in EPA attenuates the production of the pro-inflammatory cytokine interleukin (IL)-1b and improves synaptic plasticity impairment in the hippocampus of old rats [13; 14]. Above studies have shown that supplementation with PUFAs confers a certain anti-inflammatory effect just as our study has shown.

Figure 2 shows the dose-dependent relationship of the anti-granuloma activities of omega-3 oil extract. The plot showed linearity between 600 mg through 900 mg to the supplementation and standard. In table 2, granuloma formation was significantly inhibited by the oil extract and this showed synergy in activity as the control. All omega-3 doses decreased the formation of inflammatory exudates in a dose-dependent manner. This finding is consistent with some other works in literature [15]. The results showed that the larger the difference between the exudative and proliferative weights (net difference), the higher the percentage inhibition. Since utilization of animal models are considered as standard approach for therapeutic assessment, it is therefore suggestive that omega-3 has potential anti-proliferative activities because it attenuates inflammatory cascade significantly.

5. Conclusion

The positive effect observed in this study supports the use of omega-3 polyunsaturated fatty acids in treating different pathologies of inflammatory disorders. The formation of granuloma at incision sites was observed and inhibition of these granulomas was also noticed with the extract used. We therefore conclude that omega-3-polyunsaturated fatty acid showed efficacy in preventing granuloma formation associated with chronic model of inflammation.

Compliance with ethical standards

Acknowledgments

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Disclosure of conflict of interest

Authors declare no conflict of interest.

Statement of ethical approval

Animals were handled according to prescribed ethical guidelines for Care and Use of Laboratory Animals and approved by the University ethical committee with approval No CHSREC/2023/0008.

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