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Effect of dietary fish oil (Omega - 3 - fatty acid) against oxidative stress in isoproterenol induced myocardial injury in *albino wistar rats*

K Madhana Gopal*[1], M Meganathan[2], J Mohan[2], P Sasikala[2], N Gowdhaman[2], K Balamurugan[3], P Nirmala[4], Vanitha Samuel[4].

[1,2]Asst.professor, Arupadaivedu Medical College and Hospital, Kirmapakkam, Puducherry.
[3] Department of Pharmacy , Annamalai University, Annamalai Nagar, Chidambaram.
[4] Division of Pharmacology, RMMC&H, Annamalai University, Annamalai Nagar, Chidambaram.

ABSTRACT

In the present study, the protective effect of fish oil treatment on the fatty acid composition in isoproterenol (IPH)-induced myocardial infarction was studied in male albino Wistar rats. Rats were injected with Isoproterenol, IPH (8.5 mg/Kg, s.c) twice at an interval of 24hrs to induce myocardial infarction. Fish oil was administered orally at 5% v/w for 30 days after which serum and heart tissue were assayed for clinical marker enzymes in the tissue & in determination of biochemical parameters and the remaining tissue was subjected for histopathological studies.Biochemical assessment of myocardial infarction was done by measuring the activities of lipid peroxides(LPO), reduced glutathione (GSH), glutathione peroxidase(GPX), glutathione-S-transferase(GST), superoxide dismutase (SOD), Catalase(CAT), creatine kinase (CK) and serum enzymes which were significantly elevated in the rats administered with IPH. The fish oil treatment for a period of 30 days decreased the levels of cardiac markers (creatinine kinase and biochemical parameters) and reversed the biochemical lesions induced by IPH. Our study suggests that supplement of fish oil protects myocardium against oxidative stress through its antioxidant defence mechanism and anti thrombotic activity and thereby restores the structural and functional integrity of myocardium.

Keywords: Isoproterenol (IPH*),* OMEGA - 3 - FATTY ACID*,* fish oil, Antioxidant,

Corresponding author
Email: drmadhana@yahoo.co.in

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INTRODUCTION

Cardiovascular diseases form a major health concern in recent years, causing severe illness and death throughout the world. According to the statistics given by WHO (2004) about 16.7 million people around the globe die of myocardial infarction every year, which forms about one-third of the total global deaths. It is predicted that heart disease and stroke will become the leading cause of death and disability world-wide by the year 2020, with the number of fatalities projected to increase more than 20 million a year and to more than 24 million a year by 2030 [1]. A change in human behavior and life style over the last century has resulted in dramatic increase in incidence of CHD.

Prospective studies show that there is an inverse relation between fish intake and mortality from coronary heart disease [2,3]. Fish oil is most widely used as food supplements. Owing to its wide array of biological actions public and scientific interest has been directed towards the role of Fish oil in health promotion and disease prevention. Fish oil has been shown to slow or inhibit the oxidative modification of LDL that is responsible for development and progression of atherosclerosis. Recent studies have shown that Fish oil possesses a variety of cardiovascular effects including decreased platelet aggregation and arterial superoxide generation [4].

It is hypothesized that the highly unsaturated fatty acids, Eicosapentaenoic acid (EPA)and Docosahexaenoic acid (DHA) present in substantial quantities in Mediterranean diet are the active components responsible for this beneficial effect.

In recent years, accumulating evidence indicates that incidence and progression of cardiovascular disease may, to some extent, be modified by dietary means. In particular, attention has been focused on the apparent beneficial effects of Fish oil supplementation in reducing the incidence of cardiovascular disease, especially CHD associated with emotional or environmental stress [5]. Hence present study was conducted to study the cytoprotective action of dietary fish oil in myocardial stress injury induced in experimental animals.

MATERIALS AND METHODS

The animals used were albino wistar male rats with body weights in the range 140-160gm and were housed for 30 days. The rats were divided into 4 groups as follows.

Group - I	-	Control group. Rats were fed with normal diet.
Group -II	-	Isoproterenol control group
Group - III	-	5 % w/v fish oil treated group.
Group - IV	-	10% w/v fish oil treated group.

Group I & II was fed with normal diet and acts as control. Group III & IV rats were fed with 5% v/w & 10% v/w fish oil mixed with standard diet for 30days. Isoproterenol was given at a dose of 8.5mg/Kg s.c twice at an interval of 24 hrs at the end of treatment period.

After 48 hrs of the first dose of isoproterenol, all the rats were sacrificed by cervical dislocation under i.m. Ketamine. Blood samples were collected in centrifuge tubes by retro orbital puncture using sodium citrate as anticoagulant and the plasma separated was used for the determination of diagnostic marker enzymes.

The heart tissue was excised immediately and washed with chilled isotonic saline and homogenised in ice cold 0.1 M Tris-HCl buffer, pH 7.2 for various estimations. Serum glutamate pyruvate transaminase (SGPT), serum glutamate oxaloacetate transaminase (SGOT), LDH and Creatine phosphokinase (CPK) in serum, activities of Lipid peroxides (LPO), reduced glutathione (GSH), glutathione peroxidase (GPX), glutathione-S-transferase (GST), superoxide dismuatse(SOD) catalase(CAT) and malondialdehyde (MDA) in heart were estimated.

Statistical Analysis

The data were analysed by applying students "t" test. All values were reported as mean ±SEM. The statistical significance was set at $P<0.05$.

RESULTS AND DISCUSSION

In the present study the (table no : 1) (Fig No: 1-8) shows ISP administration resulted in marked rise in lipid peroxidation as indicated by increase in LPO and MDA. Following pretreatment with fish oil, (both 5% and 10% V/W of diet) there is significant reduction in the levels of lipid peroxides indicating that fish oil inhibit the lipid peroxidation. Reactive oxygen species formation need the activation of the arachidonic acid cascade via the enzyme 5-lipooxygenase (5-LOX).Fish oil which is rich in n-3 PUFAs i.e. EPA and DHA interfere with arachidonic acid cascade by inhibiting 5-LOX. Incorporation of the n-3 PUFAs with biological membrane, increased antioxidant status normalises the excited state,controls the physical status of membrane lipids and prevent rises in intracellular Ca2+ in response to oxidative stress [6].

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Table no : 1. Effect of fish oil on Cardiac lipid peroxides, antioxidant enzymes and CK-MB in cardio myocytes of rats.

Parameters	Group-I Control	Group-II ISP-treated	Group-III 5% fish oil+ ISP-treated	Group-IV 10% fish oil + ISP-treated
LPO	1.17±0.07	2.39±0.17	1.98±0.24**	1.12±0.07***
GSH	4.12±0.42	2.15±0.11	3.15±0.26**	3.86±0.22***
GPX	2.86±0.22	1.92±0.11	2.64±0.42**	3.32±0.31***
GST	1124±79	723±58	824±26*	1231±106***
SOD	5.65±0.42	2.04±0.17	3.08±0.33**	4.85±0.27***
CAT	8.72±0.52	3.15±0.19	4.64±0.86*	8.24±0.54***
CK-MB	164.2±12.6	58.4±11.2	86.7±8.6**	110.2±11.6***
MDA	67.4±8.6	98.6±8.9	64.3±11.5**	60.4±12.6***

Values expressed:

Levels of lipid peroxides (LPO)	n moles malondialdehyde released/mg of protein.
Reduced glutathione (GSH)	µ moles oxidised /min /mg of protein.
Glutathione peroxidase (GPX)	µ moles of GSH consumed /min /mg of protein.
Glutathione-S-transferase (GST)	µ moles of 1-chloro-2, 4-dinitrobenzene conjugate formed min / mg of protein.
Superoxide dismutase (SOD)	Units /mg of protein.
Catalase (CAT)	µ moles of H_2O_2 decomposed /min/ mg of protein.
Creatine kinase-MB (CK-MB) isoenzyme	IU /mg of protein.
Malondialdehyde (MDA)	n mol/g tissue

The values of tissue LPO, GSH, GPX, GST, SOD, CAT, CK-MB and MDA were compared , all values were expressed as mean ±SEM. n=6 in each group.

*** = P<0.001 highly significant, **= P<0.01 moderately significant, *= P<0.05 significant when group IV were compared with Group II , group III were compared with Group II.

GSH together with GSH dependent enzymes (GPX, GST) and CAT-SOD couple, efficiently scavange toxic free radicals .These enzymes are the first line cellular defense for the effective removal of oxygen stress in intracellular organells. (Table no:1) (FigNo: 1-8) shows reduction in glutathione dependant enzymes and other antiperoxidative enzymes (SOD and CAT) in ISP treated group.This is because all these antiperoxidative enzymes may be utilised in protecting 'SH' containing proteins from lipid peroxides [7]. These enzymes also are structurally and functionally impaired by the free radicals resulting in myocardial damage.The restoration of activities of the antioxidant enzymes in fish oil treated group could be due to its capacity to scavange the toxic free radicals and thereby preventing the myocardial damage (Kohno et al ., 1995) [8].EPA & DHA are highly lipophilic, readily pass across cellular and subcellular membranes, diffuse into intracellular compartments and protects the myocardium from toxic free radicals.

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In table ISP intoxicated rats show rise in serum ALT, AST, LDH & CPK which are well known diagnostic markers of myocardial infarction. This present observation is in line with earlier reported studies (Anandan, 200364), which have shown that the amount of diagnostic markers present in plasma is directly proportional to the number of necrotic cells present in cardiac tissue. Fish oil pretreatment shows a significant reduction in these serum enzyme markers. The cardioprotective activity of fish oil against ISP induced stress injury is also due to its antithrombotic effect mediated through release of prostacyclin (PGI3) and reduction in activity of plasminogen activator inhibitor [9]. It has been shown that the PGI3 is a valuable agent for protecting myocardial tissue during and after ischemia [10].

The results obtained from the above study indicate that supplementation of fish oil along with feed helps the myocardium to withstand the stress generated by ISP, prevents myocardial necrosis and restores the normalcy of the structural and functional integrity of the myocardium.

CONCLUSION

Fish oil supplementation with diet protects myocardium against oxidative stress through its antioxidant defence mechanism and anti thrombotic activity and thereby restores the structural and functional integrity of myocardium.This study endorses that regular consumption of fish or fish oil reduces the risk of cardiovascular diseases and also protects those with existing heart disease against further deterioration or death. So eat fish and live longer.

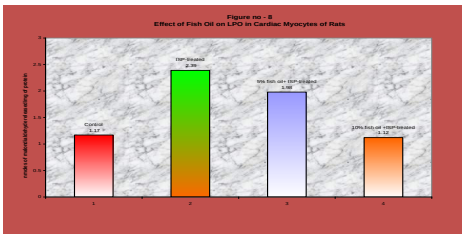

Fig No:1 Effect of fish oil on LPO in cardiac myocytes of rats

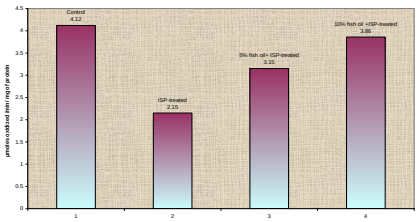

Fig No:2 Effect of fish oil on GSH in cardiac myocytes of rats

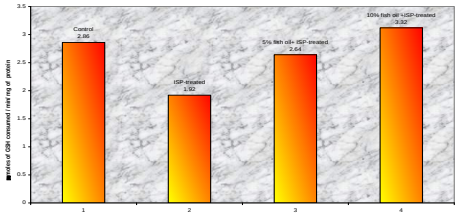

Fig No:3 Effect of fish oil on GPX in cardiac myocytes of rats

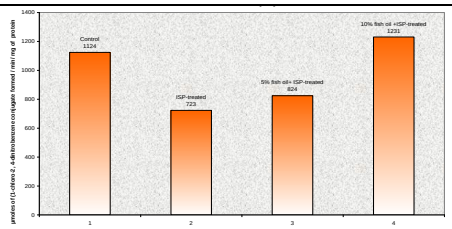

Fig No:4 Effect of fish oil on GST in cardiac myocytes of rats

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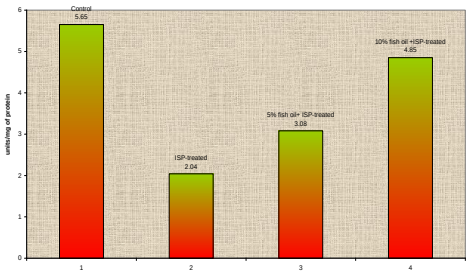

Fig No:5 Effect of fish oil on SOD in cardiac myocytes of rats

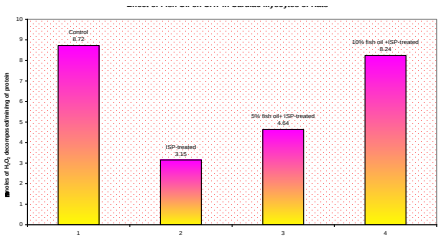

Fig No:6 Effect of fish oil on CAT in cardiac myocytes of rats

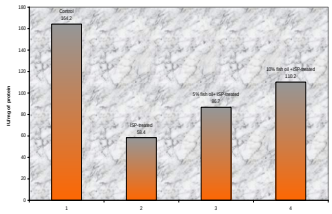

Fig No:7 Effect of fish oil on CK-MB in cardiac myocytes of rats

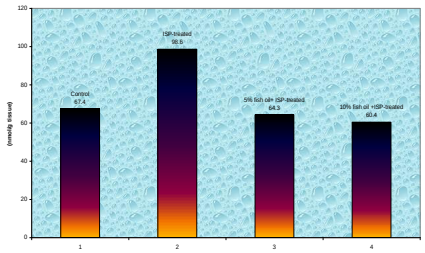

Fig No: 8 Effect of fish oil on MDA in cardiac myocytes of rats

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