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Original Research Article

Evaluation of anti-inflammatory activity of docosahexaenoic acid on carrageenan induced paw oedema in rats

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ABSTRACT

Background: Inflammation is a tissue reaction to infection, irritation of foreign substance. It is a part of the host defence mechanism but if in excess it becomes harmful. Docosahexaenoic acid (DHA) is omega-3-derivative of alpha-linolenic acid. This study was conducted to determine the anti-inflammatory activity of DHA and its comparison with standard drug diclofenac.

Methods: The study was carried out by using inflammatory models in male albino rats. Rats were divided into 4 groups of 6 animals of each. The anti-inflammatory activity was studied with carrageenan induced rat paw edema. The anti-inflammatory activity of DHA was compared with standard drug diclofenac. The study parameters for acute inflammation was assessment of reduction in inflammation and the percentage inhibition of the paw edema.

Results: DHA 200 mg/kg, DHA 300 mg/kg, and diclofenac 10 mg/kg showed 58%, 64%, and 67% reduction in paw volume, respectively. The DHA showed significant ($p < 0.05$) anti-inflammatory activity in both dosages as compared to control and was dose dependent.

Conclusions: DHA produced dose dependent anti-inflammatory activity which suggest its probable use in the treatment of inflammation.

Keywords: Docosahexaenoic acid, Carrageenan, Plethysmograph, Alpha-linolenic acid, Anti-inflammatory activity

INTRODUCTION

The inflammatory process is the response to an injurious stimulus. It can be evoked by a wide variety of noxious agents like infections, antibodies, physical injuries. It is a part of the host defense mechanism but when it is in excess it becomes harmful. The classical symptoms of inflammatory process include calor (warmth), dolor (pain), rubor (redness), and tumor (swelling).¹

There are several mechanisms that are known to cause inflammatory reaction like release of histamines, bradykinin and prostaglandins. Currently available anti-inflammatory drugs such as steroids and NSAIDs drugs are not useful in many inflammatory disorders, because of their adverse effect, economy and potency.²

Docosahexaenoic acid (DHA) is an omega-3 derivative of alpha-linolenic acid (ALA). It is high quality easily digestible nutrient mostly found in marine algae, fish oil, mother's milk and at low amount in meat and egg.³ Endogenous synthesis of DHA from ALA is very low in human due to mammalian cells lack of the specific enzymes required for the de novo synthesis of ALA, the precursor for DHA.⁴ Therefore, commercially DHA is manufactured from microalgae from genus *Cryptocodinium* and *Schizochytrium*.⁵

DHA is a primary structural component of the human brain, cerebral cortex, skin, sperm, testicles and retina.⁶ It is important for the maintenance of the brain and of learning during aging.³ DHA possess anti-inflammatory properties and may alter lymphocyte, monocyte, and

macrophage functions.⁶ It also inhibits the expression of inflammatory markers such as pro inflammatory cytokines, monocyte adhesion to endothelial cells, and cell adhesion molecules particularly vascular cell adhesion molecule-1 and E-selectin.⁷

Very few studies are conducted in the past to find the effect of pure DHA in rodents and neither has compared its effect with established anti-inflammatory activity. Therefore, present study is planned to find the effect of pure DHA supplementation in inflammation and to compared its efficacy with standard anti-inflammatory drugs.

METHODS

Chemical and drugs

DHA (IUPAC name: all cis- docosa- 4,7,10,13,16,19-hexaenoic acid, trivial name- cervonic acid, chemical formula: C₂₂H₃₂O₂, and molecular weight: 328.488) was obtained from green heaven India (an herbal manufacturing unit). Diclofenac (Tab. Voveran 50 mg- Novarits pharmaceuticals Pvt. Ltd.) were purchased from market. Distilled water and gum acacia 2% of standard quality were used. Carrageenan drug were obtained from government autonomous ayurvedic college and hospital, Gwalior. All drugs were administered as 2% gum acacia suspension.

Experimental animals

Healthy albino rats (100-150 gram) available in our institutional animal house were used for this study. Mice were housed in clean polypropylene cages and were accommodated in a controlled environment (26°-28°C) with a 12 hours light and dark cycle and provided with food and water. The experimental protocol was approved by institutional animal ethics committee (IAEC) of G.R.M.C. Gwalior registration no: 846/GO/Ere/s/04/CPCSEA.

Experimental protocol

The study was carried out in 24 male Albino rats which were randomly divided into 4 groups of 6 mice each. Gum acacia (2%, 0.5 ml) suspension was used as a

vehicle in group 2, 3, and 4. All drugs were given orally via oral gavage. Group 1 (control group) received 2% GA suspension. Group 2,3 and 4 received DHA 200 mg/kg, DHA 300 mg/kg and diclofenac 10 mg/kg. Animals were fasted for 12h before each study. All the groups received the respective treatments for a period of 30 days. On the 30th day the effect of the drug on inflammation was observed. Carrageenan induced hind paw edema is a standard experimental model of acute inflammation. Moreover, this experimental model exhibits a high degree of reproducibility.⁸ Paw volume was measured by using mercury plethysmograph.

The pre starved animals received 0.1 ml of 1% w/v carrageenan into the left hind paw one hour after drug administration. The paw volume was measured at 0, 1, 2, 3, and 4 hours after the injection of carrageenan. Anti-inflammatory effect of DHA was calculated by the following equation.

$$\text{Anti-inflammatory activity (\%)} = (1-D/C) \times 100$$

Where D= (%) difference in paw volume after the administration of drugs and C= (%) difference of volume in the control group.¹⁰

Statistical analysis

Paw volume were given as mean \pm SEM. All the groups were analyzed by one-way ANOVA followed by Tukey's multiple comparison test. P<0.05 was considered statistically significant.

RESULTS

Table 1, shows the anti-inflammatory activity of DHA. The mean difference in paw volume was calculated by subtracting final paw volume (four hours after drug administration) from initial paw volume for each group. The mean difference in paw volume was less for all doses of the DHA and standard drug as compared to control. The final paw volume was compared using one-way ANOVA (F=54.99, p<0.001).

Post hoc Tukey's test revealed that there was significant difference in reduction of paw volume as compared to the control.

Table 1: Effect of DHA on carrageenan induced paw edema in rats.

Groups	Mean normal paw volume (ml)	Mean paw volume 4 hrs after inflammation	Mean increase in paw volume (ml)	(%) paw edema
GA10	0.41 \pm 0.03	1.43 \pm 0.02	1.02 \pm 0.01	100
DHA200	0.52 \pm 0.05	1.03 \pm 0.06	0.50 \pm 0.07*	41.30
DHA300	0.52 \pm 0.05	0.89 \pm 0.07	0.36 \pm 0.04*	35.70
DCF10	0.51 \pm 0.05	0.82 \pm 0.07	0.31 \pm 0.03**†	32.51

GA10= gum acacia 10 ml/kg; DHA200 and DHA300= docosahexaenoic acid 200 mg/kg and 300 mg/kg respectively; DCF10= diclofenac 10 mg/kg (standard drug). *p<0.01 as compared to GA10, †p<0.05 as compared to test drug DHA 200 mg/kg.

Figure 1, shows the percentage inhibition of paw volume of different groups. At the dose of 200 mg/kg and 300 mg/kg DHA produced inhibition of paw volume of 50.98% and 64.71% respectively. Standard drug diclofenac produced 69.67% inhibition of paw edema and it was significant with control and DHA200. DHA produced a dose dependent increase in the percentage inhibition of paw volume; however, it was not statistically significant. The anti-inflammatory effect exhibited by DHA was not superior to that of standard drug.

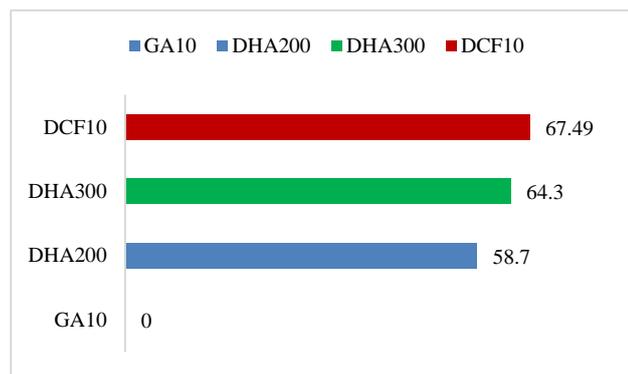


Figure 1: Percent anti-inflammatory activity of DHA.

DISCUSSION

The beneficial effect of the DHA in maintaining various bodily functions are well documented. DHA is used as dietary supplements in various food products and baby formulas to enhance their brain development and cognitive functions. Other than brain enhancement DHA also have anti-depression, anti-inflammatory, anti-cancer and immunological actions.⁹ Although the literature presents several studies on the inflammatory effects of omega-3 PUFA, there are only few studies on anti-inflammatory effect of DHA and most of the studies are in-vitro. Such findings induced us to conduct this work, relating the anti-inflammatory activity of DHA on experimental models of inflammation.

In this study DHA has been revealed a significant acute anti-inflammatory activity by inhibiting the increase in the paw volume induced by carrageenan. Carrageenan induced edema is a biphasic response. In the first phase there is release of histamine, serotonin and kinins whereas in the second phase release of PG and slow reacting substances occurs which is peak at 3 h.¹⁰ Nitric oxide (NO) also plays an important role in carrageenan induced paw inflammation. Inducible NO synthase expression and subsequent production of NO maintains the inflammation.¹¹

Present study showed DHA 300 mg/kg produced 64.3% significant paw edema inhibition. Nakamura et al study showed that administration of 4 g/kg/day of DHA as a high fat dietary supplement to rats for 5 or 15 days cause reduction of edema by 50% as compared to control in

carrageenan-induced paw edema model.¹² Nauroth et al reported that administration of a DHA- dietary supplementation in a dose of 800 mg/kg/day for 4 weeks reduced the production of the proinflammatory cytokines IL-1b and TNF-a and that DHA showed significant 30% reduction carrageenan-induced edema. Their studies also show that DHA had no effect on COX-2 expression, but PGE2 levels were reduced by DHA.¹³ Nobre et al, observed that administration of omega 3 supplementation reduced the carrageenan-induced edema by 40%. They showed that the omega-3 supplement significantly decreased MPO release which cause inhibition of COXs, leading to a reduced release of proinflammatory cytokines, such as TNF- α through in-vitro studies.¹⁴ Another in-vitro study done by Zhao et al evaluated that DHA have an inhibitory effect on LPS-stimulated inflammatory response, cause inhibition of NF- κ B activation via PPAR γ activation and concluded that these are the possible mechanism of anti-inflammatory effect of DHA.¹⁵ There are some other studies which stated that DHA have anti-inflammatory properties such as Weldon et al.¹⁶

The results of our study are in accordance with previously done studies enumerated above on anti-inflammatory effects of DHA. The mechanism of anti-inflammatory activity of DHA in carrageenan induced paw edema in our study may be due to similar action shown by them.

CONCLUSION

DHA produced dose dependent anti-inflammatory activity which suggest its probable use in the treatment of inflammation.

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Conflict of interest: None declared

Ethical approval: The study was approved by the Institutional Ethics Committee

REFERENCES

1. Brunton LL, Chabner BA, Knollmann BC. Goodman and Gilman's The Pharmacological Basis of Therapeutics. New York City. The McGraw-Hill Companies, Inc; 2011: 959-960.
2. Dhalendra G, Satapathy T, Roy A. Animal Models for Inflammation: A Review. *Asian J Pharm Res.* 2013;3(4):207-12.
3. Horrocks LA, Yeo YK. Health benefits of docosahexaenoic acid (DHA). *Pharmacol Res.* 1999;40(3):211-25.
4. Docosahexaenoic Acid (DHA): An Ancient Nutrient for the Modern Human Brain Joanne Bradbury, *Nutrients.* 2011;3:529-54.
5. Martek Biosciences Corporation (5 April 2007). 'History of Martek'. Archived from the original on February 5, 2007. Retrieved March 10, 2007.
6. Guesnet P, Alessandri JM. Docosahexaenoic acid (DHA) and the developing central nervous system

- (CNS)-Implications for dietary recommendations. *Biochimie*. 2011;93(1):7-12.
7. Docosahexanoic acid (DHA) alternative medicine review volume 14, number 4, 2009.
 8. Winter CA, Risley EA, Nuss GW. Carrageenin-induced edema in hind paw of the rat as an assay for antiinflammatory drugs. *Proceedings Society Experimental Biol Med*. 1962;111(3):544-7.
 9. Kumari J, Yadav S, Sharma V. Dcosahexaenoic Acid: wonder dietary supplement. *Int J Therapeutic Applications*. 2012;3:25-31.
 10. Vinegar R, Schreiber W, Hugo R. Biphasic development of carrageenin edema in rats. *J Pharmacol Experimental Therap*. 1969;166(1):96-103.
 11. Salvemini D, Wang ZQ, Wyatt PS, Bourdon DM, Marino MH, Manning PT, et al. Nitric oxide: a key mediator in the early and late phase of carrageenan-induced rat paw inflammation. *British J Pharmacol*. 1996;118(4):829-38.
 12. Nakamura N, Hamazaki T, Kobayashi M, Yazawa K. The effect of oral administration of eicosapentaenoic and docosahexaenoic acids on acute inflammation and fatty acid composition in rats. *J Nutritional Sci Vitaminol*. 1994;40(2):161-70.
 13. Nauroth JM, Liu YC, Van Elswyk M, Bell R, Hall EB, Chung G, et al. Docosahexaenoic acid (DHA) and docosapentaenoic acid (DPA_n-6) algal oils reduce inflammatory mediators in human peripheral mononuclear cells in vitro and paw edema in vivo. *Lipids*. 2010;45(5):375-84.
 14. Nobre ME, Correia AO, Borges BM, Sampaio TM, Chakraborty SA, Goncalves OD, et al. Eicosapentaenoic acid and docosahexaenoic acid exert anti-inflammatory and antinociceptive effects in rodents at low doses. *Nutrition Res*. 2013;33(5):422-33.
 15. Zhao G, Etherton TD, Martin KR, Heuvel JP, Gillies PJ, West SG, et al. Anti-inflammatory effects of polyunsaturated fatty acids in THP-1 cells. *Biochemical Biophysical Res Communications*. 2005;336(3):909-17.
 16. Weldon SM, Mullen AC, Loscher CE, Hurley LA, Roche HM. Docosahexaenoic acid induces an anti-inflammatory profile in lipopolysaccharide-stimulated human THP-1 macrophages more effectively than eicosapentaenoic acid. *J Nutri Biochem*. 2007;18(4):250-8.

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