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Protective Effect of Fish Oil on 4-Nitroquinoline-1-Oxide (4NQO)-Induced Oral Carcinogenesis in Rats

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The present investigation was carried out to study the chemo-preventive potential of fish oil against oxidative stress in 4-nitroquinoline-1-oxide (4NQO) induced oral carcinogenesis by measuring the levels of lipid peroxidation markers, antioxidants, and various serum enzyme activities. Fish oil was administered for 15 days, and changes in the levels of lipid peroxidation markers viz. malonaldehyde (MDA) and conjugated dienes (CD) and antioxidants like superoxide dismutase, catalase, reduced glutathione, glutathione-S-transferase and glutathione peroxidase were evaluated. The serum enzyme activities like alanine transaminase, aspartate transaminase, lactate dehydrogenase, creatine phosphokinase and alkaline phosphatase were also examined in normal and 4NQO-administered rats. A significant increase in the activities of antioxidants and a decrease in the formation of MDA and conjugated dienes were observed in oral tissues on treatment with fish oil. The administration of fish oil also significantly reduced the serum enzyme activities. The antioxidant effect of fish oil might be due to the incorporation of eicosapentaenoic acid which is the major component of fish oil, into the membrane phospholipids of oral tissue, thus protecting the cells from the oxidative stress induced by 4NQO.

Key words : 4NQO, fish oil, eicosapentaenoic acid (EPA), lipid peroxidation markers, antioxidants, serum enzyme activities.

In India, with change in life expectancy and life styles, the cancer incidences have increased steadily. Indian sub-continent accounts for one third of the cases of oral cancer in the world (Abbott *et al.,* 1994). Tobacco and alcohol use are the major risk factors in the development of oral cancer. It is now well recognized that an increased formation of oxygen radicals and other oxygen derivatives are associated with cancer etiology (Abraham *et al.,* 1992). Minimizing oxidative damage may be one of the most important approaches to the primary prevention of carcinogenesis. Epidemiological studies have demonstrated that the intake of antioxidants from food could help to maintain health and to prevent illnesses caused by oxidative stress (Block *et al.,* 1992 and Rimm *et al.,* 1996).

Death from cardiovascular disease and cancer is inversely related to the amount of marine food products consumed and this beneficial effect has been related to high content of n-3 fatty acids (Kromhuout *et al.,* 1985). The n-3 fatty acids are highly unsaturated fatty acids that are prominent constituents of marine fish oils. Biologically active n-3 fatty acids include eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). These two fatty acids are concentrated in the various commercially available forms of fish oil (20 to 60%) and may be highly purified (up to 95%) as individual ethyl esters for research or therapeutic use (Scott *et al.,* 1992). The n-3 fatty acids are important components of cell membranes and they are essential for normal human growth and development (Hardy *et al.,* 1994). Supplementation also results in a rise in the EPA to arachidonic acid ratio and some decrease in the linoleic acid level in serum (Krokan *et al.,* 1993). Studies by Kromhuout *et al.,* (1995) have suggested an antiatherogenic

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potential of n-3 fatty acids. A survival advantage of fish oil treatment has been documented in Indian patients with acute myocardial infarction (Singh *et al.,* 1997).

Nephroprotective action has been reported in animal models of adriamycin-induced (Washiq *et al.,* 1993) and gentamycin-induced (Grauer *et al.,* 1996) renal toxicity. Antimalarial property of n-3 fatty acids has been reported by Kumaratilake *et al.* (1992). Beneficial effects of EPA and DHA in breast cancer (Noguchi *et al.,* 1995; Craig-Schmidt *et al.,* 1993; Shao *et al.,* 1995) and lung cancer were also reported (Abbott *et al.,* 1994). 4-nitroquinoline-1-oxide (4NQO) induced rat tongue carcinogenesis is a suitable animal model for studying cancer chemoprevention. Administration of 4NQO, a water-soluble quinoline derivative, to experimental animals produces a spectrum of preneoplastic and neoplastic lesions in the oral cavity (Tanaka *et al.,* 1991). Various agents, both naturally occurring and synthetic compounds, have been studied for their chemopreventive effects against 4NQO-induced oral carcinogenesis (Balasenthil *et al.,* 2000).

In the present investigation, the fish oil has been chosen as a chemoprotective agent against 4NQO - induced oxidative stress during oral carcinogenesis in rats.

Materials and Methods

Adult male albino Wistar rats, (weighing 80-200g each) were used in this study. The rats were maintained in a controlled environment under standard conditions of temperature and humidity with an alternating light and dark cycle. Throughout the experimental period, the animals were fed with a balanced commercial diet (Hindustan Lever Ltd., Mumbai, India) and water *ad libitum*. The animals used in the present study were maintained in accordance with the guidelines prescribed by the National Institute of Nutrition, Indian Council of Medical Research, Hyderabad, India and approved by the Institutional Animal Ethics Committee.

4NQO was obtained from Fluka, Switzerland. Menhaden fish oil was obtained from Sigma Chemical Company, St. Louis Missouri, USA. All other chemicals used were of analytical grade.

A total of 24 rats were used and were divided into 4 groups of 6 rats each, and treated as below.

Group I: Normal untreated rats

Group II: Normal rats administered with 0.05 ml of menhaden fish oil per animal orally by gastric intubation

Group III: 4NQO administered rats

Group IV: 4NQO treatment followed by administration with fish oil (0.05 ml of menhaden fish oil per animal).

Third and fourth group animals were given 4NQO (0.5% in olive oil) treatments by applying to the oral cavity on daily basis for three months. After three months, the 4NQO treatment was stopped. Second and fourth group animals were given 0.05 ml of menhaden fish oil orally by gastric intubation for 15 days. No visible irritation or restlessness was observed following the administration of the oil. No noticeable adverse effects were observed in any of the animal after the treatment. On the 16th day, the animals were deprived of food overnight and sacrificed by decapitation. Blood was collected from jugular vein and serum was separated.

The serum was used for the assay of alanine transaminase (ALT), aspartate transaminase (AST) (Reitman and Frankel, 1959), lactate dehydrogenase (LDH) (Nieland, 1959) and creatine phosphokinase (CPK) (Okinaka *et al.,* 1961). The tissue homogenate from the oral tissue was used for the assay of thiobarbituric acid reactive substances (TBARS) (Moore & Roberts., 1998), conjugated dienes (Nichans & Samuelson, 1968),

superoxide dismutase (SOD) (Kakkar *et al.,* 1984), catalase (CAT) (Sinha, 1972), glutathione peroxidase (GPx) (Rotruck *et al.,* 1984), glutathione-S-transferase (GST) (Habig *et al.,* 1974) and glutathione (Ellman, 1959).

All data were expressed as mean ± SD. The statistical significance was evaluated by one-way analysis of variance (ANOVA) using SPSS version 9.0 (SPSS, Cary, NC, USA) Student's t test.

Results and Discussion

Table 1 depicts the levels of serum diagnostic marker enzymes (ALT, AST and LDH, ALP and CPK) in normal and experimental rats. 4NQO-induced oral carcinogenic rats showed a significant increase in the level of these marker enzymes. No significant alterations were observed in rats treated with fish oil alone; whereas fish oil administration in carcinogenic rats significantly decreased these enzyme activities.

The levels of lipid peroxidation markers and antioxidant levels are presented in Table 2. The lipid peroxidation marker (MDA and CD) levels significantly increased in the oral tissue of 4NQO-induced rats. Treatment with menhaden fish oil significantly decreased the levels of lipid peroxidation markers. No significant difference in the levels was observed in rats treated with fish oil alone.

The activities of SOD, CAT, GPx, GST and GSH in the oral tissue of experimental animals are shown in Table 2. Reduced levels of antioxidants were observed in 4NQO-treated rats. Oral administration of menhaden fish oil elevated the antioxidant levels in the oral tissue. The antioxidant levels in normal rats treated with fish oil alone were not significant when compared to control rats.

Involvement of free radicals and the role of these toxic agents in lipid peroxidation and antioxidant defense system during carcinogenesis have been studied extensively (Devasagayam *et al.,* 2004 and Collins, 2005). In the present study, it was observed that pretreatment with fish oil offered protection against 4NQO - induced lipid peroxidation during oral carcinogenesis in albino rats.

Increased concentration of the markers was observed in the serum of 4NQO-treated rats. The increased activity of these enzymes in serum could have been due to the leakage of the enzymes from the damaged liver into the circulation. This shows that chronic treatment with 4NQO for induction of oral carcinogenesis might have caused liver damage. Similar results were reported earlier by Balasendhil *et al.* (2001). In the present study, administration of fish oil tends to bring the activity of marker enzymes back to near normal. This clearly indicates that fish oil may inhibit oxidative stress of hepatic tissues and protects liver from the 4NQO-induced damage, which might be attributed to the presence of antioxidants, such as vitamin E and squalene.

Table 1. Effect of fish oil on the serum enzyme activities of 4-NQO treated rats

Groups	AST	ALT	LDH	CPK	ALP
Group I	27.9 ± 2.1	29.6 ± 1.8	39.4 ± 5.3	19.9 ± 2.7	47.8 ± 4.7
Group II	26.4 ± 2.2^{NS}	30.4 ± 1.9 ^{NS}	37.9 ± 4.8 ^{NS}	17.1 ± 2.9 ^{NS}	45.9 ± 5.1 ^{NS}
Group III	58.9 ± 2.3***	61.8 ± 2.2***	82.9 ± 5.3***	45.6 ± 2.5***	79.7 ± 4.3*
Group IV	31.2 ± 1.9***	37.5 ± 1.7***	41.4 ± 3.9***	22.6 ± 2.9***	43.9 ± 5.1**

Group I- Normal untreated rats; Group II- treated with fish oil; Group III-4NQO treated rats; Group IV-4NQO + fish oil. Values are given as mean ± S.D for 6 rats in each group. Values are expressed as follows: serum AST, ALT, LDH, CPK and ALP-IU/L. AST- aspartate transaminase;ALT- alanine transaminase; LDH- lactate dehydrogenase. CPK-creatine phosphokinase; ALP-alkaline phosphatase.

(*** $p<0.001$, ** $p<0.01$, * $p<0.05$, NS - Not significant}

Table 2. Protective effect of fish oil on 4NQO - induced oxidative stress in oral tissue in experimental rats.

Parameters	Group I	Group II	Group III	Group IV
MDA	2.7 ± 0.6	2.9 ± 0.5 ^{NS}	5.68 ± 0.9**	3.1 ± 0.4***
Conjugated dienes	17.9 ± 1.5	17.2 ± 1.6 ^{NS}	39.3 ± 1.4 ***	19.3 ± 1.2 ***
SOD	6.4 ± 0.9	6.1 ± 0.7 ^{NS}	4.2 ± 0.6***	5.9 ± 0.4***
Catalase	1.2 ± 0.3	1.4 ± 0.4 ^{NS}	0.68 ± 0.2***	1.19 ± 0.3***
Glutathione	43.3 ± 2.3	42.5 ± 3.2 ^{NS}	31.7 ± 2.2***	39.7 ± 2.2*
GST	1.83 ± 0.4	1.85 ± 0.6 ^{NS}	0.96 ± 0.4***	1.68 ± 0.3***
GPX	9.1 ± 0.6	9.8 ± 0.7 ^{NS}	5.8 ± 0.4***	7.89 ± 0.7***

Group I- Normal untreated rats; Group II- Treated with fish oil; Group III-4NQO treated rats; Group IV-4NQO + fish oil. Values are expressed as mean ± S.D for 6 rats in each group. Values are expressed as follows: MDA- picomoles/dl; Conjugated dienes- nmoles/mg protein; SOD (superoxide dismutase)-units/mg protein/min; CAT (catalase)- μ mole of H_2O_2 utilized/min/mg protein; GSH (gluthione)- n moles /g of tissue; GST (glutathione-S-transferase)- μ mole of CDNB (1-chloro-2,4-dinitrobenzene) conjugated/min/mg protein; GPX (gluthione peroxidase)- nmoles of GSH oxidized/min/mg protein. (*** p< 0.001., ** p<0.01., * p<0.05, NS- Not significant}

Increased concentration of the lipid peroxidation markers TBARS and CD were observed in oral tissue of 4NQO - treated rats. Associated with the changes in lipid peroxidation, the 4NQO - treated rat tissues showed decreased activities of the key antioxidants such as SOD, CAT, GPx, GST and the level of GSH, which play an important role in scavenging of free radicals. The decrease in these activities may lead to accumulation of superoxide anion and hydrogen peroxide, resulting in the initiation of lipid peroxidation reaction (Henrikson, 1989). In the present study, administration of fish oil tends to bring the levels of oral tissue peroxidation markers back to near normal. Fish oil pretreatment increased the activity of antioxidant enzymes and may help to control free radicals, as fish oil has been reported to be rich in eicosapentaenoic acid, which stimulates the antioxidant system (Kresium *et al.,* 1996). In addition, in the animals which received fish oil pretreatment alone, no significant alteration was observed in lipid peroxidation levels with slightly higher activities of antioxidant enzymes. It is suggested that the ability of the n-3 fatty acids to modulate the properties of the biological membranes might account for the above effects (Kresium *et al.,* 1996). Fish oil modifies the composition of membrane phospholipids and increases both n-3/n-6 ratio and the double bond index (Lamers *et al.,* 1987). Abraham *et al.* (1992) reported significant increase in the activities of catalase, glutathione-S-transferase and glutathione peroxidase after EPA feeding. They also reported lower levels of lipid peroxides and an increase in reduced glutathione content.

The results of the present study substantiate the protective property of fish oil against oxidative stress induced by 4NQO during oral carcinogenesis. Further studies are required to elucidate the exact mechanism behind possible protective property of fish oil.

References

Abbott, W. G., Tezabwala, B., Bennet, M. and Grundy, D. M. (1994) Melanoma Lung Metastases and Cytolytic Effector Cells in Mice Fed Antioxidant-Balanced Corn Oil or Fish Oil Diets. *Nat. Immun*. **13**, pp 15-28

Abraham, D., Nina, W., Rolf, Kristian. and Berg. (1992) Eicosapentaenoic Acid at Hypotriglyceridemic Dose Enhances the Hepatic Antioxidant Defence in Mice. *Lipids* **27**, pp 968-971

Balasenthil, S., Ramachandran, C. R. and Nagini, S. (2001) Prevention of 4-

Nitroquinoline 1-Oxide-induced Rat Tongue Carcinogenesis by Garlic. *Fitoterapia* **72**, pp 524-531

Balasenthil, S., Arivazhagan, S. and Nagini, S. (2000) Effect of Garlic on Circulatory Oxidant and Antioxidant Status During 4-Nitroquinoline 1-Oxide-Induced Rat Oral Carcinogenesis. *Nutr. Res.* **20**, pp 1581-1589

Block, G., Patterson, B. and Subar, A. (1992) Fruits, Vegetables and Cancer Prevention: a Review of the Epidemiological Evidence. *Nutr. and Cancer* **18**, pp 1-29

Collins, A. R. (2005) Antioxidant Intervention as a Route to Cancer Prevention. *Eur. J. Cancer* **41**, pp 1923-1930

Craig-Schmidt, M., White, M. T., Teer, P., Johnson, J. and Lane, H. W. (1993) Menhaden, Coconut and Corn oils and Mammary Tumor Incidence in BALB/C Virgin Female Mice Treated with DMBA. *Nutr. and Cancer* **20**, pp 99-106

Devasagayam, T. P., Tilak, J. C., Boloor, K. K., Sane, K. S., Ghaskadbi, S. S. and Lele, R. D. (2004) Free Radicals and Antioxidants in Human Health: Current Status and Future Prospects. *J. Ass. Physicians India* **52**, pp 794-804

Ellman, G. L. (1959) Tissue Sulfhydryl groups. *Arch. Biochem. Biophys.* **82**, pp 7077

Grauer, G. F., Greco, D. S., Bhrend, E. N., Fettmen, M. J., Mani, I. and Getzy, D. M. (1996) Effects of Dietary n-3 Fatty Acid Supplementation Versus Thromboxane Synthetase Inhibition on Gentamycin-induced Nephrotoxicosis in Healthy Male Dogs. *Am. J. Vet. Res*. **57**, pp 948-956

Habig, W. R., Pbst, M. J. and Jakpoly, W. B. (1974) Glutathione Transferase. A First Enzymatic Step in Mercaturic acid Formation. *J. Biol. Chem.* **249**, pp 7130-7139

Hardy, S. C. and Kleinsman, R. E. (1994) Fat and Cholesterol in the Diet of Infants and Young Childrens; Implications for Growth, Development and Longterm Health. *J. Pediatr*. **125**, pp S69-S77

Henrikson, R. (1989) *Earth* Food Spirulina. Cited from Recolina ltd. Renore enterprises. Inc. Launa Beach. *California* pp 27-65

Kakkar, P., Das, B. and Viswanathan, P. N. (1984) A Modified Spectrophotometric Assay of Superoxide Dismutase. *Ind .J. Biochem. Biophy.* **21**, pp 130-132

Kresium, S. D., Milner, P. C. and Martin, J. F. (1996) Bleeding Time and Platelet Volume in Acute Myocardial Infarction- a 2 Year Follow-up Study. *Thromb. Haemostas*. **59**, pp 49-52

Krokan, H. E., Bjerv, K.S. and Mork, E. (1993) The Enteral Bioavailability of Eicosapentaenoic acid and Docosahexaenoic acid is as Good from Ethylesters as from Glyceryl Esters in Spiteof Lower Hydrolytic Rates by Pancreatic Lipase *in vitro. Biochim. Biophys. Acta*. 1168, pp 59-67

Kromhuout, D., Bosschieter, E. B. and Coulander, C. D. L. (1985) The Inverse Relation Between Fish Consumption and 20 Year Mortality from Coronary Heart Disease. *N. Eng. J. Me.* **312**, pp 1201-1209

Kromhuout, D., Feskens, E.J. and Bowles, C.H. (1995) The Protective Effect of a Small Amount of Fish on Coronary Heart Disease Mortality in an Elderly Population. *Int. J. Epidemiolet.* **24**, pp 340-345

Kumaratilake, L. M., Robinson, B. S., Ferrante, A. and Poulos, A. (1992) Antimalarial Properties of n-3 and n-6 Polyunsaturated Fatty Acids: *in vitro* Effects on *Plasmodium falciparum* and in Vivo Effects on *P. berghei. J. Clin. Invest.* **89**, pp 961-967

Lamers, J. M. J., Hartog, J. M., Verdouw, P. D. and Hulsmann, W. C. (1987) Dietary Fatty Acids and Myocardial Function. *Basic. Res. Cardiol*. **82**, pp 209-221

Moore, K. and Roberts, L. J. (1998) Measurement of Lipid Peroxidation. *Free Radic. Res.* **28,** pp 659-71

Nichans, W. G. and Samuelson, D. (1968) Formation of Malondialdehyde from Phospholipid Arachidonate during Microsomal Lipid Peroxidation. *Eur. J. Biochem.* **6**, pp 126-130

Nieland, A. (1959) Lactic Dehydrogenase of Heart Muscle. *Methods in Enzymology*, 1, 449-420

Noguchi, P. G., Rose, D. P., Earashi, M., Miyazaki, I. (1995) The Role of Fatty Acids and Eicosanoid Synthesis Inhibitors in Breast Carcinoma. *Oncology* **52**, pp 265-71

Okinaka, S., Kumogai, H., Ebashi, S., Sugita, H., Momoi, H., Toyokura, Y. and Fujie. (1961) Serum Creatine Phosphokinase Activity in Progressive Muscular Dystrophy and Neuromuscular Diseases. *Arch. Neurol*. **4**, pp 520-525

Reitman, S. and Frankel, S. A. (1959) Colorimetric Method for Determination of Serum Glutamate Oxalo Acetate and Glutamic Pyruvic Transaminases. *Am. J. Clin. Pathol.* **28**, pp 56

Rimm, E. B., Ascherio, A., Giovanucci, E., Speigelman, D., Stampfer, M. J. and Willet, D. C. (1996) Vegetable, Fruit and Cereal Fiber Intake and Risk of Coronary Heart Disease Among Men. *J. Amer. med. Assoc.* **275**, pp 447-451

Rotruck, J. T., Pope, A. L., Ganther, H. E. and Swanson, A. B. (1984) Selenium Biochemical Roles as a Component of Glutathione Peroxidase. Science, **179**, pp 588-590

Scott, H. G., John, A. C., Marc, F. and Garret, A. F. (1992) Assessment of the Therapeutic Use of n-3 Fatty Acids in Vascular Disease and Thrombosis. *Chest* **102**, pp 374S-384S

Shao, Y. and Pardini, R. S. (1995) Dietary Menhaden Oil Enhances Mitomycin C Antitumor Activity toward Human Mammary Carcinoma. MS-1. *Lipids* **30**, pp 1035-45

Singh, R. B., Niaz, M. A., Sharma. J. P., Kumar. R., Rastogi, V. and Moshiri, M. (1997) Randomized, Double Blind, Placebo-Controlled Trial of Fish Oil and Mustard Oil in Patients with Suspected Acute Myocardial Infarction: Indian Experiment of Infarct Survival-4. *Cardiovasc. Drugs Ther.* **11**, pp 485-491

Sinha K. A. (1972) Colorimetric Assay of Catalase. *Anal. Biochem.* **47**, pp 389-394

Tanaka, T., Kojima, T., Okumura, A., Yoshimi, N. and Mori, H. (1991) Alterations of the Nuclear Organizer Regions during 4-Nitroquinoline 1-Oxide-Induced Tongue Carcinogenesis in Rats. *Carcinogenesis* **12**, pp 329–333

Washiq, M., Nanishi, F. and Onoyama, K. (1993) Effect of Fish Oil Rich in Eicosopentaenoic Acid on Focal Glomerulosclerosis of Adriamycin-Induced Nephropathy in Rats. *Curr. Therapeutic Res.* **53**, pp 35-41